



## RESEARCH PAPER

# Pathogens associated with grain discolouration and their management in paddy (*Oryza sativa* L.)

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**Abstract :** Grain discoloration complex disease of rice is an emerging threat to rice crop all over the world and it acquires particular importance as it results in qualitative loss of harvested crop. So the present study was carried out to identify different fungi associated with disease and their management. The discoloured seed samples were collected from different farmer's field during survey. Totally seven fungi including both saprophytic as well as pathogenic were isolated and identified from both blotter and potato dextrose agar method viz., *Curvularia lunata* (36.30 %), *Helminthosporium oryzae* (25.6 %), *Fusarium moniliformae* (19.6 %), *Colletotrichum gloeosporioides* (8.1 %) and *Alternaria* spp. (7.01 %) were predominantly associated with discoloured paddy seeds. Other saprophytic fungi like *Aspergillus* spp. (16.6 %) and *Rhizopus* spp. (12.1 %) were frequently observed. Similarly among the eleven treatment including control, seed treatment with carbendazim 50 WP (2 g kg<sup>-1</sup>) with two sprays of tebuconazole 250 EC at 0.1 per cent concentration and tricyclazole 18 % + mancozeb 62 % WP at 0.2 per cent first spray during flowering stage and second spray during 15 days after first spray reducing discoloured panicle by 61.46 per cent and 59.92 per cent, respectively.

**Key Words :** Paddy, Pathogens, Chemical control, Discoloration, Qualitative, Treatment

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## INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crop of the world both in terms of area and production. About 90 per cent of world rice is grown and consumed in Asian countries and 60 per cent of world population also depends on rice for their half of the calorie intake from this crop. After China, India is the second largest producer of rice in the world.

In rice some of the diseases which were less

significant earlier, are now gradually gaining importance and posing serious threat to the crop production (Gupta *et al.*, 2015). Grain discolouration is prevalent in almost all parts of the world where paddy is grown. It was earlier considered to be a minor disease and it is now gaining more importance due to its severity in tropical rice growing areas. In many regions of India particularly during wet seasons due to high humidity and warm environmental condition during flowering and post

flowering stages which significantly induce the disease.

Seed discolouration is an early indication of poor seed germination or grain quality which is complex disease and cannot be diagnosed prior to harvest, in nature disease is generally associated with many pathogens, among them fungi predominates. Bodalkar and Awadhiya (2014) reported that the seed borne inoculum of *Alternaria alternata* is responsible for grey discolouration and *Cochilobolus miyabeanus* for black discolouration, these pathogens are present in seed coat and endosperm of discoloured seed, whereas *Curvularia lunata* (eye shaped spots), *Fusarium moniliformae* produces pinkish discolouration and *Sarocladium oryzae* is responsible for light brown discolouration which are found in the seed coat, endosperm and embryo of discoloured seed (Sachan and Agrawal, 1995). *Nigrospora oryzae*, *Aspergillus niger*, *Bipolaris oryzae*, *Alternaria padwickii*, *Burkoldaria oryzae* are other pathogens which are responsible for discolouration.

Although grain discolouration diseases has been a minor problem in the past. But in recent years it is becoming a serious problem if no attention is given to the disease. Keeping in view all the factors a field experiment was conducted to evaluate some commercially available fungicides for the effective management of the disease.

## MATERIAL AND METHODS

### Detection of seed mycoflora:

Discoloured grains were collected from different rice growing areas, the seed mycoflora was isolated by using different methods such as Standard blotter paper method and method, Potato dextrose agar method, as recommended by International Seed Testing Association ISTA (1966). Observations were recorded in percent seed infection.

### Standard blotter paper method:

Sterile Petriplates (9 cm) were used in the study, three discs of blotter paper were moistened with sterilized water, excess of water was drained and were placed at the bottom of each Petri plates 25 seeds were placed in each Petri plate with forceps and were incubated at room temperature. For better growth and sporulation of fungal flora 12 h artificial light was provided by placing the plates below the Phillips 40W tubes by alternated with 12 h darkness. After incubation of the plates for seven days, the seeds were examined under stereo binocular

microscope. The pathogens were identified by preparing slides and examining them under compound microscope, following the procedure of Mew and Mishra (1994).

