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Seed Discoloration and their Effect on Seedlings Growth of Egyptian Hybrid Rice

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ABSTRACT

The experiments were conducted at the laboratory of Seed Technology Research Unit, Mansoura, Seed Technology Research Department, ARC, to investigate the effect of seed discoloration on seedling vigor of two Egyptian hybrid rice (EHR1 and EHR2). Eight categories of discoloration seeds were tested by Blotter method and percentage of seed-borne fungi were recorded. The main results could be summarized as follows: *Bipolaris oryzae* associated with light brown, black dots, dark brown and brown spot discoloration with high percent followed by *Alternaria padwickii* associated with ash gray, dark brown and light pink discoloration, *Fusarium* spp. responsible for light pink and pale yellow, *A. alternata* associated with ash gray, *Curvularia oryzae* associated brown spot and dark brown discoloration, while *Sarocladium oryzae* lead to light brown discoloration, respectively. *Bipolaris oryzae*, *Fusarium* spp. and *A. padwickii* were most prevalent in the seed coat followed by husks and embryo, while *S. oryzae* recorded the highest value in the embryo. Light brown, dark brown, brown spot and black dots discoloration gave maximum presence in abnormal seedlings followed by pale yellow, light pink and ash gray, respectively. On the other hand, light brown and light pink discoloration were found principally in rot seeds when compared with healthy seeds. All pathogens of fungi were isolated from seed rot and blight seedlings, while isolated *B. oryzae* and *S. oryzae* were found in stunted seedlings.

Key words: Egyptian hybrid rice, seed discoloration, seed-borne fungi, seedlings

INTRODUCTION

Rice (*Oryza sativa* L.) is an important cereal crop in Egypt. Seed-borne diseases cause enormous losses to our crop. The infected seed may fail to germinate, transmit disease from seed to seedling and from seedling to growing plants (Fakir *et al*., 2002).

There are possibilities of higher seed infection in hybrids as compared to conventional varieties because of the parental lines having open flower type and exposure of flowers for a longer period of time. There is very little information available on seed-borne fungi and seed discoloration in hybrid rice. Grain discoloration of rice is a complex disease due to infection by certain microorganisms on the glumes, kernels or both. The fungi that are reported to be associated with discoloration of grains are *Bipolaris oryzae*, *Alternaria padwickii*, *Pyricularia oryzae*, *Fusarium moniliforme*, *F. graminearum*, *Ngrospora oryzae* and *Curvularia* spp. (Ou, 1985; Arshad *et al*., 2009). The split glumes nature of hybrid rice seed has a deleterious effect on the quality of seed lots as they manifest poor field emergence, seedlings survival and serve as an easy

avenue for their attack by pests and diseases (Zhou *et al*., 1989). Devaraju *et al*. (2012) stated that fungi, *P. oryzae*, *Rhizopus*, *Alternaria*, *B. oryzae*, *Curvularia* sp., *Epiccum* sp., *Negrospora* sp., *Rhizoctonia oryzae*, *A. padwickii* and *Fusarium* were isolated from discolored seed hybrid rice by blotter method and they were found to be associated with the seed externally as well as internally and acts as a source of infection. Vachspati *et al*. (2000) on hybrid rice, showed that seed-borne fungi *A. padwickii*, *B. oryzae*, *Fusarium* spp. and *Magnaporthe grisea* were detected only from seed coats and endosperms, while *D. oryzae*, *Curvularia* spp., *F. graminearum*, *N. oryzae* and *S. oryzae* were detected from all parts of seed (seed coat, endosperm and embryo). They also found that seed infection with *M. salvinii* resulted in seedling rot and seedling mortality. The maximum seedling mortality was found in seeds with discoloration on both endosperm plus embryo regions. Abdel-Monem *et al*. (1995) stated that there was an indication of direct relationship between seed infection and seed rot or seedling rot in case of both fungi *D. oryzae* and *P. oryzae*, while *D. oryzae*, *F. moniliforme*, *F. semitectum*, *P. oryzae* and *A. padwickii* were detected in the pericarp and the embryo. Tateishi (1998) isolated frequencies of *F. moniliforme*, the causal fungus of bakana disease from infected, untreated rice seeds were 75, 25 and 15% from hulls, endosperm and embryo, respectively. Duraiswamy and Marippan (1983) found that grain discoloration caused by fungi such as *B. oryzae*, *A. padwickii*, *M. grisea* (*P. oryzae*), *F. moniliforme*, *F. graminearum*, *S. oryzea* and *C. oryzea* caused reduction in seed viability and such grain seeds on planting usually exhibit pre-or post-emergence death of seedlings.

