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SEED HEALTH TESTING OF RICE AND EFFECT OF SOME FUNGICIDAL SEED TREATMENTS ON SEED - BORNE PATHOGENIC FUNGI

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Abstract

Forty rice seed samples of five varieties obtained from lots designated for seed production were used in this investigation. Seed-borne fungi, germination, seedling abnormality, and chemical control have been studied. According to the mean percentage of infected seed with different seed-borne fungi, the varieties could be arranged as follow: Riho (14.36%), Giza 171 (11.45%), IR 28 (8.14%), Giza 172 (2.45%) and IR 1626 (2.09%). The most common pathogens were *Drechslera oryzae* (28.0%) followed by *Trichoconiella padwikii* (18.8%) and *Pyricularia oryzae* (11.4%). Meanwhile, *Sarocladium oryzae*, the sheath rot pathogen of rice, had been observed for the first time in Egypt. Moreover, it has been observed that the aforementioned pathogens caused four different kinds of seedling abnormality, i.e. decay in shoot and root, decay in shoot only, twisted shoots and weak spindly primary root. However, the last type of abnormality was associated with some saprophytic fungi. Therefore, samples of lots showing high germination but infected with important pathogens should be rejected.

Elimination of seed-borne fungi inocula has been obtained effectively by soaking seeds in the fungicide Hinosan 50% (Edifenphos) at concentration of 1000 ppm for 24hr. Besides, germination of treated rice seed was increased 8.43% more than the control.

INTRODUCTION

Rice (*Oryzae sativa* L.) is grown, in summer, with a total of 1/3 out of the total area in Egypt. Most rice pathogens have been proved to be seed transmitted. There are about 50 seed-borne diseases known to occur on rice crop (Richardson, 1979). Those diseases are one of many factors which contribute to the low yield and heavy losses of rice crop. *Drechslera oryzae* (Van Breda de Haan) Subram & Jain-Perf. St. *Cochliobohus miyabeanus* (Ito & Kuribay) the brown spot disease and *Pyricularia oryzae* Cav., the blast disease were regarded as the most serious biologi-

cal menace to rice production. Besides, *Fusarium moniliforme* Sheld. - Perf. St. *Gibberella fujikuroi* (Saw.) Wollen weber the foot rot disease; *F.semitectum* Berk & Ray, the dry rot of grains and *F.graminearum* Schwabe-Teleomorph *Gibberella Zeae* (Schw.) Petch - the scab disease may be severe under favorable condition (Ou, 1985). Also, *Alternaria padwickii* (Ganguly) M.B. Ellis [syns. *Trichoconis padwickii* Ganguly; *Trichoconiella padwickii* (Ganguly) Jain], the stalk burn disease; *Nigrospora oryzae* (Berk & Br.) Patch-Perf. St. *Khuskia oryzae* Hudon, producing grain spots and *Sarocladium oryzae* (Sawada) W. Games & D. Hawksw (syn. *Acrocylindrium oryzae* Sawada), the sheath rot disease were reported as minor diseases (Singh and Mathur, 1992). Moreover, some fungi, i.e. *Cephalosporium* spp., *Verticillium* sp. and *D.rostorata* (Drechsl.) Richardson & Fraser were collectively reported associated with rice seed (Neergaard, 1970; Kang *et al.*, 1972 and Chidambaram *et al.*, 1973). Under Egyptian conditions, sixteen fungi were observed on blotter from 118 Egyptian rice seed samples of 6 cultivars and they vary greatly according to the cultivar, locality and yearly variation of climatic condition (Abdel-Moneim *et al.*, 1995). However, the great majority of rice diseases, which seem to be seed-borne in nature, are disseminated from different sources and by means other than seed (Neergaard, 1970).

On the other hand, it has been reported that seedling abnormality may be caused by inoculum of some seed-borne fungi, i.e. *D.oryzae, P.oryzae, T.padwickii* .. etc. Such seedlings usually develop into weak plants (Guerrero *et al.*, 1972).

