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Effect of Grain Discolouration on Rice Grain Biochemical Constituents and Seed Germination and Seedling Vigour

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ABSTRACT

The studies on rice grain discolouration were carried out in the Agricultural College, Bapatla, Guntur district, Andhra Pradesh during 2013-14. Significantly higher concentration of starch, total sugar, reducing and non reducing sugars and lower concentrations of total phenols were observed in healthy grains than in discoloured grains. Changes in biochemical constituents between healthy and discoloured grains were significant except non reducing sugars. Seed germination and seedling vigour decreased significantly with increase in the grade of discolouration and storage period. Seed germination was 88.40 to 92.20% in seeds with 1 – 5% discolouration from the four districts and was found to be lesser; 85.80 to 89.40% in seeds with 6-25% discolouration, 81.60 to 86.20% in seeds with 25-50% discolouration and 79.40 to 80.80% bin seeds with >50% discolouration in the first month of collection of samples. Seed germination decreased as an obvious consequence of increased discolouration with increasing storage period; four months after storage seed germination in seeds with 1-5% discolouration was 84 to 86.60% and in seeds with >50% discolouration it was 64.60 to 69.4 %. Seedling vigour index was found to be in the trend as that of seed germination *i.e.*, an inverse relationship with level of grain discolouration and storage period.

Key words : Biochemical constituents, Grain discolouration, Rice seed germination, Seedling vigour.

Rice is one of the most important cereal crops having highest cultivated area after wheat in the world. It is the staple food crop of Andhra Pradesh (India) and is extensively cultivated both in *kharif* and *rabi* seasons occupying an area of 4.10 M ha with 12.90 M t production and 3146 kg ha^{-1} productivity (Directorate of Economics and Statistics, 2013). In recent past, grain discolouration has become a serious problem and is a bottleneck during seed certification. Discolouration occurs in moderate to severe form every year with variations in the colour of mature seed from that of normal that may lead to seed abortion, seed necrosis coleoptile or radical decay, seedling mortality, seed rotting and seed toxification (Mohana *et al*., 2011). It not only reduces seed germination and vigour, but also results in kernel rotting or seedling blight. It is an early indication of poor quality seeds (Bodalkar and Awadhiya, 2014). Farmers are realising a lesser price for their produce as discolouration is lowering the fair average quality of the grains. Considering the importance of grain discolouration, the present investigation was taken up to study the effect of grain discolouration on seed quality, seed germination and seedling growth.

MATERIAL AND METHODS

The investigation was carried out in the Agricultural College, Bapatla, Guntur district, Andhra Pradesh during 2013-14. Seed samples were collected from the four selected major rice growing districts of Andhra Pradesh during *kharif*, 2013. Seed samples were pooled variety wise and separated as healthy and discoloured samples for biochemical analysis. Starch and total sugars were estimated following Anthrone method (Hodge and Hofreiter, 1962), reducing sugars by Dinitrosalicylic acid method (Miller, 1959), and total phenols by Folin-Ciocalteau Reagent method (Malick and Singh,1980).

Germination percentage was determined by following rolling paper towel method described by International Rules for Seed Testing (ISTA, 1993). Four replicates of 25 discoloured seeds were drawn grade wise (1-5%, 6-25%, 26-50%, 51-100% and healthy sample) from each sample and the seeds were incubated by placing them on moistened germination paper towel which were rolled along one edge with ample space for the germinating seed. Germination percentage was calculated after seven days using the formula,

$$\text{Germination percentage} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds}} \times 100$$

Randomly selected samples from different discoloured grades were used for determining vigour index. Twenty five seeds from each grade were sown in the pots in four replicates to determine vigour index using the formula described by Abdulbaki and Anderson (1976).

