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SIGNIFICANCE OF PRE HARVEST SEEDBORNE FUNGI OF PADDY VAR. SITA

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Altogether 19 spp of fungi belonging to 12 genera of Dematiaceae with varying frequency were isolated from paddy var. Sita seeds 15-20 days ahead of harvesting. Two spp of *Fusarium* were also present in this stage. *Helminthosporium gramineum, H. sativum, Curvularia lunata, Alternaria alternata, A. tenuissima, Cladosporium herbarum, Memnoniella echinata* and *Fusarium moniliforme* were dominant. Both the spp of *Alternaria* and *Helminthosporium, C. geniculata, Periconia minutissima, M. echinata* and *D. australiensis* inflicted brown spot symptom on the seeds when artificially inoculated in pre harvest stage and incubated at 70 and 80 % RH. Most of the fungi caused off-colouration of considerably high per cent of seeds in comparable condition. 2-4% of the seedling raised from the inoculated seeds stored at 60% RH for six month had brown lesions on the root due to a few fungi while in 3-5% seedlings raised from the seeds stored at 70% RH, two additional types of symptoms of disease i.e. water soaked lesion on the root and the foot were observed. The seeds stored with fungi at 80% RH produced more number of seedling affected with above diseases. Besides the above symptoms, the number of fibrous roots were less. Smaller oval brown spots were inflicted on the leaves by *H. gramineum* and *H. sativum. F. moniliforme* produced more number of seedlings affected with water soaked lesions. The length of the root and the shoot of the seedlings were significantly (P= 0.001) less due to the pre harvest fungi.

Keywords : Diseases in seeds and seedlings; Paddy; Pre harvest fungi; Root and shoot length.

Introduction

The mycodeterioration of seeds during storage has been extensively studied [1-4] but the significance of pre harvest fungi of seeds has not yet been appreciably investigated except some pioneering works [1,5-8]. Narain[9] has reported that dematiaceous hyphomycetes and *Fusarium* spp cause discolouration and shrivelling of seeds, death of embryo and other anomalies in the developing seeds. The present paper deals with the isolation of pre harvest seedborne fungi of paddy var. Sita and their significance with respect to infliction of diseases in the seeds and the seedlings, and the length of root and shoot in the next cropping season.

Materials and Methods

Seeds of paddy (*Oryza sativa* L) var. Sita were collected 15-20 days ahead of harvesting in sterilized polyethylene pockets from various parts of Bihar state in December 2001 and stored at 4-5°C in a refrigerator, if needed.

Isolation of fungi from pre harvest seeds : The seeds were surface sterilized with 0.1% $HgCl_2$ for 1 min and washed thoroughly with tap water and three times with autoclaved distilled water. Twenty seeds were placed aseptically on sterilized moist blotter and ten on potato dextrose agar (PDA) medium. These were incubated at 25 ± 1°C for 10 days and under 12 hr tube light of 5000 lux and 12 hr darkness. The isolated fungi were identified [10,11] and their frequency was recorded (Table 1). The fungi were preserved in pure form on PDA slants.

Inoculation of the seeds : The fungi (Table 1) were grown on PDA slants at 25±1°C for 10 days and spore suspension was prepared using 10% aq. Tween 20. The number of spores was adjusted to 500 approx / ml suspension with the help of heamaçytometer.

In the next December, the inflorescences of the crop were surface sterilized as noted for the seeds in the field nearly 20 days ahead of harvesting and washed thoroughly with tap water and finally with sterilized distilled water with the help of sprayer. The adhering water with seeds was soaked with sterilized dry towel and the seeds were inoculated with 10 ml of spore suspension per

inflorescence by spraying with glass atomizer.

Each inflorescence was covered with transparent and colourless polyethylene pocket separately filled with 100 ml of glycerol solution to maintain 60,70 and 80% RH[12]. The inoculated inflorescence was hanged in the pocket over glycerol solution by covering the neck of the inflorescence with sterilized absorbant cotton pad and tying lightly with thread . Each polyethylene pocket was supported by three sticks pitched in the soil. Triplicate of the inflorescence was incubated for each fungus and each RH level for 20 days. Uninoculated inflorescences were maintained as the control.