Chemical seed treatment as one of controlling measures for eliminating important seed-borne fungal inocula has been recommended by many investigators (Froyd *et al.*, 1976; Mogi, 1979; Dharam Vir *et al.*, 1971; Park & Cho, 1972; Vidhyasekaran, 1980 and Fuji, 1983). However, some of the aforementioned investigators recommended application of fungicides as foliar spray, soil drenching to kill the other sources of rice diseases inocula.

The aims of the work were to see whether any of the important pathogens of rice occur more frequently on seeds of rice and the effect of their inocula on producing abnormal seedlings and whether pathogens could be eradicated by chemical seed treatment.

MATERIALS AND METHODS

Seed health testing:

Forty seed samples of different rice varieties i.e. Giza 171, IR 28, Riho, IR

1626 and Giza 172 from different localities in Egypt were used in this investigation. The seed samples were obtained from lots designated for seeding. Samples were tested by using the standard blotter method (ISTA, 1966). Two hundred seeds of each sample were plated in Petri dishes on three layers of well moistened blotters at the rate of 25 seeds / dish and then incubated in alternating cycles of 12 hrs light and darkness at 20°C for 8 days. Seeds were then examined using stereobinocular and compound microscope for the presence of seed-borne fungi.

Germination test:

This test was carried out simultaneously with the blotter method. Samples were tested using rolled towel method (Guerrero *et al.*, 1972). In all four hundred seeds of each sample in four replicates, were spaced on a double layer of moistened filter paper sheets (size 25 x 18 cm) and covered with a single sheet. The sheets were folded along one edge and then rolled up. The rolls were placed in poly-ethylene bags in a dark room at 28°C. After 8 days of incubation the seedlings were evaluated. Normal and abnormal seedlings were counted according to ISTA (1966). A second count of abnormal seedlings was made on completion of the test after 14 days. Abnormal seedlings were examined on both occasions for the presence of seed-borne fungi.

Elimination of seed-borne pathogens of rice by chemical treatment:

Two seed lots (Riho variety) were selected based on high value of their infection level with some important fungi ,i.e. *P.oryzae*, *D.oryzae*, *T.padwickii*, *F.semitectum* and *N.oryzae*. A working sample (3200 seeds) was taken from each lot and divided into eight subsamples (400 seeds, each). The two fungicides Hinosan 50% (Edifenphos) and Beam 75% (Tricyclazole), were diluted with distilled sterilized water to give the desired concentration, i.e. 500, 1000 and 1500 ppm. of Hinosan 50% EC and 2256, 4511 and 6767 ppm of Beam 75%. These concentrations equal 50, 100 and 150% of the recomended dose. Each 400 seeds were soaked in the aforementioned concentrations for 24 hrs. The remaining subsamples were used as control (one soaked in distilled sterilized water for 24 hrs and another without any treatment). The test was repeated twice with each seed lot.

Evaluation of treated seed with both fungicides at the aforementioned concentrations determined using the blotter method (ISTA, 1966) to record the efficacy of treatments on seed-borne fungi and germination as mentioned above. Average of

treatments (mean of 2 seed lots); and efficacy of each treatment on different seed-borne fungi and germination were calculated according to Anon. (1993).

RESULTS AND DISCUSSION

Seed borne fungi of rice :

Data of 40 seed samples collected from different localities in Egypt representing 5 varieties are summarised in Table (1). Eleven fungi, *Viz.P.oryzae, D.oryzae, T.padwickii, F.graminearum, F.moniliforme, F.semitectum, N.oryzae, S.oryzae, Verticillium sp., Cephalosphorium* sp. and *D.rostorata* were isolated by blotter method. These fungi, observed in the present investigation, are in accordance with findings recorded by a number of authors as mentioned in the introduction except for *S.oryzae.*

Table 1. Seed - borne fungi of different rice varieties collected from different localities of Egypt.