Vigour index = (Mean of root length + Mean of shoot length) × Germination percentage

RESULTS AND DISCUSSION

Starch content was higher in healthy grains (46.80 – 67.05 mg/g) than in discoloured grains (41.10 - 61.95 mg/g). Healthy grains of MTU-1010 contained significantly the highest starch content of 67.05 mg/g while it was 61.95 mg/g in discoloured grains resulting in a decrease of 7.61% which was the lowest among the genotypes analyzed. Starch content in the other genotypes varied between 46.80 mg to 57.75 mg/g whereas in discoloured grains it was 41.10 to 52.05 mg/g with the decrease in content of discoloured grains from healthy ones ranging from 9.87 to 18.18%. Depletion in starch content in grains due to discolouration was found to be the highest in NLR 34449 (Fig 1.). Grain discolouration was reported to have an adverse effect on starch content in rice grains infected by *H. oryzae* and *S. oryzae* (Vidhyasekaran *et al.*, 1973 and Sachan and Agarwal, 1995). Decrease in starch content may be due to rapid enzymatic degradation by the pathogens for their utilization in building biomass.

Significant differences among rice genotypes for total phenols content in both healthy and discoloured grains were observed. It was significantly higher in discoloured grains than in healthy grains of all the genotypes. In discoloured grains it varied from 56.33 (MTU-1010) to 98 mg/100 g (MTU-7029) while in healthy grains it ranged between 39.67 (MTU-1010) and 66.66 mg/100 g (MTU-7029). The rise in total phenol content in discoloured grains was the highest in MTU 7029 (47.01%) and the lowest was in MTU 1121 (29.27%) (Fig 2.). Total phenol content was reported to increase in *S. oryzae* infected compared to healthy grains and the increase ranged from 7.67% to 70.78% (Gopalakrishnan *et al.*, 2010). A significant increase in total phenols was reported in *S. oryzae* inoculated rice plants of all age groups as the plants probably mobilized all its defence mechanisms to the site of infection resulting in an increase in total phenols (Velazhahan and Ramabadran, 1993). Increased phenol content was coupled with increased activity of peroxidase and polyphenol oxidase which mediate the oxidation of phenols that are highly toxic to the pathogen (Sequeira, 1983). Higher level of phenolics would be a result of activation of host defence system triggered by the fungi resulting in discolouration or necrosis (Gopalakrishnan *et al.*, 2010).

Healthy grains contained 20.07-25.50 mg/g of total sugar whereas in discoloured grains it ranged between 15.13-21.07 mg/g. Maximum sugar content of 25.50 (healthy) and 21.07 mg/g (discoloured) was recorded in MTU-1010 and minimum of 20.07 (healthy) and 15.13 mg/g (discoloured) was present in MTU-7029 (Fig 3.). Per cent decrease of total sugars over healthy ranged from 11.35-24.60. Total differences in sugar content in healthy and discoloured grains among rice varieties were statistically significant.

A similar trend as that of total sugar content was observed in the content of reducing sugars, *i.e.,* lower reducing sugars (15.13 - 21.07 mg/g) in discoloured grains than in healthy ones (20.07 - 25.50 mg/g) in all the genotypes and the difference was statistically significant. Reducing sugars were high in MTU-1010 (9.73, healthy and 5.80 mg/g, discoloured) and were minimum in MTU-7029 (6.67 mg/g, healthy and 2.57 mg/g, discoloured) (Fig 4.). Per cent decrease of reducing sugars over healthy ranged from 39.54 (MTU-1112) - 61.45 (MTU-7029). Non reducing sugars content in healthy grains among rice genotypes varied between 12.50 (MTU 1166) and 16.50 mg/g (MTU 1112) and the content in discoloured grains was in the range of 12.13 (BPT 2270) and 15.27 mg/g (MTU (1010). Decrease in non reducing sugar content of discoloured relative to healthy grains in some varieties (MTU 1010, MTU 1061, MTU 7029, MTU 1112 and MTU 2270) and increase in other varieties (NLR 34449, BPT 5204, MTU 2067, MTU 1121, MTU 1166 and MTU 1112) was observed. However, the difference in non reducing sugar contents of healthy and discoloured grains was marginal and non significant.