Seeds were observed after the expiry of incubation for infliction of symptom of disease, if any. Total seeds and those with symptoms of disease in the three inflorescences for one fungus and three RH levels were separately counted and the per cent value of diseased seeds was recorded as whole number (fraction above 0.5 was considered as 1 and below 0.5% as zero) (Table 2).Then the seeds were extracted as normal harvest, and of the three inflorescences they were mixed together. The moisture content of the harvested seedlot weighing 30 g each was reduced by placing it over fused $CaCl_2$ in sealed desiccators for 96 hr. Per cent moisture content of desiccated seedlot was determined by drying 5g seedlot of each in triplicate in an oven initially at 80°C for 48 hr and finally desiccating over fused $CaCl_2$ for next 72 hr. The moisture content of the seedlot varied from 7.12 to 7.39%. The seedlots treated as above, were stored in polyethylene pockets at room temperature from January to June 2003, the normal storage period.

Raising the seedlings from the fungus stored seeds :The seedlings were raised in July 2003 in the garden soil autoclaved at 20 psi for 20 min for two consecutive days. The autoclaved soil was filled in earthen pots having top diameter 20 cm, base diameter 15 cm and depth 15cm. The stored seedlots kept in the pockets, were surface sterilized as stated earlier and 10 seeds per pot, at equal distance, were sown nearly 0.5 cm deep in the soil which was covered with 0.5 cm thick sterilized cotton wool. Five such pots were prepared for each fungus and RH level. Watering with autoclaved tap water was maintained every alternate day for a period of 30 days and symptoms of disease were observed on the leaf and the root and foot by rooting out and washing the seedlings. Mean of total length of fibrous roots and shoots of fifty seedlings was recorded (Table 4) and statistically analysed.

Results and Discussion

Altogether 19 spp of fungi belonging to 12 genera were isolated from paddy var. Sita seeds in pre harvest stage (Table 1). Except *Fusarium* spp all other fungi with varying frequency, belonged to Dematiaceae. Among them *H. gramineum, H. sativum, C.lunata, A. alternata, A. tenuissima, C.herbarum* and *M. echinata* were dominant. *Fusarium moniliforme* was also equally dominant.

On artificial inoculation, the seeds incubated at 60% RH and the control of all the RH levels were symptomless. 3-9% of the seeds of the inflorescence inoculated with *A. alternata, A. tenuissima, C. geniculata, H. sativum, H. gramineum, P. minutissima, M. echinata and D. australiensis* and stored at 70 and 80% RH were found inflicted with brown spots. Most of the fungi caused off- colouration of the seeds more at 80% RH than 70% (Table 2). 2-4% of the seedlings raised from the inoculated seeds stored at 60% RH had brown lesions on the root due to some fungi (Table 3) while in those seedlings raised from the seeds stored at 70% RH, two additional types of symptom of disease i.e. water soaked lesions on the root and foot were added in 3-5% of the seedlings. The inoculated seeds stored at 80% RH produced more number of seedlings affected with the above symptoms of disease besides decrease in the number (Table 3) and length of fibrous roots and shoots (Table 4). Smaller oval brown spots were inflicted on the leaves by *H.gramineum* and *H. sativum.* Water soaked lesions in the root of maximum number of seedlings was observed due to *F. moniliforme.*The control seedlings were symptomless.

Pre harvest seedborne fungi have earlier been reported[6-8]. The authors have observed the association of dematiaceous hyphomycetes, and occasionally very few *Aspergillus* and *Fusarium* spp.

The occurrence of fungi in pre harvest stage of the seeds depends upon their parasitic nature, growth at the prevailing temp and tolerance of the sun rays besides their ability of secreting enzymes for facilitating the entry into the seed. Working on the microbial decay of grass shoot, Narayan[13] observed the weak parasitic tendency of most of the fungi enlisted in Table 1. These have also been reported in pre harvest seeds of garden plants of Asteraceae. The compact inflorescence of this family containing more moisture and fading floral parts as suitable substrate, were held responsible[7] for the occurrence of per harvest fungi. The same author, earlier reported[6] pre harvest seedborne endophytic fungi in Umbelliferae. The optimum temperature for the growth of these fungi has been observed to be between 25-30°C[8] but it is also evident from the present work that they tolerate the conditions of storage and cause diseases in the seedlings. *In vitro* high activity of cellulolytic and pectolytic enzymes of these fungi have been observed[8]. Cutinolytic activity of

Table 1. Frequency of the fungi isolated from the pre harvest seeds of paddy var. Sita.