Pathogen	Maximum rice seed infection with some associated fungi recorded on different varieties					
	Giza 171	IR28	Riho	IRI626	Giza 172	Mean
P.oryzae	15.00	12.00	25.00	00.00	05.00	11.40
D.oryzae	59.00	21.00	45.00	06.00	09.00	28.00
T.padwickii	07.00	36.00	45.00	06.00	00.00	18.80
F.graminearum	06.00	01.00	00.00	00.00	00.00	01.40
F.moniliforme	02.00	01.00	00.00	00.00	01.00	00.80
F.semitectum	12.00	07.00	13.00	01.00	03.00	07.20
N.oryzae	07.00	03.00	15.00	07.00	02.00	06.80
S.oryzae	02.00	02.50	00.00	01.00	00.50	01.20
Verticillium sp.	02.00	03.00	00.00	00.00	00.00	01.00
Cephalosporium sp.	12.00	02.00	14.00	02.00	07.00	07.40
D.rostorata	02.00	01.00	01.00	00.00	00.00	00.80
Total	126.00	89.50	158.00	23.00	27.50	--
Mean	11.45	08.14	14.36	02.09	02.45	

The varieties could be arranged according to their total number of infected seed with different seed-borne fungi as follows: Riho (158); Giza 171 (126); IR 28 (89.50); Giza 172 (27.50) and IR 1626 (23.00). It also, can be arranged according

to the mean percentage of infection with different seed-borne fungi as follow: Riho (14.36); Giza 171 (11.45); IR 28 (8.14). Giza 172 (2.45) and IR 1626 (2.09). Moreover, *D.oryzae* (28.00) was generally the most common pathogen followed by *T.padwickii* (18.80) and *P.oryzae* (11.40). Of the other pathogenic or weak pathogenic species of fungi, *F.semitectum* (07.20) was the most frequent, followed by *N.oryzae* (06.80). The remaining fungi, i.e. *F.graminearum* and *F.moniliforme* occurred at lower frequencies. Besides, other saprophytic genera of associated fungi are not considered further as they probably do not play any pathological role.

Sheath rot caused by *Sarocladium oryzae* has been found for the first time in some rice seed lots collected from different localities of Egypt, representing four varieties, i.e. IR 28, (2.50), Giza 171 (2.00), IR 1626 (1.00) and Giza 172, (0.50). The disease had been recorded from India, Japan, Taiwan, Thailand, United states and Vietnam (Shahjaham *et al.*, 1977 and Ou, 1985). Seed transmission of the disease and quarantine significance were studied by Singh and Mathur (1992). Growth of the fungus on infected seeds developed on different parts of seed including the embryonal end in the blotter test. The fungus was examined carefully to see the angle of the conidiophores and shape of conidia to avoid confusion with *Verticillium* spp. The observed symptoms on seeds and the plants in fields were confirmed with typical symptom of sheath rot described by some investigators (Chuke, 1983 and Milagrosa, 1987). However, economic importance of the disease in Egypt is under observation.

Germination test:

Data of germination and abnormal seedlings of the three varieties Riho, Giza 171 and IR 28 are recorded in Table (2). The three aforementioned varieties were selected for this study based on their data in survey. They generally have more inocula of associated fungi than the other varieties. Four different kinds of abnormality, i.e. decay in shoot and root (1.58%), decay in shoot (1.08%), twisted shoot (0.42%) and weak spindly primary root (0.42%) are encountered. *D. oryzae* was the most frequent fungus isolated from abnormal seedling of the three varieties, followed by *T.padwickii*, decay in shoot phase; *P.oryzae* and *F.moniliforme*, decay in shoot and root phase; and *Cephalosporium* sp. twisted shoot.

The weak spindly primary root phase was correlated with saprophytic some fungi, i.e. *D.rosotrata, Cephalosporium* sp. and *Verticillium* sp. Therefore, observation on varieties abnormality percentage was confirmed in our finding of survey of infection with different associated fungi. The varieties could be arranged according

Table 2. Types abnormalities of rice plants associated with some seed - borne fungi (Rolled towel method).