Sachan and Agarwal (1995) demonstrated that seed-borne inoculum of fungi associated with ash gray discoloration (*A. alternata*), light brown (S. *oryzae*), black, dark purple, dark brown spots and light to dark brown dot like spots caused by *B. oryzae* were found in seed coat and endosperm, of discolored seeds. *F. equiset*, *F. graminearum* and *F. moniliforme* were responsible for light pink discoloration. These seed-borne pathogens may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plan growth by systemic or local infection (Bateman and Kwasna, 1999) and (Khanzada *et al*., 2002). Moreover, Danquah *et al*. (1976) reported that infected seeds are often discolored, an indication of poor seed quality.

Abnormal and diseased seeds in seed lot also indicate poor health. Abnormal seed conditions include discolored, spotted, deformed, unfilled, partially filled, smutted and insect damaged seeds. Symptoms of grain discoloration appears externally on the glumes or internally on the kernels or both, the dots varied in size, shape and colors were caused by a large number of fungi and bacteria association (Ou, 1983; Nghiem and Hoang, 1993). Neergaard (1973) reported several types of abnormalities on the seeds, which mainly include seed discoloration, necrosis, seed abortion, seed toxification seed rotting, etc. He further reported that these types of abnormalities occurred due to dominate fungi like *Aspergillus*, *Curvularia*, *Drechslera*, *Fusarium*, *Penicillum*, *Rhizoctonia*, *Verticilastum* and *Alternaria* species.

This researcher aims to study the discoloration by seed-borne fungi, seed transmission including the location on three regions of the seed production and their effect on types of abnormalities on seedlings of hybrid rice.

MATERIALS AND METHODS

This study was performed in the years of 2012 and 2013 at the Laboratory of Seed Technology Research Unit, Mansoura, Egypt on freshly harvested seed samples of cultivars Egyptian hybrid rice 1 (EHR1) and Egyptian hybrid rice 2 (EHR2) from Rice Research Center in Sakha, Field Crops Research Institute, A.R.C.

Discolored seeds were separated and divided into eight types of discoloration namely, ash gray, black dots, dark brown, light pink, light brown, brown spots, pale yellow and healthy seeds (Vachspati *et al*., 2000).

Seed discoloration and location of inoculum by blotter test: One hundred seeds of each category of discoloration were tested by standard blotter method as recommended by ISTA (1999). Pieces of filter papers were soaked in sterilized water and then placed at the bottom of a 9 cm plate (glass Petri dishes). Seeds were surface sterilized with 2% sodium hypochlorite solution for 2 min. Twenty-five seeds were planted in each plate, four plates were used for each sample. The Petri dishes containing seeds were incubated at 25±2°C under alternating cycles of 12 h near Ultraviolet (NUV) light and darkness for 7 days. After incubation, the seeds were examined under stero-binocular microscope in order to record the percentage incidence of different seed-borne fungi. Observations were also made on percentage of location and seed transmission of these fungi in different regions for seed, seed coat, husk and embryo.

Seedlings test by rolled towel method: Seedling test was carried out using in-between blotter paper method (ISTA, 1999). Two sheets of square blotter paper (23×26.5 cm) were moistened in distilled water leaving adequate margins. One hundred seeds for each cultivar and discolored seeds were surface sterilized with 2% sodium hypochlorite solution for 2 min and sown evenly (25 seeds in 1 replicate) between blotters in four replicates. The sheets were folded along one edge and then rolled up. The rolls were placed up right inside a plastic bag to avoid drying during incubation. The incubation was at 28°C, which alternated between 12 h near Ultraviolet (NUV) light and 12 h darkness using day light fluorescent tubes as the light source. After 14 days of incubation, the seedlings were evaluated number of normal seedlings, seed rot and abnormal seedlings which were classified to stunted and blighted. The data were later converted to percentages.

