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Detection of mycoflora associated with rice grain discolouration

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Abstract
Rice samples collected from Guntur and Nellore districts to analyse the location of inoculum in the grain and biochemical changes observed in storage period. Fungal genera of *Alternaria* sp., *Aspergillus* sp., *Curvularia* sp., *Bipolaris* sp. and *Rhizopus* sp. were isolated from discoloured glumes, endosperm and embryo of discoloured grains by component plating method. In order to assess the portion of the grain colonized by the mycoflora, component plating method was followed. Among the fungi isolated from glumes, *Aspergillus* sp. (37.83%) was predominant followed by *Alternaria* sp. (20.83%), *Curvularia* sp. (17.83%), *Bipolaris* sp. (17.17%) and *Rhizopus* sp. (6.33%). Similarly, *Aspergillus* sp., (48.33%) was the most predominant followed by *Bipolaris* sp. (17.33%), *Alternaria* sp. (15.50%), *Curvularia* sp. (14.33%) and *Rhizopus* sp. (4.50%) in embryo. Similarly in endosperm, *Aspergillus* sp., (50.50%) has dominated followed by *Bipolaris* sp. (22.17%), *Curvularia* sp. (17.17%), *Alternaria* sp. (8.83%) and *Rhizopus* sp. (1.33%).

Keywords: Discolouration, fungi, grains and samples

Introduction
Rice is one of the most important staple foods in the world. Rice in Andhra Pradesh occupies an area of 3.89 M ha with 11.56 M t production and productivity of 2856 kg ha^{-1} (www. indiastat.com, 2014-2015). Grain discolouration is a common problem throughout the rice growing areas which is caused due to various fungal and bacterial pathogens (Ou, 1985) [6]. Prevalence of fungi in freshly harvested and stored paddy were reported to cause discolouration in moderate to severe form thus reducing the fair average quality and market value. Neninger *et al*. (2003) [4] reported *Alternaria* sp. as a field fungi more or less parasitic and infect the grains before harvest and is frequently found on glumes but may cause black spots on the endosperm and may contribute to decrease in grain quality (Notteghem *et al*., 1995) [5]. Halgekar and Giri (2015) [1] observed maximum colonization of seed borne fungi in the seed coat (0-3.30%) and endosperm (0-1.65%). The maximum association of seed borne fungi (*Fusarium* spp.) were reported to be present in endosperm and other fungi found in seed coat (Zare, 2013) [10]. Fungi were isolated from the endosperm and seed coat of black pointed bread wheat kernels at higher rates than from the embryo. *A. alternata* was isolated at higher frequency from endosperm of infected kernel in all cultivars (Ozer, 2004) [7]
In the present scenario of rice cultivation grain discolouration may become a potential threat for rice production. There is need to investigate grain discolouration for better grain and seed quality.

Material and Methods
Rice grain samples collected from Guntur and Nellore districts of Andhra Pradesh were observed for the incidence of grain discolouration by separating the grains into discoloured and healthy (No discolouration). The observations revealed that the discolouration incidence increased with increase in storage period irrespective of the variety used and type of storage. In Tenali division of Guntur district BPT-5204 was collected from two villages at two different locations (Nalluri Palem (NP-1 and NP-2) and Ponna Palle (PP-1) (PP-2) Similarly, in the variety Ankursona, the rice grain samles were collected from Singu Palem (SP-1and SP-2). In Narasaraopet division of Guntur district, BPT 5204 was collected from two villages at two different locations Rentachintala (RE-1 and RE-2) and Gurajala (GU-1 and GU-2). In Nellore district, variety NLR 34449, was collected from four villages (Atmakur- AK, Vegur VE Cheserla- CH and ARS- AR).

Component plating method: Discoloured grains were surface sterilized and soaked individually in sterilized water for 16 h at 25 °C. Each grain was dissected aseptically into different parts *i.e.,* glume, kernel, endosperm and embryo with the help of sterilized lancet and needle. Different parts of each grain were placed on a PDA plate and was incubated at 25 °C. Plates were examined at regular intervals and initial growth of the pathogen was sub-cultured into agar slants (Halgekar and Giri, (2015) [1].

Results and Discussion

Isolation of Mycoflora from Glumes

Among the fungi isolated from glumes *Aspergillus* sp. was found to be most predominant (37.83%) followed by *Alternaria* sp. (20.83%). Mixed infection was observed in the samples of Tenali division where PM-1 sample was found to be dominated with *Alternaria* sp., while PM-2 sample been associated with *Curvularia* sp. However, Narasaraopet division samples were highly contaminated with *Aspergillus* spp. except RE-1 which was associated with *Bipolaris* sp.

The samples from Nellore district showed wide variation in their association with mycoflora, where AK sample was recorded with 53.33% *Aspergillus* infection, while, VE and CH samples with *Alternaria* sp. and AR sample with *Curvularia* sp. (46.67%) infection (Table 1).