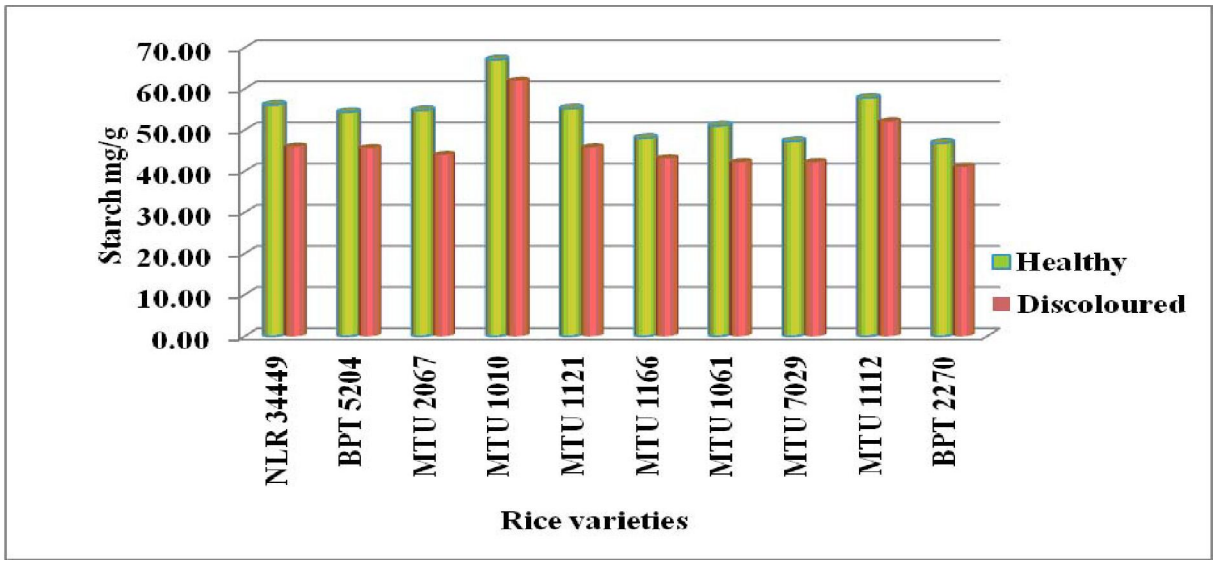


Fig 1. Starch content in healthy and discoloured grains of different rice varieties.