### Potato dextrose agar method:

In this method, seeds were soaked for 5 minute at sodium hypochlorite (1 %) solution and immediately washed with distilled water 3 to 5 times in order to prevent fungal pathogens infection. To detect the mycoflora of the seeds, totally 400 seeds were placed. Each Petri dish was poured with some prepared PDA then with sterile forceps under the hood 10 seeds were placed on PDA. After six days Petri dishes were placed at a temperature ( $28 \pm 1^\circ\text{C}$ ) and periodically for the growth of colonies were observed. The plates were examined for the mycoflora under stereo binocular microscope and compound microscope. Mycelial growth and sporulation were studied. Pure culture and pure colonies which developing from the grains will be transfer to PDA slants and incubating at  $28 \pm 1^\circ\text{C}$  for 10 days. Then such slants will be used for the study the pathogen characters.

### Identification of fungi:

The fungi were identified based on spore morphology and colony characters as suggested by Barnett and Hunter (2007). The per cent seed infection of different pathogens was calculated as under;

$$\text{Per cent seed infection} = \frac{\text{No. of seed infected with the pathogen}}{\text{Total number of seed plate}} \times 100$$

### Management of grain discolouration of paddy under field condition:

The field experiment was conducted in farmer's field with 11 treatments and three replications during *Kharif* 2017 at Medleri village of Ranebennur taluk on Haveri district under irrigated condition to find out the suitable fungicide for managing grain discolouration of paddy. The fungicides which performed well under *in vitro* were selected for field evaluation. Variety was grown as per package of practices. A control plot was maintained. The fungicide solutions were prepared by dissolving known quantity of fungicide in water to get desired concentration. The first spray was taken up at the time of anthesis and second spray at 15 days after first spray. In each replication of the treatment, ten plants were tagged, severity of grain discolouration was recorded one day before the first spray and 15 days after

first spray using 0-9 scale and yield per plot was calculated at harvest of crop. Data was statistically analyzed. Per cent increase in yield was calculated.

**Details of the experiment:**

- Design : Randomized completely block design (RCBD)
- Treatments : 11
- Replications : 3
- Plot size : 4 m × 3 m
- Spacing : 20 cm × 10 cm.
- Genotype : MTU-1010
- Date of sowing : 21/06/2017

**Note:**

First spraying was done at the time of anthesis and second spray at 15 days after first spray. The severity of grain discolouration was recorded by counting the number of infected grains from 10 panicles per plot. The thousand grain weight, yield, seed germination and vigour index was also recorded after harvest.

**RESULTS AND DISCUSSION**

The discoloured seed samples were collected from different farmer’s field during survey. Totally seven fungi including both saprophytic as well as pathogenic were isolated and identified from both blotter and potato dextrose agar method. From the Table 1 it is clear that *Curvularia lunata* (36.30 %), *Helminthosporium oryzae* (25.6 %), *Fusarium moniliformae* (19.6 %), *Colletotrichum gloeosporioids* (8.1 %) and *Alternaria* spp. (7.01 %) were predominantly associated with discoloured paddy seeds. Other saprophytic fungi like *Aspergillus* spp. (16.6 %) and *Rhizopus* spp. (12.1 %) were frequently observed. Mycoflora was more in blotter method as compared to potato dextrose agar method. Based on these findings grain discolouration is complex in which different pathogens are involved. This results

List of treatment combinations for management of paddy grain discolouration	
T <sub>1</sub>	Seed treatment with carbendazim (2 g kg <sup>-1</sup> seed)
T <sub>2</sub>	T <sub>1</sub> + Spray with thiophanate methyl (0.1 %)
T <sub>3</sub>	T <sub>1</sub> + Spray with tebuconazole (0.1 %)
T <sub>4</sub>	T <sub>1</sub> + Spray with chlorothalonil (0.2 %)
T <sub>5</sub>	T <sub>1</sub> + Spray with mancozeb (0.2 %)
T <sub>6</sub>	T <sub>1</sub> + Spray with captan + hexaconazole (0.2 %)
T <sub>7</sub>	T <sub>1</sub> + Spray with tricyclazole + mancozeb (0.2 %)
T <sub>8</sub>	Spray with panchagavya (5 %)
T <sub>9</sub>	Spray with <i>Pseudomonas fluorescens</i> (10 g/ lit)
T <sub>10</sub>	Spray of KNO <sub>3</sub> (0.5 %)
T <sub>11</sub>	Untreated check (control)