Rhizopus infection was completely absent in Nellore district while, a sample from Tenali division has maximum infection due to *Rhizopus* sp. Therefore glumes were found to be highly vulnarable to microbial attack. Among the mycoflora isolated from glumes the individual interactions between *Bipolaris* sp. and other genera *i.e., Aspergillus* spp., *Alternaria* sp., *Curvularia* sp., and *Rhizopus* sp. was found to have significant impact on the grain discolouration.

Table 1: Frequency of mycoflora associated with glumes of discoloured grains (%)

S. No.	Sample	Frequency of mycoflora in the Glumes (%)				
		***Aspergillus* sp. (A)**	***Bipolaris* sp. (B)**	***Alternaria* sp. (C)**	***Curvularia* sp. (D)**	***Rhizopus* sp. (E)**
1	NP1	53.33	26.67	3.33	13.33	3.33
2	NP2	66.67	6.67	26.67	0.00	0.00
3	PP1	26.67	13.33	0.00	20.00	40.00
4	PP2	60.00	20.00	3.33	13.33	3.33
5	SP1	63.33	13.33	16.67	6.67	0.00
6	SP2	46.67	16.67	13.33	23.33	0.00
7	PM1	46.67	33.33	0.00	6.67	13.33
8	PM2	20.00	20.00	0.00	46.67	13.33
9	GU1	60.00	13.33	16.67	0.00	10.00
10	GU2	0.00	13.33	40.00	20.00	26.67
11	RE1	26.67	46.67	20.00	3.33	3.33
12	RE2	40.00	13.33	26.67	20.00	0.00
13	NK1	53.33	6.67	13.33	26.67	0.00
14	NK2	0.00	20.00	66.67	13.33	0.00
15	PR1	46.67	0.00	20.00	20.00	13.33
16	PR2	60.00	0.00	13.33	26.67	0.00
17	AK	3.33	20.00	63.33	13.33	0.00
18	VE	20.00	20.00	0.00	60.00	0.00
19	CH	16.67	20.00	53.33	10.00	0.00
20	AR	46.67	20.00	20.00	13.33	0.00
	Mean	37.83	17.17	20.83	17.83	6.33

Isolation of Mycoflora from Endosperm

Endosperm was found to be concentrated with the inoculum of *Aspergillus* spp. in most of the samples tested. Among the samples tested, frequency of *Aspergillus* spp. ranged from 6.67 (AR) to 73.33% followed by *Bipolaris* sp. infection (NK-2) (22.17%). *Curvularia* sp. was found to be dominant only in Nellore sample (AR) with 53.33% while *Bipolaris* sp. was observed to be more prevalent in CH (46.67%) and PM-2 (40%). Effect of *Rhizopus* sp. was comparatively low in endosperm compared to glumes as it was being traced only in two samples. PP-1 and AR each with 13.33% occurrence.

Occurrence of *Aspergillus* spp. was comparatively higher (50.5% mean frequency) in endosperm than that in glumes (37.8% mean frequency). Similarly occurrence of *Bipolaris* sp. was also high in endosperm (22.17% frequency) than that in glumes (17.2). Association of *Curvularia* sp. was similar in both glumes (17.8%) and endosperm (17.17%). In case of *Alternaria* and *Rhizopus* sp. frequency of association decreased in endoisperm compared to that in glumes (Table 2). Among the mycoflora isolated from endosperm the association of *Bipolaris* sp. with *Aspergillus* spp. *Alternaria* sp., *Curvularia* sp., and *Rhizopus* sp. in different combinationas was found to be highly significant in causing discoloration.

Table 2: Frequency of mycoflora associated with endosperm of discoloured grains (%)

S. No.	Sample	Frequency of mycoflora in the Endosperm (%)				
		***Aspergillus* sp.**	***Bipolaris* sp.**	***Alternaria* sp.**	***Curvularia* sp.**	***Rhizopus* sp.**
1	NP1	46.67	13.33	13.33	26.67	0.00
2	NP2	60.00	33.33	0.00	6.67	0.00
3	PP1	46.67	20.00	6.67	13.33	13.33
4	PP2	53.33	33.33	0.00	13.33	0.00
5	SP1	56.67	10.00	13.33	20.00	0.00
6	SP2	53.33	20.00	20.00	6.67	0.00
7	PM1	46.67	33.33	3.33	16.67	0.00
8	PM2	23.33	40.00	20.00	16.67	0.00
9	GU1	56.67	20.00	13.33	10.00	0.00
10	GU2	60.00	26.67	13.33	0.00	0.00
11	RE1	66.67	26.67	6.67	0.00	0.00
12	RE2	60.00	6.67	20.00	13.33	0.00
13	NK1	53.33	13.33	13.33	20.00	0.00
14	NK2	73.33	6.67	0.00	20.00	0.00
15	PR1	66.67	16.67	6.67	10.00	0.00
16	PR2	53.33	20.00	0.00	26.67	0.00
17	AK	60.00	23.33	6.67	10.00	0.00
18	VE	46.67	6.67	20.00	26.67	0.00
19	CH	20.00	46.67	0.00	33.33	0.00
20	AR	6.67	26.67	0.00	53.33	13.33
	Mean	50.50	22.17	8.83	17.17	1.33