Isolation and identification of pathogenic fungi: The seedlings test were separated into seed rot, stunted and blighted seedlings and the causal fungi examined using a stereoscopic microscope. The pathogen was initially identified morphologically on the basis of colony color, shape and size. Later Microscopic examination were performed to confirm the pathogens on the basis of mycelial growth, spore shape and size as described in the technical bulletin on seed-borne diseases and seed health testing of rice (Agarwal *et al*., 1989; Ellis, 1980). Hyphal-tips from the fungi were transferred on to Potato Dextrose Agar (PDA) plates using the tips of heat-stretched capillary tubes. Pure cultures of the isolated fungi were obtained and all isolates were maintained on slants of PDA at 5°C for further studies.

Statistical analysis: Data collected were subjected to statistical analysis ANOVA according to Gomez and Gomez (1984).

RESULTS

Seed discoloration and location of inoculums by Blotter test: The results in (Table 1 and 2) showed that fungi lead to seed discoloration in various degrees. Also, different fungi are present on different parts of seed.

***Alternaria alternata* (*A. tenuis*):** The average percent incidence of *A. tenuis* in seeds showing ash grey and black dots discoloration was (6-8%) and (6-5%) on cvs (EHR 1) and (EHR 2), respectively. This fungus was the most on seed coat compared with husk and embryo.

Table 1: Percentage of seed-borne fungi associated with eight different types of discoloration in hybrid rice seeds

Types of discoloration	*A. alternata*	*A. padwickii*	*B. oryzae*	*C. oryzae*	*F. graminearum*	*F. moniliforme*	*F. semitectum*	*S. oryzae*	*Trichothecium* sp.	Total fungi (%)
Ash gray										
EHR 1	6	13.0	0	0.0	1	2.0	0.0	3	2	27.0
EHR 2	8	15.0	0	0.0	0	2.0	0.0	4	0	29.0
Black dots										
EHR 1	6	0.0	15	0.5	3	0.0	0.0	0	0	24.5
EHR 2	5	1.0	13	1.0	2	0.0	0.0	0	2	24.0
Brown spots										
EHR 1	1	0.5	13	6.0	0	0.0	0.0	0	0	20.5
EHR 2	0	0.0	12	3.0	0	0.0	0.0	0	0	15.0
Dark brown										
EHR 1	0	10.0	14	4.0	0	0.0	0.0	0	0	28.0
EHR 2	0	6.0	12	3.0	0	0.0	0.0	0	0	21.0
Light brown										
EHR 1	0	2.0	16	3.0	2	0.5	0.0	6	0	29.5
EHR 2	0	5.0	14	0.0	3	0.0	0.0	5	0	27.0
Light pink										
EHR 1	0	4.0	0	0.0	6	9.0	4.0	2	0	25.0
EHR 2	0	5.0	0	0.0	8	10.0	3.0	0	0	26.0
Pale yellow										
EHR 1	0	4.0	6	0.0	10	3.0	0.5	0	1	24.5
EHR 2	0	4.0	3	0.0	8	6.0	0.0	0	2	23.0
Healthy										
EHR 1	3	3.0	2	0.0	1	2.0	0.0	1	0	12.0
EHR 2	2	5.0	0	0.0	0	3.0	0.0	0	0	10.0
Total										
EHR 1	16	36.5	66	13.5	23	16.5	4.5	12	3	191.0
EHR 2	15	41.0	54	7.0	21	21.0	3.0	9	4	175.0

Test was carried out using Blotter technique, one hundred seeds were tested to each category of discoloration

Table 2: Percentage of location and seed transmission of seed-borne fungi on three regions of hybrid rice

Fungi (%)	Seed coat	Husks	Embryo
A. alternata			
EHR 1	15	10	2
EHR 2	16	9	2
A. padwickii			
EHR 1	12	10	5
EHR 2	8	10	3
B. oryzae			
EHR 1	22	11	4
EHR 2	12	9	5
C. oryzae			
EHR 1	16	3	4
EHR 2	10	5	5
F. graminearum			
EHR 1	20	8	2
EHR 2	15	7	3
F. moniliforme			
EHR 1	20	5	8
EHR 2	13	4	6
F. semitectum			
EHR 1	15	0	2
EHR 2	12	0	3
S. oryzae			
EHR 1	4	3	12
EHR 2	3	2	10
***Trichothecium* sp.**			
EHR 1	4	3	10
EHR 2	5	3	8

***Alternaria padwickii*:** The average percent incidence of *A. padwickii* seeds showing ash grey as highest percentage with both two cvs (EHR1) and (EHR2) was 13-15% followed by dark brown 10-6%, light pink 4-5%, light brown 2-5% and pale yellow 4-4%, respectively while detected on seed coat, husks and embryo with the rate 12, 10 and 5%, respectively on cv. (EHR1), while on cv. (EHR2) was 8, 10 and 3%, respectively.