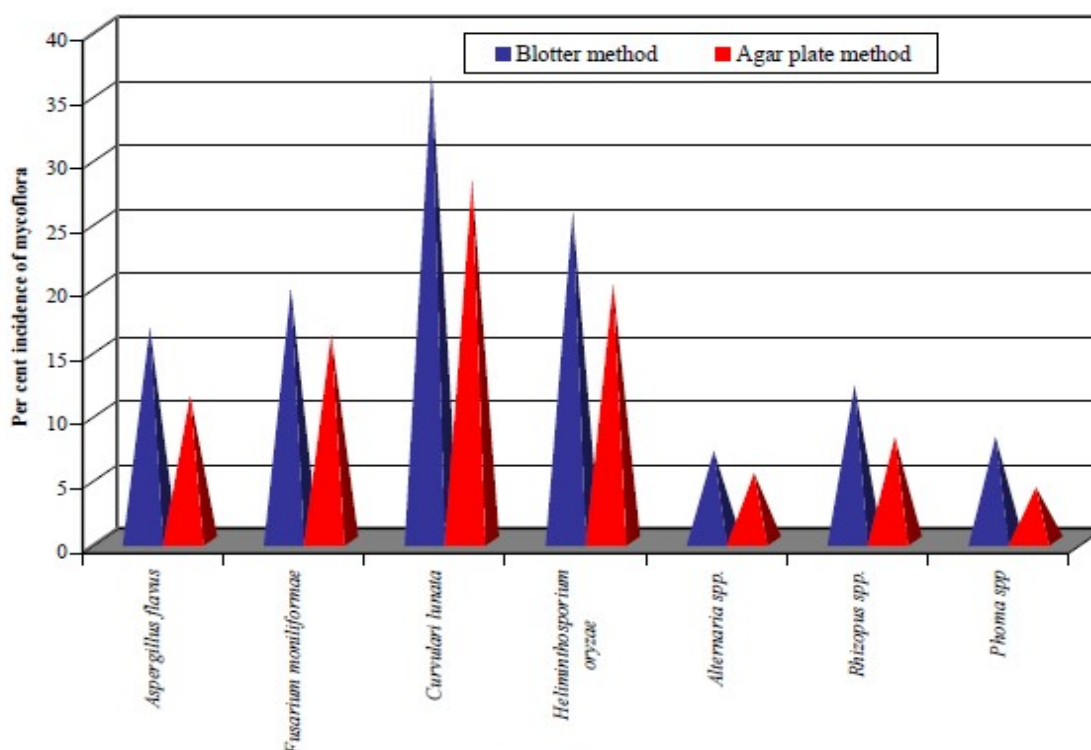


Fig. 1 : Seed mycoflora of paddy detected by seed health testing methods

**Table 1 : Detection of seed mycoflora by seed health testing methods**

Sr. No.	Identified fungi	Per cent seed infection	
		Blotter method	Agar plate method
1.	<i>Aspergillus</i> spp.	16.6	11.3
2.	<i>Fusarium moniliformae</i>	19.6	16.1
3.	<i>Curvularia lunata</i>	36.30	28.1
4.	<i>Helminthosporium oryzae</i>	25.6	20.0
5.	<i>Alternaria</i> spp.	7.01	5.3
6.	<i>Rhizopus</i> spp.	12.1	8.1
7.	<i>Colletotrichum gloeosporioides</i>	8.1	4.2

an agreement with Habib *et al.* (2012) reported rice panicles infested with grain discolouration disease were collected from different rice areas and eight pathogens namely *Bipolaris oryzae*, *Alternaria alternata*, *Alternaria padwickii*, *Drechslera oryzae*, *Fusarium moniliforme*, *Curvularia oryzae*, *Nigrospora oryzae* and *Aspergillus niger* were isolated from diseased

samples.

### Identification of fungi from paddy seeds:

Identification of the associated fungi was carried out by studying the morphological characters with respect to colony, pigmentation and spore morphology. *Fusarium moniliformae* produced white cottony aerial growth on the seed. Blackish grey mycelia covered only part of the seed in case of *C. lunata*. Black mycelial growth on the seed in *H. oryzae* and spores were produced in chain in case of *Alternaria* sp. In *Aspergillus* spp. grew on the hilum region of the seed, later covered the entire seed bearing heavy sporulation without allowing the seed to germinate.