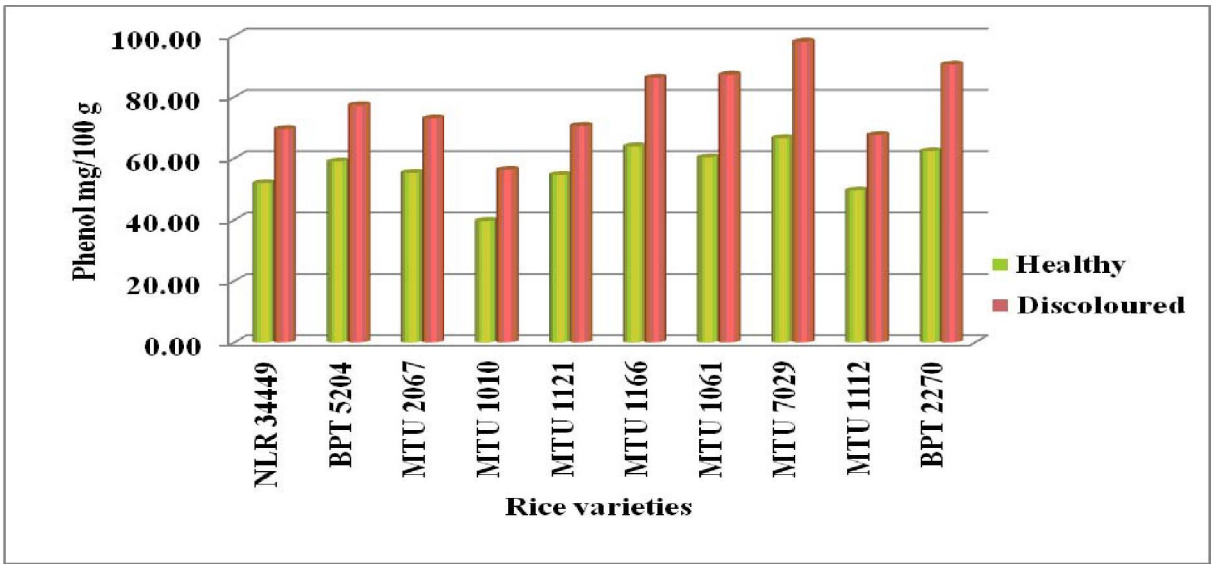


Fig 2. Phenol content in healthy and discoloured grains of different rice varieties.

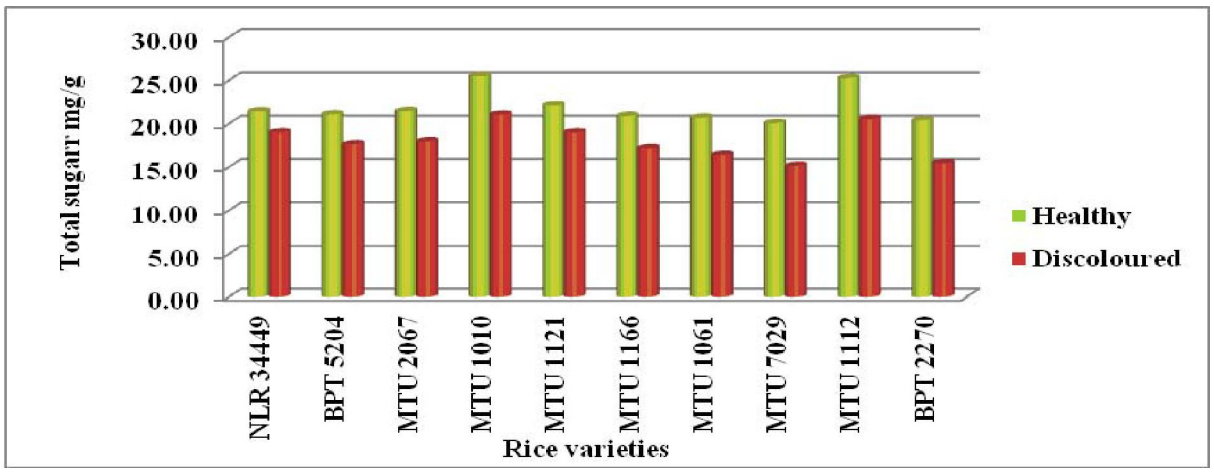


Fig 3. Total sugars content in healthy and discoloured grains of different rice varieties.