Fungus spp	Frequency (%)
Alternaria alternata (Fr) Keissler	11
A. tenuissima (Kumzet ex. Pers) Wiltshire	12
Cladosporium cladosporioides (Fresen) de Vries	08
C. herbarum (Pers) Link ex Gray	12
Curvularia geniculata (Tracy & Earle) Boedijn	10
C. lunata (Wakker) Boedijn	15
C. pallescens Boedijn	10
Dictyoarthrinium quadratum Hughes	04
Epicoccum nigrum Link Schoo – Schwarz	10
Helminthosporium sativum Pammel. King & Bakke	12
H. gramineum (Rabenh ex Schlecht) Shoemaker	17
Trichoconis padwickii Ganguli	09
Drechslera australiensis (Buqnicourt) Subram & Jain	07
Periconia minutissima Corda	08
Memnoniella echinata (Rivolts) Galloway	11
Nigrospora oryzae (Berk & Br) Petch	05
N. shaerica (Saccardo) Nason	05
Fusarium moniliforme Scheldon	14
F. roseum Link ex Fries	08
	12 genera and 19 spp

Table 2. Symptoms of disease in the seeds of paddy var. Sita following inoculation of inflorescence with pre harvest fungi incubated at varying RH (%) for 20 days.

Pre harvest fungi	RH (%) of incubation		
	60	70	80
A. alternata	-	BS(3)	BS(5)
A. tenuissima	-	BS(4)	BS(9)
C. cladosporioides	-	-	OC(61)
C. herbarum	-	-	OC(69)
C. geniculata	-	BS(3)	BS(6)
C. pallescens	-	-	OC(70)
D. quadratum	-	OC(10)	OC(81)
E. nigrum	-	OC(7)	BS(2), OC(57)
H. gramineum	-	BS(6)	BS(9)
H. sativum	-	BS(5)	BS(7)
T. padwickii	-	OC(6)	OC(69)
D. australiensis	-	OC(6)	BS (3), OC(72)
P. minutissima	-	MBS(4)	BS(6), MBS(10)
M. echinata	-	MBS(3)	BS(7), MBS(12)
N. oryzae	-	OC(7)	OC(59)
N. sphaerica	-	OC(8)	OC (72)
F. moniliforme	-	OC(13)	OC(98)
F. roseum	-	OC(11)	OC(85)
Control	-	-	-

- = No Symptoms of disease, BS = Brown spot, MBS = Minute brown spot, OC = off – colouration. Figures in parenthesis indicate the per cent seeds affected.

Table 3. Diseases in the seedling of paddy var. Sita raised from the seeds stored with pre harvest fungi at varying RH (%).

Pre harvest fungi	Symptoms of diseases in the seedlings	Per cent seedlings affected		
		60%RH	70%RH	80%RH
A. alternata	1. Number of fibrous roots 3-5	-	-	8
	2. Water soaked lesions on the foot	-	4	9
	3. Brown lesions on the root	4	5	10
A. tenuissima	1. Number of fibrous roots 3-5	-	-	12
	2. Water soaked lesions on the foot	-	2	10
	3. Brown lesions on the root	3	6	11
C. herbarum	1. Number of fibrous roots 3-5	-	-	6
	2. Water soaked lesions on the foot	-	2	4
C. lunata	1. Number of fibrous roots 3-5	-	-	5
	2. Water soaked lesions on the foot	-	2	6
C. pallercens	1. Number of fibrous roots 3-5	-	-	4
	2. Water soaked lesions on the foot	-	3	9
	3. Brown lesions on the root	-	-	6
E. nigrum	1. Number of fibrous roots 3-5	-	-	5
	2. Water soaked lesions on the foot	-	4	6
	3. Brown lesions on the root	2	3	6
N. oryzae	1. Number of fibrous roots 3-5	-	-	7
	2. Water soaked lesions on the foot	-	3	7
	3. Brown lesions on the root	3	4	6
F. moniliforme	1. Number of fibrous roots 3-5	-	-	8
	2. Water soaked lesions on the root	3	4	15
	3. Brown lesions on the root	-	2	8
	4. Water soaked lesions on the foot	-	4	9
H. gramineum	1. Number of fibrous roots 3-5	-	2	6
	2. Brown lesions on the root	3	7	11
	3. Water soaked lesions on the foot	-	6	10
	4. Smaller oval brown spots on the leaves	-	-	5
H. sativum	1. Number of fibrous roots 3-5	-	3	8
	2. Brown lesions on the root	-	6	10
	3. Water soaked lesions on the foot	-	6	8
	4. Smaller oval brown spots on the leaves	-	-	4
M. echinata	1. Number of fibrous roots 3-5	-	-	6
	2. Brown lesions on the root	-	4	8
	3. Water soaked lesions on the foot	-	5	7
N. sphaerica	1. Number of fibrous roots 3-5	-	-	4
	2. Brown lesions on the root	-	5	9
	3. Water soaked lesions on the foot	-	4	7
Control	1. Number of fibrous roots 7-10			
	2. No symptom of disease on the foot and root			