***Bipolaris oryzae*:** The discoloration types by *B. oryzae* were light brown, black dots, dark brown, brown spots and pale yellow at the rate of 16,15,14,13 and 6%, respectively on cv. EHR 1, while was on cv. EHR 2 the rates were 14, 13, 12, 12 and 3%, respectively. The infection of *B. oryzae* was detected from all parts of seed coat, husks and embryo with average of 22, 11 and 4% on cv. EHR 1 and 12, 9 and 5% on cv. EHR 2, respectively. Overall, the percent incidence of *B. oryzae*in seeds of both cultivars showed the following trends in magnitude light brown followed by black dots, dark brown and brown spots. Fungus percent on seed coat was the highest in both cultivars.

***Curvularia oryzae*:** Infection of *C. oryzae* was detected from seeds exhibiting dark brown and brown spots discoloration with average of 4 and 6% on cv. EHR 1, while was on cv. EHR 2, 3 and 3% were found, respectively. The average percent incidence was 16 and 10% from seed coat of the two cultivars, husks was 3 and 5%, embryo 4 and 5% of EHR 1and EHR 2, respectively. On the other hand, *C. oryzae*, brown spots discoloration was highest on seed coat of EHR 1.

Table 3: Effect of cultivars and types of discoloration on seed and seedling characters percentage

		**Abnormal seedlings		
Types of discolored	*Normal seedlings	Stunted	Blighted	***Seed rot
EHR 1	41.80	15.50	20.20	21.20
EHR 2	39.60	14.00	21.10	25.30
F. test	*	*	ns	**
LSD 5 (%)	1.18	1.20	-	1.20
Ash gray	35.50	10.00	26.00	28.50
Black dots	54.50	13.00	22.50	10.00
Brown spots	47.50	23.50	14.00	15.50
Dark brown	40.00	20.00	14.00	25.50
Light brown	23.50	12.00	32.50	32.00
Light pink	33.50	14.00	20.00	32.70
Pale yellow	23.00	15.50	33.00	28.50
Healthy	68.50	9.50	8.50	13.50
F. test	**	**	**	**
LSD 5 (%)	2.65	2.35	4.41	2.40

*Normal seedlings: Plumule, roots well developed, **Abnormal seedlings: Decay in shoot and root, primary root showing browning no branching or secondary development shoot usually appearing weak, ***Seed rot: Consisted of non-germinated seeds covered with hyphal growth of the tested pathogen (Guerrero *et al*., 1972)

***Fusarium* spp.:** *F. graminearum* recorded the highest percentage on pale yellow discoloration seeds with rate (10 and 8%) and light pink (6 and 8%) on cvs (EHR 1) and (EHR 2), respectively followed by *F. moniliforme* and *F. semitectum*. *Fusarium* genus was detected from seed coat with rat the highest from husks and embryo.

***Sarocladium oryzae*:** The infection of *S. oryzae* was exhibited as light brown with percent (6-5% and ash grey 3-4%) on cvs EHR 1 and EHR 2, respectively. The average detected from the embryo was 12 and 10% on EHR 1 and EHR 2, respectively.

Figure 1 illustrated that *B. oryzae* is the most effect on seed discoloration (66) and (54) of both two cultivars followed by *A. padwickii* (36.5) and (41), *Fusarium graminearum* (23) and (12), *F. moniliforme* (16.5) and (21), *A. alternata* (16) and (15) and *S. oryzae* (12) and (9) to cvs EHR 1 and EHR 2, respectively.

Figure 2 shows that light brown and ash gray gave highest values of total fungi percentage followed by dark brown, light pink, black dots, pale yellow and brown spots in both cultivars.

Seedlings disease test by Rolled Towel Method: Table 3 shows that hybrids 1 and 2 gave percent normal seedlings of 41.8 and 39.6%, respectively while the abnormal seedlings (stunted and blighted) gave 15.5 and 20.2% in cv. EHR 1 with 14 and 21.1% in cv. EHR 2. Seed rot was significantly high (25.3%) in EHR 1 and 21.3% in EHR 2.