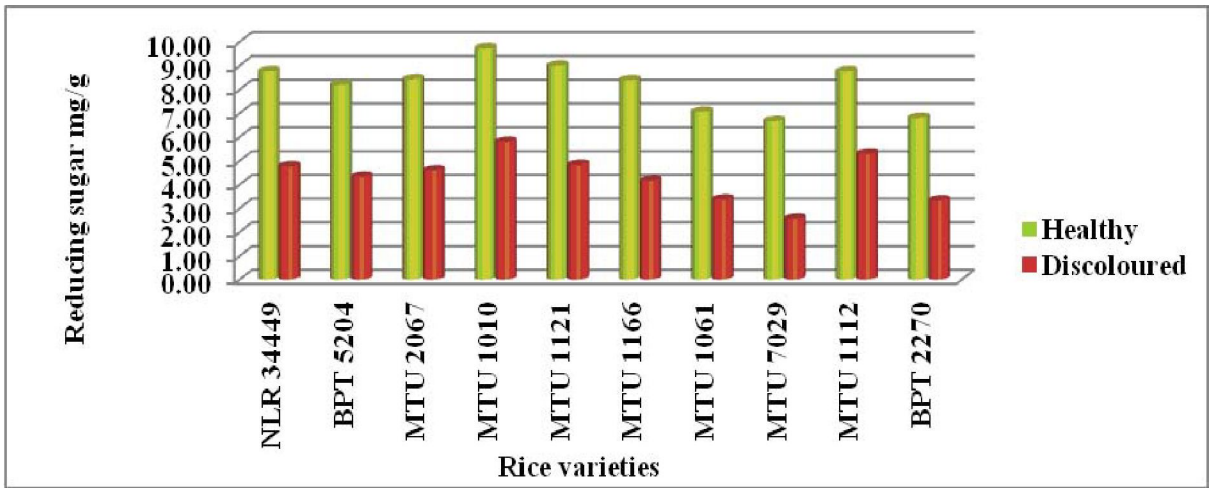


Fig 4. Reducing sugars content in healthy and discoloured grains of different rice varieties.

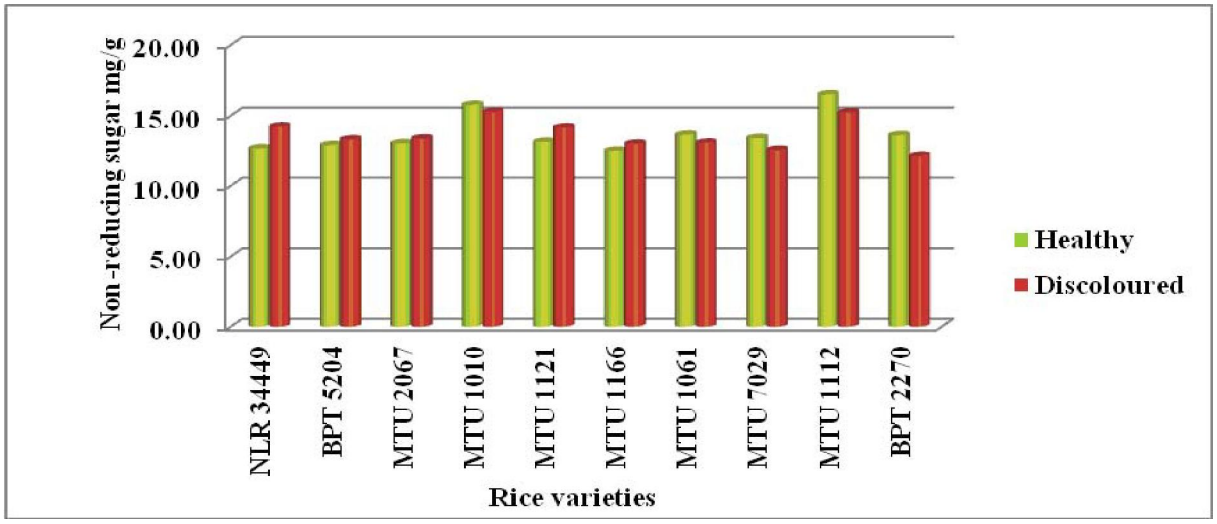


Fig 5. Non-reducing sugars content in healthy and discoloured grains of different varieties.

(Fig 5.). Total sugars, reducing and non-reducing sugars were reported to be reduced by artificial inoculation of *S. oryzae* by 26.73, 25.35 and 28.56%, respectively (Raja and Syamala, 2012). Total sugar content was reduced by 28% on *S. oryzae* infection of rice (Gopalakrishnan *et al*., 2010). Reduction in total sugars, reducing and non-reducing sugars observed in the present study corroborate the findings of earlier studies (Vidhyasekaran *et al.,* 1973) and fluctuations in the amount of non reducing sugars may reflect the genetic makeup of the respective variety resulting in variations in deterioration. Rice grains show decreased biosynthetic activity due to biochemical changes during pathogenesis, resulting in seed deterioration. Pathogens utilize carbon from the sugars for their biomass development that might have led to reduction in sugar content in discoloured grains.