### Management of grain discolouration of paddy under field condition:

Results indicated statistically significant differences among treatments for per cent disease index compared

**Table 2 : Management of grain discolouration of paddy under field conditions during Kharif 2017**

Treatments	PDI		Per cent disease reduction over control	Yield (q/ha)	Per cent increase in yield over control	1000 grain weight (g)	Per cent increase in grain weight over control	B:C ratio
	10 days after first spray	10 days after second spray						
T <sub>1</sub> Seed treatment with carbendazim (2 g kg <sup>-1</sup> seed)	33.86 (35.57)*	31.57 (34.17)	28.05	29.44	13.99	21.67	18.40	1.42
T <sub>2</sub> T <sub>1</sub> + Spray with thiophanate methyl (0.1 %)	26.06 (30.68)	24.57 (29.70)	44.02	28.61	10.77	21.33	16.58	1.33
T <sub>3</sub> T <sub>1</sub> + Spray with tebuconazole (0.1 %)	21.06 (27.30)	16.91 (19.82)	61.46	36.11	39.80	24.77	35.34	1.70
T <sub>4</sub> T <sub>1</sub> + Spray with chlorothalonil (0.2 %)	31.23 (33.96)	29.44 (32.85)	32.90	30.00	16.14	21.23	16.03	1.42
T <sub>5</sub> T <sub>1</sub> + Spray with mancozeb (0.2 %)	24.86 (29.89)	22.67 (28.42)	49.10	32.50	25.82	22.93	25.32	1.54
T <sub>6</sub> T <sub>1</sub> + Spray with captan + hexaconazole (0.2 %)	28.13 (32.01)	25.34 (30.21)	42.25	29.72	15.07	22.40	22.40	1.38
T <sub>7</sub> T <sub>1</sub> + Spray with tricyclazole + mancozeb (0.2 %)	21.34 (27.50)	17.59 (24.78)	59.92	34.44	33.35	23.67	29.33	1.61
T <sub>8</sub> Spray with panchagavya (5 %)	36.53 (37.17)	35.11 (36.32)	20.00	26.94	4.31	19.37	5.83	1.28
T <sub>9</sub> Spray with <i>Pseudomonas fluorescens</i> (10 g/ lit)	35.13 (36.33)	33.70 (35.47)	23.21	27.50	6.47	20.10	9.84	1.33
T <sub>10</sub> Spray of KNO <sub>3</sub> (0.5 %)	30.10 (33.26)	27.02 (31.30)	38.44	27.78	7.54	20.00	9.29	1.00
T <sub>11</sub> Untreated control	39.04 (38.65)	43.89 (41.47)	0.0	25.83	0.00	18.30	0.00	1.25
S.E. ±	1.53	1.64		0.23		1.00		
C.D. (P=0.05)	4.51	4.86		0.70		2.97		