Isolation of Mycoflora from Embryo

Embryo's of the grain samples separated from Guntur district were dominated with *Aspergillus* spp. occurrance with an exception, where embryos of PP-1 sample from Tenali division was found dominated with *Rhizopus* sp. Similarly, Nellore district samples differed in their embryo mycoflora as the sample AK being associated with *Bipolaris* sp. (46.67), VE with *Aspergillus* spp. (46.67%), CH with *Curvularia* sp. (46.67%) and AR with *Bipolaris* sp. The frequency of *Aspergillus* spp. in the embryo's of Guntur district rice samples ranged from 20.00% to 66.67% with an average of 48.33%.

Aspergillus sp. prevalence in embryo (48.36%) was more or less similar to that in endosperm (50.5%) and comparatively higher than that in glumes (37.8%). In case of *Bipolaris* sp. its occurrence (17.33%) was similar to that in glumes (17.2%) but less than that in endosperm (22.17%). *Curvularia* sp. was comparatively less in embryo (14.33%) than that in glumes or endosperm. In case of *Alternaria* sp. its prevalence increased in embryo (15.5%) compared to endosperm (8.83%) at still lesser than that in glumes (20.8%). Similar observation was made with *Rhizopus* sp. which was found in higher frequency in embryo (4.5%) than that in endosperm (1.3%) (Table 3).

Table 3: Frequency of mycoflora associated with embryo of discoloured grains (%)

S. No.		Frequency of mycoflora in the Embryo (%)				
	Sample	***Aspergillus* sp.**	***Bipolaris* sp.**	***Alternaria* sp.**	***Curvularia* sp.**	***Rhizopus* sp.**
1	NP1	43.33	33.33	6.67	6.67	10.00
2	NP2	53.33	0.00	26.67	20.00	0.00
3	PP1	10.00	16.67	26.67	6.67	40.00
4	PP2	56.67	20.00	10.00	0.00	13.33
5	SP1	46.67	6.67	33.33	13.33	0.00
6	SP2	46.67	20.00	10.00	23.33	0.00
7	PM1	60.00	0.00	23.33	16.67	0.00
8	PM2	46.67	20.00	6.67	26.67	0.00
9	GU1	56.67	13.33	16.67	0.00	13.33
10	GU2	66.67	0.00	13.33	20.00	0.00
11	RE1	60.00	13.33	26.67	0.00	0.00
12	RE2	66.67	20.00	0.00	13.33	0.00
13	NK1	60.00	13.33	0.00	26.67	0.00
14	NK2	53.33	13.33	10.00	23.33	0.00
15	PR1	33.33	46.67	0.00	20.00	0.00
16	PR2	56.67	20.00	13.33	10.00	0.00
17	AK	56.67	16.67	0.00	26.67	0.00
18	VE	46.67	20.00	20.00	13.33	0.00
19	CH	20.00	6.66	46.67	16.67	10.00
20	AR	26.67	46.67	20.00	3.33	3.33
	Mean	48.33	17.33	15.50	14.33	4.50

Thus, the present investigation revealed the prevalence of *Aspergillus* spp. as the most predominant mycoflora in all parts *viz*., glumes, endosperm and embryo of rice grain followed by *Alternaria* sp., *Bipolaris* sp., *Curvularia* and *Rhizopus* sp. in the descending order of their prevalence. It was also observed that glumes acted as the primary source for the fungal association as all the pathogens had more or less equally affected glumes with the frequency that ranged

between 6.33 and 37.83% and later was followed by endosperm and embryo region. However, the interactions between the different genera isolated from the grains revealed that the presence of *Aspergilus* spp. in healthy grain and embryo had significantly enhanced discolouration on interacting with other associated genera. The role of *Bipolaris* sp. was much when it was seated on glumes and endosperm wherein it showed synergistic effect in bringing out grain deterioration.

The reports on higher prevalence of fungi in glumes and embryo were published earlier. Halgekar and Giri, (2015) [1] reported maximum colonization of seed borne fungi in seed coat followed by endosperm where, *D. oryzae*, *B. oryzae* and *C. lunata* (1.65-3.30%) were reported to be located in all parts of seed in all varieties. Ibrahim and Dahab (2014) [2] reported the location of inoculum of *B. oryzae*, *Fusarium* sp. and *A. padwickii* more on seed coat, followed by embryo. Pandey *et al*. (2000) [8] recovered higher percentage of the pathogens from both the embryo and the endosperm regions. Similarly, Sachan and Agarwal (1995) [9] reported the frequency of fungi to be predominantly located in seed coat followed by endosperm and embryo. The average incidence of *A. alternata* was 57.4 and 20.7% in seed coat and endosperm, respectively.

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