Seed discoloration of pale yellow gave the lowest normal seedlings percentage (23%) followed by light brown (23.5%). Also, light brown seeds recorded 32% seed rot and 32.5% Blighted seedlings when compared with other types of discoloration seeds and the healthy seeds.

In Table 4, the Egyptian hybrid rice 1 was found to have light brown seed discoloration on abnormal seedlings caused by fungi *B. oryzae*, *S. oryzae* and *A. padwickii* and also gave 54% abnormal seedlings (14% stunted and 40% blighted) and seed rot 20%. This was followed by pale

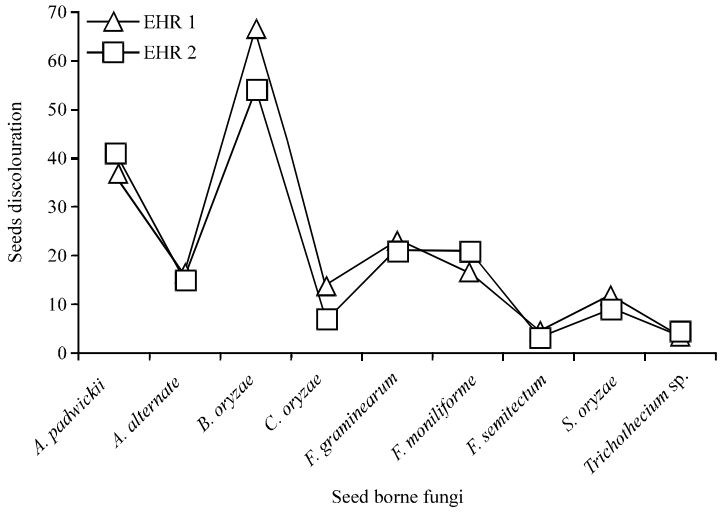

Fig. 1: Seed-borne fungi relation to seed discoloration of hybrid rice

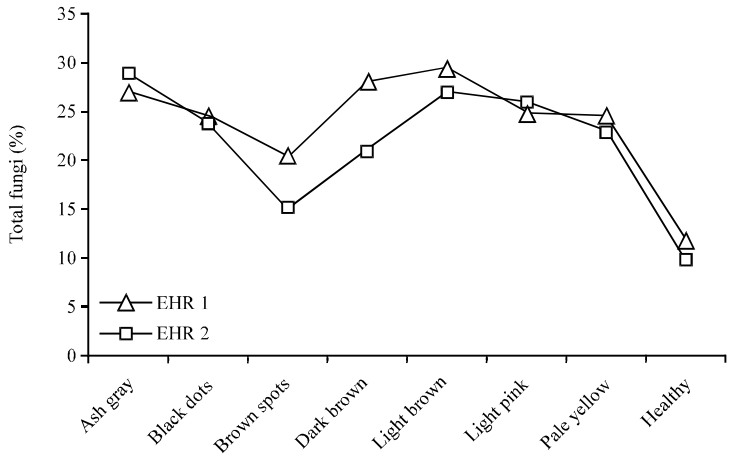

Fig. 2: Total fungi percentage in relation to types of seed discoloration

yellow discoloration of 50% abnormal seedlings (15% stunted and 35% blighted) and seed rot 35%. The least percent normal seedlings 15% was pale yellow caused by number fungi *F. graminearum*, *B. oryzae* and *F. moniliforme*. Light pink discoloration seeds caused by genus *Fusarium* and *A. padwickii* gave 38% abnormal seedlings, 23% seed rot and 39% normal seedlings. Black dots discoloration caused by fungi *B. oryzae* and *A. alternata* gave 36% of abnormal seedlings (13% stunted and 23% blighted), 6% seed rot while it gave the highest percentage of normal seedlings (58%) as compared with other types of discoloration. *B. oryzae*, *A. padwickii* and *C. oryzae* were responsible for dark brown, the major pathogens that caused seedling disease, these gave 35% abnormal seedlings (25% stunted and 10% blighted) and 16% seed rot.

Ash grey discoloration caused by fungi *A. padwickii* and *S. oryzae* lead to 33% abnormal seedlings (8% stunted and 25% blighted) and 32% seed rot, while brown spots discoloration caused by fungi *B. oryzae* and *C. oryzae* recorded 28% abnormal seedlings and 32% seed rot.