Table 4 . Lenth of root and shoot (in cm) of the seedlings of paddy var. Sita raisad from the seeds stored with pre harvest fungi at 60,70,80% RH.

Pre harvest fungi	RH (%)	Root length	Shoot length
A. alternata	60	49	17
	70	43	15
	80	31	11
A. tenuissima	60	48	16
	70	44	14
	80	32	11
C. herbarum	60	54	18
	70	51	16
	80	40	13
C. lunata	60	55	18
	70	33	16
	80	41	12
C. pallescens	60	53	19
	70	49	16
	80	42	12
E. nigrum	60	54	18
	70	50	17
	80	41	12
N. oryzae	60	54	18
	70	51	17
	80	42	13
F. moniliforme	60	53	18
	70	49	16
	80	40	11
H. gramineum	60	52	18
	70	50	16
	80	38	10
H. sativum	60	52	17
	70	49	16
	80	37	10
M. echinata	60	54	19
	70	52	18
	80	45	12
N. sphaerica	60	55	19
	70	54	17
	80	44	13
Control	60	72	29
	70	70	27
	80	66	24

F Table 4 A (For root length)

Sources of variation	SS	df	ms	F	P
Between treatments	1684.54	12	140.378	47.58	0.001
Between replicates	1154.65	2	577.325	196.23	0.001
Residual	70.61	24	2.924		

F Table 4 B (For shoot length)

Sources of variation	SS	df	ms	F	P
Between treatments	517.99	12	43.165	99.688	0.001
Between replicates	260.97	2	130.485	301.351	0.001
Residual	10.40	24	0.433		

pathogenic fungi has seen reported earlier[14]. This activity is highly expected to help colonize on the seeds and inflict brown spots. Off-colouration of the seed is expected due to corrosion of the seed surface by cutinolytic enzyme which is expected to impart depreciating salability.

The increase in the number of diseased seedlings raised from the seeds stored with pre harvest fungi at high RH points out that this facilitates the fungi in establishing in the seed tissue. Parasitic nature of *H. gramineum* and *H. sativum* producing leaf spot becomes evident. The water soaked lesions on the root and foot might appear due to colonization of fungi producing enzymes to hydrolyse the cell wall constituents. Brown lesions on the root surface are expected due to oxidation of phenolics at the exposed tissue. Less number of fibrous roots and their smaller length are manifestation of disturbance in growth physiology due to destruction of IAA by IAA oxidase as observed recently[15]. Besides this, the mitotic index of the root tip of wheat, gram and mustard has been found lower due to the metabolite of *Aspergillus flavus* in Richard solution and acetone extract of the fungus-stored seeds[4]. Decreased number of roots, their shorter length and shorter shoot can be explained in the light of above noted facts.

Pre harvest seedborne fungi have been observed to inflict shrivelling of seeds, their blighting, staining and death of the embryo besides degrading the market value of the seeds[1,9]. Observing the load of pre harvest seedborne fungi, pre harvest fungicidal spray to the maturing seeds can be attempted to destroy them following the suggestions of Beraha *et al.*[16] for the fruits and vegetables and success gained in case of finger-millet seed using 0.4% Captan[17].

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