Germination was found to decrease significantly with increasing grade of discolouration and storage period (*i.e*., from one to four months) (Table1, Plate1). Germination percentage of seeds with 1-5% discolouration (84.00 - 92.20%) was more or less equal to that of healthy seeds without discolouration (85.60 - 93.20%) which was in accordance to Phat *et al*. (2005) who did not record any significant difference between control and 1-5% discoloured seeds. Nevertheless, as seed discolouration increased from lower grade (6-25%) to higher grade (51-100%) an obvious reduction was observed *i.e*., from 75.60 - 89.40% to 64.60 - 80.80%, respectively.

Seed germination in samples collected from different locations when stored for four months was lesser than germination in healthy

Table 1. Germination of rice seeds with different degrees of discolouration collected from different locations (%).

Location	West Godavari district				Krishna district				Guntur district				Nellore district			
	Storage															
Discolouration grade	1st month	2nd month	3rd month	4th month	1st month	2nd month	3rd month	4th month	1st month	2nd month	3rd month	4th month	1st month	2nd month	3rd month	4th month
1-5%	90.60	89.00	87.20	84.20	92.20	89.80	88.20	85.80	91.60	90.20	87.20	86.60	88.40	87.40	85.60	84.00
6-25%	88.40	86.00	84.00	82.00	88.60	86.80	84.00	81.00	89.40	85.00	81.80	79.00	85.80	81.60	79.00	75.60
26-50%	86.20	83.00	78.60	74.60	85.40	82.80	79.00	72.40	86.00	82.00	77.60	73.00	81.60	77.80	74.20	70.20
51-100%	80.80	76.80	73.20	69.40	80.20	77.80	72.80	66.00	80.80	77.60	72.60	67.00	79.40	73.60	69.00	64.60
Control	90.80	90.60	88.20	86.20	92.40	91.40	89.00	86.80	93.20	91.00	88.80	87.60	89.60	89.00	87.60	85.60
SEm±	0.74	0.94	1.28	1.11	0.70	0.71	1.03	0.80	1.41	1.46	1.40	1.25	2.01	1.76	1.43	1.58
CD (Pd"0.05)	2.12	2.69	3.67	3.20	2.01	2.04	2.95	2.31	4.06	4.18	4.01	3.61	5.78	5.06	4.09	4.54
CV %	1.89	2.47	3.48	3.14	1.79	1.85	2.78	2.30	3.59	3.82	3.82	3.58	5.30	4.81	4.03	4.66

Data represents mean of four replications of five samples

samples. Germination in healthy seed sample from West Godavari district was 90.80% during first month of storage, decreased to 86.20% after four months of storage which accounted for 5.06% reduction over first month of storage. When the extent of discolouration was 6-25%, after four months of storage 7.20, 8.57, 11.63 and 11.88% reduction in germination was recorded for samples from West Godavari, Krishna, Guntur and Nellore districts, respectively. Similarly, when the discolouration ranged from 51-100%, 14.10, 17.70, 17.07 and 18.63% reduction in germination has occurred after a storage period of four months in the samples collected from the respective districts. The results elucidate that grain germination was much affected by discolouration (Phat *et al*., 2005) and it was directly proportional to the severity of discolouration (Kheroda and Chhetry, 2008) which may be due to the invasion of storage fungi (Purushotham *et al*., 1996).

The least vigour index (647.70 to 1379.84) at all the storage periods was recorded in seedlings germinated from seed exhibiting a high grade of discolouration (51-100%) collected from different locations (Table 2). A decline in seedling vigour index from 1777.06 to 1054.32 was observed when seeds from West Godavari with 1-5% discolouration were stored for four months which corresponded to an increase in discolouration in storage. Similarly, young plants developed from seeds with 51-100% discolouration showed a reduction in vigour index from 1292.84 to 647.70 after four months of storage as against 2083.36 to 1224.43 in healthy. Among all seed samples collected, the lowest vigour index was recorded in seedlings germinated from seeds with 51-100% grain discolouration collected from West Godavari. Seedling vigour index was found to be of a similar pattern with respect to grade of discolouration and length of storage for seed collected from different districts though seed from Krishna district produced seedlings with greater vigour than that from other districts. Seedling vigour index (1965.06) of young plants from seeds with 1-5% discolouration stored for a month decreased on further storage for four months to 1143.57. During first month of storage, seedlings developed from 1-5% discoloured seed of Guntur district had a vigour index of 1916.97 which was reduced to 1145.20 upon storage for four months. When discolouration of seed was 51-100% seedling vigour index has reduced from 1379.84 to 749.54 after four months of seed storage (Plate 2). Young plants developed from healthy seed samples of Nellore

Table 2. Vigour index of rice seedlings raised from seed with different degrees of discolouration collected from different locations.