\* Figures in parenthesis indicate arcsine transformed values

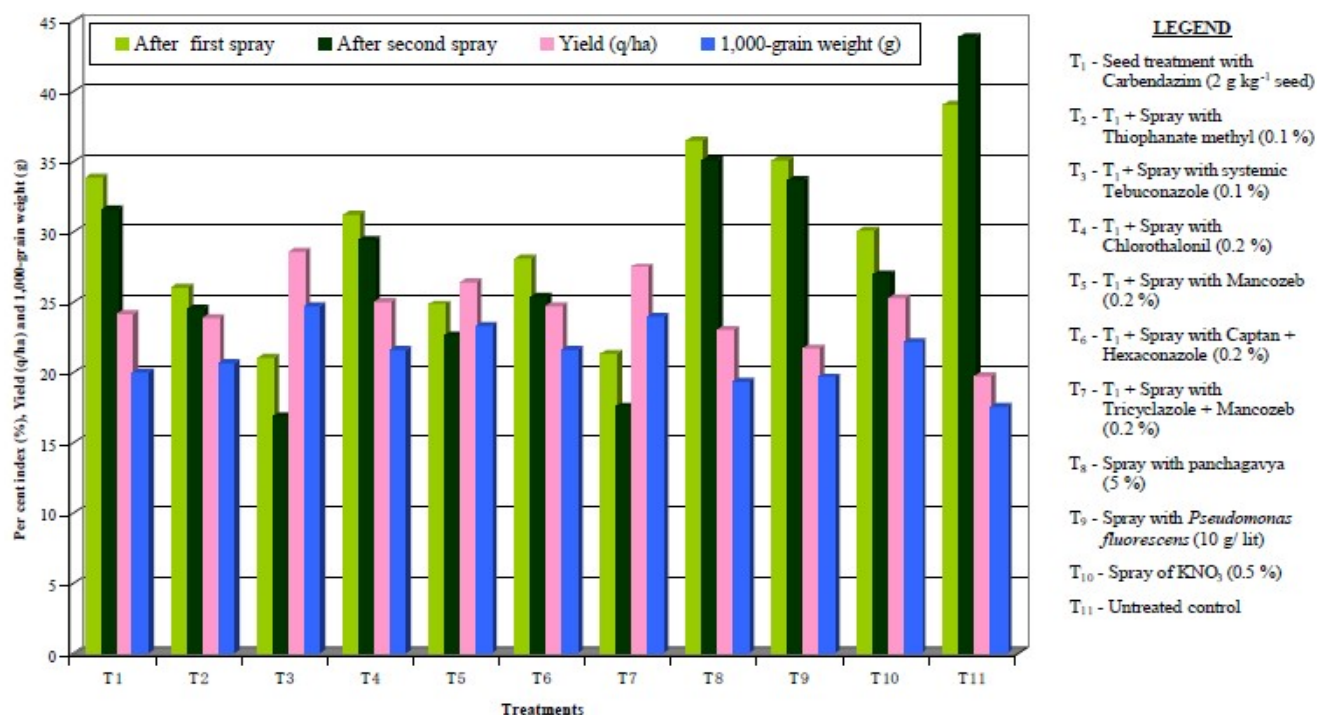


Fig. 2 : Management of grain discolouration of paddy under field conditions

to untreated control (43.89 %). Among the 11 treatments formulated and evaluated treatment (T<sub>3</sub>)- seed treatment with carbendazim 50 WP (2 g kg<sup>-1</sup>) with two sprays of tebuconazole 250 EC at 0.1 per cent concentration, first spray during flowering stage and second spray during 15 days after first spray was showed least per cent disease index (16.91 %) with 61.46 per cent reduction of disease over control and which was on par with tricyclazole 18 % + mancozeb 62 % WP spray at 0.2 per cent was showed least per cent disease index of 17.59 with 59.92 per cent reduction of disease over control and followed by mancozeb 75 WP at 0.2 per cent with per cent disease index of 22.34 with 49.10 per cent reduction of disease over control was recorded. Panchagavya at 5.0 per cent was found maximum per cent disease index (35.11 %). Similar results were reported by Manaskumar and Malaykumar (2010) reported that tebuconazole, propineb, mancozeb were found effective against grain discolouration in the field experiment and improved paddy yield and combination of fungicides (carbendazim + mancozeb) which reduced panicle infection and improve paddy yield.

### Conclusion:

Seven fungi were isolated and identified from both

blotter and potato dextrose agar method. The results revealed that *Curvularia lunata*, *Helminthosporium oryzae*, *Fusarium moniliformae*, *Colletotrichum gloeosporioides* and *Alternaria* spp. were predominantly associated with discolored paddy seeds. Other saprophytic fungi like *Aspergillus* spp. and *Rhizopus* spp. were frequently observed. So, grain discolouration is a complex in which different pathogens are involved. For management of grain discoloration field studies were conducted. seed treatment with carbendazim (2 g/kg) with two sprays of tebuconazole 250 EC (0.1 %) first spray during flowering stage and second spray during 15 days after first spray recorded less PDI with increase yield (36.11 q/ha) and grain weight (24.77 g) with 61.46 per cent reduction of disease over control and which was on par with tricyclazole 18 % + mancozeb 62 % WP spray at 0.2 per cent was showed least per cent disease index of 17.59 with 59.92 per cent reduction of disease over control these two fungicides are best for management of grain discolouration in paddy.

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