Table 4: Effect of discoloration on seedlings symptomatology percentage of hybrid rice by rolled towel method

		Abnormal seedlings			
Types of discolored	Normal seedlings	Stunted	Blighted	Total abnormal	Seed rot
Ash gray					
EHR 1	35.00	8.00	25.0	33	32.00
EHR 2	36.00	12.00	27.0	39	25.00
Black dots					
EHR 1	58.00	13.00	23.0	36	6.00
EHR 2	51.00	13.00	22.0	35	14.00
Brown spots					
EHR 1	40.00	14.00	14.0	28	32.00
EHR 2	26.00	15.00	26.0	41	33.00
Dark brown					
EHR 1	49.00	25.00	10.0	35	16.00
EHR 2	46.00	22.00	18.0	40	14.00
Light brown					
EHR 1	26.00	14.00	40.0	54	20.00
EHR 2	21.00	10.00	25.0	35	44.00
Light pink					
EHR 1	39.00	26.00	12.0	38	23.00
EHR 2	42.00	14.00	16.0	30	28.00
Pale yellow					
EHR 1	15.00	15.00	35.0	50	35.00
EHR 2	31.00	16.00	31.0	47	22.00
Healthy					
EHR 1	73.00	9.00	13.0	22	5.00
EHR 2	64.00	10.00	4.0	14	22.00
F. test	**	**	**	-	**
LSD 5 (%)	3.53	3.52	6.2	-	3.53

Egyptian hybrid rice 2 was found that effect pale yellow discoloration seeds on abnormal seedlings caused by fungi *Fusarium* spp. and *A. padwickii* where gave 47% abnormal seedlings (16% stunted and 31% blighted) and 22% seed rot, followed by brown spot seed discoloration caused by fungi *B. oryzae, C. oryzae* gave 41% abnormal seedlings and 33% seed rot. Dark brown seed discoloration caused by fungi *B. oryzae, A. padwickii* and *C. oryzae* lead to 40% abnormal seedlings and 14% seed rot, while ash grey discoloration caused by *Alternaria* genus gave 39% abnormal seedlings (12% stunted and 27% blighted) and 25% seed rot. Black dots and light brown seed discoloration recorded both 35% abnormal seedlings, while light brown caused by fungi *B. oryzae* and *A. padwickii* recorded the maximum value 44% seed rot and minimum value 21% normal seedlings.

Isolation and identification of seed transmission of abnormal seedlings: Table 5 illustrated that *A. padwickii, A. tenuis, B. oryzae, Curvularia oryzae, F. graminearum* and *F. moniliforme* were isolated from seed rot of both cultivars. Also, *A. padwickii, B. oryzae, F. graminearum* and *S. oryzae* were isolated from blight seedlings while *B. oryzae* and *S. oryzae* were isolated from stunted seedlings. Most common pathogens were isolated from diseased seedlings of *A. padwickii, B. oryzae* and *Fusarium* spp.

Table 5: Pathogenic isolated fungi from seed rot and abnormal seedlings in two cultivars

Seedling diseases	Isolated pathogens
Seed rot	
EHR 1	*A. padwickii, A. tenuis, B. oryzae, C. oryzae, F. graminearum* and *F. moniliforme*
EHR 2	*B. oryzae, C. oryzae, F. graminearum, F. moniliforme* and *S. oryzae*
Blighted seedlings	
EHR 1	*A. padwickii, B. oryzae, F. graminearum* and *S. oryzae*
EHR 2	*A. padwickii, B. oryzae, F. graminearum* and *S. oryzae*
Stunted seedlings	
EHR 1	*B. oryzae* and *S. oryzae*
EHR 2	*B. oryzae* and *S. oryzae*