Phase of abnormal seedling	Isolated fungi	Frequency% of isolated fungi from abnormal seedlings			Mean
		Riho	Giza 171	IR28	
Decay in shoot	*D.oryzae*	05.05	07.39	01.30	04.58
	D.rostorata	00.65	01.22	00.00	00.62
	P.oryzae	00.65	00.00	00.00	00.22
	T.padwickii	03.15	02.55	00.65	02.12
	N.oryzae	02.55	01.30	01.30	01.72
Percentage of Abnormality		01.00	01.75	00.50	01.08
Decay in shoot and root	*D.oryzae*	01.90	02.55	01.30	01.92
	T.padwickii	00.65	00.00	00.00	00.22
	P.oryzae	00.65	01.30	00.00	00.65
	F,moniliforme	01.90	00.00	00.00	00.63
	D.rostorata	01.30	00.00	00.00	00.43
	Cephalosporium sp.	00.30	00.30	00.30	00.30
	F.graminearum	00.00	01.25	00.00	00.42
Percentage of Abnormality		02.75	01.75	00.25	01.58
Twisted shoot	*D.oryzae*	02.55	01.90	01.30	01.92
	T.padwickii	00.00	00.00	00.65	00.22
	Cephalosporium sp.	00.65	00.65	00.00	00.43
Percentage of Abnormality		00.50	00.50	00.25	00.42
Weak spindly primary root	*D.rostorata*	01.30	01.30	00.65	01.08
	Cephalosporium sp.	00.00	00.65	00.00	00.22
	Verticillium sp.	00.00	00.00	00.25	00.08
Percentage of Abnormality		00.50	00.25	00.50	00.42
Total Abnormality %		04.75	04.25	1.50	03.50
No germination	*Cephalosprium sp.*	00.25	2.65	1.30	01.40
	N.oryzae	00.65	1.90	0.65	01.07
Total of ungerminated seed%		05.25	04.75	05.50	5.17
Germination %		90.00	91.00	93.00	91.33

to their percentages of abnormality as follows: Riho (4.75%); Giza 171 (4.25%) and IR 29 (1.5%).

In the case of ungerminated seed, different fungi has been investigated, i.e. *Cephalosporium* sp. and *N.oryzae*. However, many saprophytic fungi could be seen on ungerminated seed, i.e. *Aspergillus* spp., *Penicillium* sp., *Rhizopus nigricans* and Tri-*choderma viridae* (data not shown). therefore, the observation of the pathological aspect concerning abnormal seedlings during germination test proved that *D.orzyae* and *T.padwickii* were found associated with 3 out of the 4 abnormal phases, while *P.oryzae* occured with 2 abnormal phases. Also, *F.moniliforme* and *F.graminearum* were recorded in only 1 of the abnormal seedling phases. Data indicated that seed lots with highest percentages of pathogenic fungal inocula would be rejected for export and even for local sowing. These results are in accordance with those obtained by Guerrero *et al.*, (1972).

However, the saparophytic genera associated with abnormalities might have a litte role to cause abnormality and BP test permited to encourage their occurrence. Moreover, a number of normal seedlings counted on the eight day in rolled towel were picked up at random and examined under stereobinocular microscope. Some of these were found to carry T.padwickii. Such normal seedling may become abnormal later. Hence, the pathogenic fungi of rice which might be seed transmitted caused seedling abnormality and that reflect their effect at earlier stage of rice to give weak plants. Thereby, samples of lots showing high germination but infected with important pathogns should be rejected.

Elimination of some seed-borne pathogens by chemical seed treatment:

Data of soaking 2 seed lots of rice in different concentrations i.e. 000; 500 ; 1000 and 1500 ppm of Hinosan 50% and 0000; 2256; 4511 and 6767 ppm of Beam 75% for 24 hr are summarised in Table (3). The best fungicidal seed treatment which killed all seed-borne fungal inocula and increased seed germination by 8.34% more than the control (soaking in water). was hinosan at 1000 ppm The following seed treatment (at 4511 ppm, Beam 75) gave complete control of *D.oryzae* and *P.oryzae*, whereas its efficacy on other fungi ranged from 70.10 to 91.67% and increased seed germination by 3.93% more than the control.

However, soaking rice seed in 1500 ppm Hinosan 50% and 6767 ppm Beam 75% killed effectively all seed-borne inocula but seed germination has been de-

Table 3. Efficacy of different concentrations of fungicides on rice seed - borne fungi and seed germination.