Location	West Godavari district				Krishna district			
	Storage							
Discolouration grade	1st month	2nd month	3rd month	4th month	1st month	2nd month	3rd month	4th month
1-5%	1777.06	1621.38	1295.54	1054.32	1965.06	1651.83	1443.74	1143.57
6-25%	1609.50	1457.10	1186.83	938.20	1739.50	1462.94	1269.45	1005.57
26-50%	1434.34	1275.02	1033.32	802.54	1528.70	1325.14	1059.64	841.87
51-100%	1292.84	1137.52	883.85	647.70	1305.74	1094.68	907.00	657.40
Control	2083.36	1885.21	1481.31	1224.43	2136.86	1852.08	1660.50	1293.10
SEm±	77.20	43.24	62.76	56.76	106.83	69.18	44.72	50.61
CD (Pd”0.05)	221.59	124.10	180.16	162.92	306.65	198.59	128.37	145.27
CV %	10.53	6.55	11.93	13.60	13.77	10.47	7.89	11.45

Table 2. cont.....

Location	Guntur district				Nellore district			
	Storage							
Discolouration grade	1st month	2nd month	3rd month	4th month	1st month	2nd month	3rd month	4th month
1-5%	1916.97	1724.62	1402.23	1145.20	1791.16	1682.30	1522.48	1056.24
6-25%	1739.51	1517.90	1233.33	1053.67	1641.02	1514.70	1385.10	954.09
26-50%	1564.92	1373.28	1089.89	894.32	1458.63	1278.48	1127.68	784.49
51-100%	1379.84	1131.20	905.33	749.54	1220.10	1068.22	879.64	665.01
Control	2020.90	1886.26	1627.40	1287.49	1997.96	1877.74	1701.36	1244.06
SEm±	41.74	64.64	70.70	47.87	87.37	52.74	45.49	45.55
CD (Pd”0.05)	119.80	185.54	202.93	137.42	250.78	151.38	130.59	130.74
CV %	5.41	9.47	12.55	10.43	12.05	7.95	7.69	10.83

Data represents mean of four replications of five samples

district recorded a seedling vigour index of 1997.96 during first month of storage which has reduced to 1244.06 after a storage period of four months. Seeds with 1-5% discolouration after four months of storage had a vigour index that has reduced from 1791.16 to 1056.24. Similarly, 51-100% discoloured seed, after four months of storage resulted in reduction of seedling vigour index (1220.10 to 665.01).

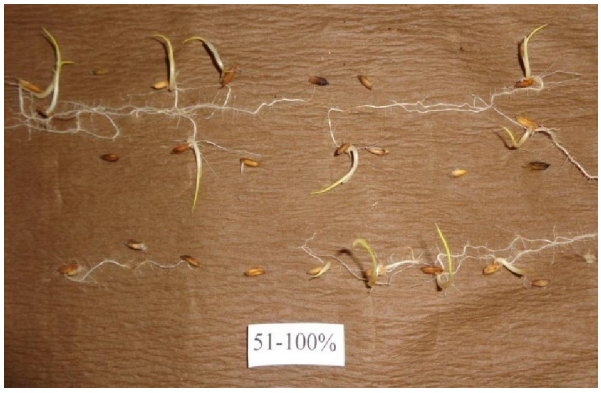
The result revealed that seedling vigour index of seeds with lesser discolouration was higher than that of seeds with higher levels of discolouration and that the vigour index was found to decrease with the length of storage from one to four months for seed samples of all the four districts. As discolouration was found to hinder seed germination, it consequently had a negative effect on seedling vigour index. Pandey *et al*. (2000) reported that seedling mortality increased with increase in discolouration and storage fungi associated with seed inhibited seedling growth (Purushotham *et al*., 1996), resulting in abnormal seedlings (Nghiep and Gaur, 2004). Fluctuations in vigour of discoloured grains could result from the depletion of the reserve food in rice kernel due the activities of seed borne fungi (Ora *et al*., 2011).