DISCUSSIONS

The present study showed that *Bipolaris oryzae* was the most prevalent fungi causing seed discoloration in Egyptian hybrid rice, responsible for light brown, black dots, dark brown and brown spots in both cultivars, followed by *A. padwickii* responsible for ash gray, dark brown, light pink, light brown and pale yellow, while the genus *Fusarium* was responsible for light pink and pale yellow; *A. alternate* led to ash gray, while *C. oryzae* led to brown spot and dark brown; *S. oryzae* leading to light brown. The results showed also that light brown and ash gray gave the highest values of total fungi followed by dark brown, light pink, black dots, pale yellow and brown spot in the two cultivars, respectively. These results were consistent with previous studies by Ou (1983), Nghiem and Hoang (1993) and Sachan and Agarwal (1995). Also, Agarwal *et al.* (1989) found that *A. alternate* caused brown discoloration in conventional rice seeds. Imolehin (1983) reported that *Curvularia lunata* caused glume and kernel discoloration in rice. Roy and Baruah (1972) found that *F. moniliforme* caused discoloration of rice seeds and poor seed germination. Results indicated that location of inoculum on seed parts as followed: *B. oryzae, Fusarium* spp. and *A. padwickii* were more on seed coat, followed by on husks and embryo, while *S. oryzae* recorded highest value on embryo. These results are agreement with earlier studies by Vachspati *et al.* (2000), Puglia (2004) and Tateishi (1998). Ou (1985) found that the condia of *A. padwickii* may be present on the seed surface and the resting mycelium in the tissues of endosperm, embryo, bran layers and glumes. Milagrosa (1987) isolated *S. oryzae* from the husks and kernels of both discolored an non- discolored seeds from panicles of sheath rot affected plants. Sachan and Agarwal (1995) also reported a higher percentage of seed-borne fungi on conventional rice varieties, when the degree of discoloration was higher. Vachspati *et al.* (2000), also found a higher percentage recovery of the pathogens were found on both the embryos and the endosperm regions which were more discolored as compared to lower of discoloration in hybrid rice. With respect to the effect of discoloration seeds by pathogenic fungi on seedlings, the results illustrated that *B. oryzae, A. padwickii, S. oryzae* and *C. oryzae* gave light brown, black dots, dark brown, brown spots and ash gray discoloration with the highest effect on percentage of abnormal seedlings and seed rot followed by *Fusarium* spp. that produced light pink and pale yellow discoloration seeds giving 15% normal seedlings, 50% abnormal seedlings and 30% seed rot. Earlier studies by Neergaard (1973), Abdel-Monem *et al.* (1995). Ou (1985) and Agarwal *et al.* (1989) found seedling mortality, root browning, color, necrosis and coleoptile spots due to *B. oryzae* infection in conventional rice.

B. oryzae has been reported to reduce seed germination and has also been shown to cause seedling blight (Guerrero *et al.*, 1972; Rath, 1974). Narain (1992) showed that seed rot and seedling mortality due to *S. oryzae* infection may be the result of toxin production. Toxic metabolites

of *S. oryzae* caused chlorosis and growth retardation of rice seedlings (Ghosh *et al*., 2002; Sakthivel *et al*., 2002). Mathur *et al*. (1972) noted *Alternaria padwickii* caused decay of rice seed resulting in the death of seedlings. Guerrero *et al*. (1972) also observed *A. padwickii*in 23% of abnormal seedlings and 15% of seedlings with decaying roots and shoots. Ora *et al*. (2011) found that *B. oryzae* and *F. moniliforme* were predominant on all tested hybrid rice varieties, also rotten seed and post-emergence mortality of seedlings were directly associated with seed-borne pathogenic infection. Isolation and identification pathogenic fungi, *A. padwickii*, *B. oryzae*, *C. oryzae*, *F. moniliforme* and *F. graminearum* from seed rot. *A. padwickii*, *B. oryzae*, *F. graminearum* and *S. oryzae* were isolated from blighted seedlings, while *B. oryzae* and *S. oryzae* were isolated from stunted seedlings in earlier studies conducted by Vachspati *et al*. (2000) and Duraiswamy and Marippan (1983).

Results of this work showed that *B. oryzae* was more fungi associated for light brown, black dots, dark brown and brown spot discoloration; *A. padwickii* was associated with ash gray, dark brown, light pink, light brown and pale yellow and *Fusarium* spp. for light pink and pale yellow discoloration. *B. oryzae*, *Fusarium* spp. and *A. padwickii* were more present in seed coat, while *S. oryzae* may be present in the embryo. Also, *B. oryzae*, *A. padwickii* and *S. oryzae* had the highest effect on abnormal seedlings and seed rot followed by *Fusarium* spp. on Egyptian hybrid rice. Finally, it could be concluded that using healthy seed of Egyptian hybrid rice were better in producing good seedlings compared with discolored seeds. Also, types of discolored seed were considered as good indicator for negative characters of seedlings regarding the high cost of their production.

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