Fungicides	Concentration ppm	*Alternaria spp.*	*D.oryzae*	*Fusarium spp.*	*N.oryzae*	*P.oryzae*	*T.padwickii*	Germination
Water	000	08.33	59.09	37.50	33.33	59.09	61.86	00.00
Hinosan	500	100.00	91.67	87.50	50.00	100.00	100.00	01.69 (+)
50% Ec	1000	100.00	100.00	100.00	100.00	100.00	100.00	08.43 (+)
	1500	100.00	100.00	100.00	100.00	100.00	100.00	12.36 (-)
Beam 75%	2256	33.33	91.67	75.00	33.33	100.00	61.86	00.00
	4511	91.67	100.00	91.67	83.33	100.00	70.10	03.9 (+)
	6767	100.00	100.00	100.00	100.00	100.00	84.50	15.17 (-)

creased by 12.36% and 15.17%, respectively, of the control (soaking in water). Therefore, chemical rice seed treatment with Hinosan 50% at 1000 ppm should be recommended to eliminate inocula of pathogenic fungi which are seed transmitted. In addition, it has been noticed that soaking seed in water gave some efficacy in reducing some seed-borne pathogens. In this case, water plays a role to wash inocula adhering loosly onto seed surface.

These results are in accordance with findings reported by a number of authors as stated in the introduction. In addition, it has been recommended spraying both systemic fungicides Edifenphos and Tricyclazole to control rice blast disease of rice (Anon., 1992). However, more chemical control may be required to kill inocula which dissiminate by other means of transmission, i.e. soil and air.

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دراسة سلامة حبوب الأرز وتأثير استخدام المبيدات الفطرية على الفطريات الممرضة

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أجريت هذه الدراسة على ٤٠ عينه أرز من لوطات معدة كتقاوى للزراعة من خمسة أصناف مختلفة، وقد شملت الدراسة تقدير نسب الإصابة بالفطريات المختلفة، وتقدير نسب الإنبات ، وتقدير نسب التشوهات فى البادرات المصابة ونوع هذه التشوهات وعلاقاتها بنسب الإصابة بالفطريات المختلفة. كما تم دراسة تأثير المعاملة ببعض المبيدات الفطرية لمقاومة الفطريات المصاحبة للتقاوى عن طريق النقع قبل الزراعة.

أوضحت النتائج أن متوسط نسب الإصابة بالفطريات المختلفة على الأصناف المستخدمة هى كالتالى : ريهو (١٤,٣٦٪)، جيزة ١٧١ (١١,٤٥٪)، IR28 (٨,١٤)، جيزة ١٧٢ (٢,٤٥٪)، IR1626 (٢,٠٩٪) ووجد أن فطر *Drechslera oryzae* هو المرض الشائع على البذور وسجل بنسبة (٢٨٪) يليه الفطر *Trichoconiella padwickii* بنسبة (١٨,٨٪) ثم الفطر *Pyricularia oryzae* بنسبة (١١,٤٪) وقد تم تسجيل الفطر *Sarocladium* oryzae لأول مرة فى جمهورية مصر العربية. وقد وجد أن الفطريات الممرضة السابقة سببت أربعة أنواع مختلفة لتشوهات البادرات أثناء الانبات وهى : تحلل فى الريشة والجذير، تحلل الريشة فقط، التواء الريشة، خروج جذير منتفخ ضعيف ولو أن الحالة الإخيرة من التشوه إقترنت مع ظهور الفطريات الرمية. ومن هنا فإن نسبة الإنبات فى اللوطات المعدة لإنتاة التقاوى يجب ألا تكون الحكم الوحيد على قبولها للزراعة.

وبدراسة تأثير بعض المطهرات الفطرية على قتل اللقاحات الموجودة على حبوب الأرز، وجد أن نقع حبوب الأرز فى محلول مبيد الهينوسان ٥٠٪ بتركيز ١٠٠٠ جزء فى المليون لمدة ٢٤ ساعة قبل الزراعة أدى إلى القضاء على جميع اللقاحات الموجودة فى الحبوب بكفاءة عالية مع زيادة نسبة الإنبات بدرجة موجبة تساوى ٨,٤٣٪.