A

B

C

D



E

Plate 1 Germination of rice seed with different grades of discolouration

A- Control B- 1-5% discolouration C- 6-25% discolouration

D- 26-50% discolouration E- 51-100% discolouration

LITERATURE CITED

Abdulbaki A A and Anderson J D 1976 Vigour determination in soybean seed by multiple criteria. *Crop Science*, 13: 630-633.

Bodalkar C and Awadhiya G K 2014 Assessment of percent grain discolouration in important rice varieties. *International Journal of Current Research in Biosciences and Plant Biology*, 1: 61-64.

Directorate of Economics and Statistics 2013 Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India, New Delhi.

Gopalakrishnan C, Kamalakannan A and Valluvaparidasan V 2010 Effect of seed borne *Sarocladium oryzae*, the incitant of rice sheath rot on rice seed quality. *Journal of Plant Protection Research*, 50: 98-101.

Hodge J E and Hofreiter B T 1962 In: *Methods in Carbohydrate Chemistry*. (eds.,) Whistler, R.L. and Be Miller, J.N., Academic Press, New York, 420pp.

ISTA 1993 *International Rules for Seed Testing*. Seed Science and Technology, 21: 141-146.

Kheroda L and Chhetry G K N 2008 Fungi associated with discoloured rice grains and their effect on germination. *Journal of Mycopathological Research*, 46: 125-126.

Malick C P and Singh M B 1980 Quantification of total phenol content. *In : Plant Enzymology and Histo Enzymology*. Kalyani publishers, New Delhi, 286pp.

Miller G L 1959 Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry,* 31: 426-428.

Mohana D C, Prasad P and Vijaykumar V 2011 Plant extract effect on seed-borne pathogenic fungi from seeds of paddy grown in southern India. *Journal of Plant Protection Research*, 51: 101-106.

Nghiep H V and Gaur A 2004 Role of *Bipolaris oryzae* on producing abnormal seedlings of rice. *Omonrice*, 12: 102-108.

Ora N, Faruq A N, Islam M T, Akhtar N and Rahman M M 2011 Detection and identification of seed borne pathogens from some cultivated hybrid rice varieties in Bangladesh. *Middle-East Journal of Scientific Research*, 10: 482-488.

Pandey V, Agarwal V K and Pandey M P 2000 Location and seed transmission of fungi in discoloured seeds of hybrid rice. *Indian Phytopathology*, 53: 45-49.

Phat C T, Duong NT and Du L T 2005 Influence of grain discolouration to seed quality. *Omonrice*, 13: 139-144.

Purushotham S P, Patkar K L, Prakash H S and Shetty H S 1996 Storage fungi and their influence on rice seed quality. *Indian Phytopathology*, 49: 152-156.

Raja I Y and Syamala M 2012 Physiological alterations induced by plant extracts in rice plants inoculated with Saroc Ladium Oryzae. *Madras Agricultural Journal,* 99: 107-115.

Sachan I and Agarwal V K 1995 Seed discolouration of rice: Location of inoculum and influence on nutritional value. *Indian Phytopathology*, 48: 14-20.

Sequeira L 1983 Mechanism of induced resistance in plants. *Annual Review of Microbiology*, 37: 51-79.

Velazhahan R and Ramabadran R 1993 Changes in phenolic contents of rice during pathogenesis of *Sarocladium oryzae*. *Madras Agricultural Journal*, 80: 50-51.

Vidhyasekaran P, Ramadoss N and Srinivasulu N 1973 Quantitative and qualitative losses in paddy due to Helminthosporiose epidemic. *Indian Phytopathology*, 37: 487-493.

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