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STORAGE FUNGI OF CROP SEEDS AND THEIR SIGNIFICANCE

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Altogether 16 spp of fungi were isolated from stored maize, 27 spp from gram and 22 spp from mustard seeds. Deuteromycotina outnumbered the others. The frequency of *Aspergillus flavus*, *A. niger* and *Fusarium moniliforme* was maximum. The extract of the seeds stored with these fungi suppressed the seed germination. Considerable per cent of the radicles were curved and seedlings were smaller and the mitotic index was lowered due to these fungi besides the diameter of the nuclei in the cells of gram radicle tip was bigger due to *A. niger* and smaller due to *A. flavus* and *F. moniliforme* than the control. The activity of amylase, protease and lipase was found to be suppressed due to the metabolite of these fungi in Richard solution. The root and shoot length and dry weight of the seedlings were found to be less due to the metabolite. Also, the metabolite reduced the amount of total chlorophyll, total soluble sugar, total free amino acid and Ca, Mg and Zn in the seedlings.

Keywords : Abnormal seedlings; Biochemicals; Crop seeds; Germination; Hydrolytic enzymes; Metaboliite; Seedling growth; Storage fungi.

Introduction

Storage fungi of the crop deteriorate the seeds in many ways that include the suppression of seed germination, seed decay, alteration in the seed physiology and producing seedlings with crippled physiology and biochemistry, production of abnormal seedlings and inflicting seedling diseases[1-7].

The present paper deals with the isolation of seedborne storage fungi of maize, gram and mustard, their effect on seed germination, production of abnormal seedlings, mitotic index, size of the nuclei in the cell of root tip, total chlorophyll (TC), total soluble sugar (TSS), total free amino acid (TFAA) and Ca, Mg and Zn in the seedlings besides the length of the root and shoot and dry weight of the seedlings.

Materials and Methods

Collection of seeds and isolation of storge fungi - Seeds of maize (*Zea mays* L.) Var shaktiman-1, gram (*Cicer arietinum* L.) var local and mustard *(Brassica campestris* L.) Var Varuna stored with farmers of different parts of Bihar state were collected in sterlized polyethylene pockets and fungi were isolated adopting blotter technique[8]. The fungi were cultured on potato dextrose agar medium at 28±2^0 C for facilitating isolation in pure form and identification. Frequency of the isolates was calculated. Based on the frequency, *Aspergillus flavus* Link ex Fries, (87-92%), *A. niger* Van Tieghem (56-61%) and *Fusarium moniliforme* Sheldon (24-49%) were selected for observing their effect on aspects of seeds and seedlings of the enlisted crops.

Infestation and storage of the seeds and observation of germination and seedling abnormalities- Fifty g of seeds of above crops were surface sterilized separately with 0.1% $HgCl_2$ for 1 min and washed thrice with tap water and twice with sterilized distilled water and infested with the spores of the fungi selected above[5] and stored for 45 days over saturated ammonium sulphate solution (80%RH) at 30±1^{0}C. The control lot was uninfested. The stored seedlots were extracted each with 100 ml of acetone, filtered, centrifuged at 5,000 rpm for 10 min and the filtrate was dried at the room temperature under vacuum. The residue was dissolved in 25 ml of sterilized conductivity water.

The total volume of the extract was divided into five parts each of 5ml. A lot of 10 seeds each of five replicates of maize, gram and mustard possessing 98, 99 and 97% germinability respectively were soaked in the extract for 18 hr at 30±0.5C. Such treated seeds were germinated on sterilized moist blotter at 30±0.5^{0}C for 5 days and the germination was recorded. Abnormalites as curved and distinctly small radicle were also

recorded besides observing the mitotic index in the cells of the root tips using acetocarmine stain. The diameter of the resting nuclei of 100 cells of the smear prepared was measured in μm after camera lucida sketch and the mean was culculated.

Assay of hydrolytic enzymes of seeds-The hydrolytic enzymes such as amylase[9] in maize and gram, protease[10] in gram, and lipase[11] in mustard were assayed soaking the seeds possessing noted germinability in the metabolite of the fungi in Richard solution. Twenty g of the seeds of the above crop was soaked in 20 ml of metabolite prepared in 100 ml solution at 28°C for 10 days.

Observation of the characteristics of the seedlings-The seeds soaked as above in the metabolite was germinated in sterilized moist blotter as noted earlier and cultured in pure and sterilized sand impregnated with Hoagland solution[12] taking in plastic jars of 10 cm depth and 5 cm diameter. Altogether 10 pots for maize and 5 pots each for gram and mustard were used for raising 50 seedlings each for 15 days at 22-30°C and RH 70-80% and 12 hr light (10,000 lux) and 12 hr darkness. The control was maintained of the seedlings cultured in Hoagland solution on soaking the seeds in the Richard solution only.

Root and shoot length of the seedlings were measured in cm and dry weight was taken on drying them at 70°C for 48 hr and cooling them for next 72 hr over fused $CaCl_2$ in sealed desiccaters. TC[13], TSS[14] and TFAA[15] were estimated in first leaf. The mineral matter of the seedlings of maize only was determined on drying, grinding and ashing them in Muffle furnace at 600°C for 2hr. Ca, Mg and Zn were estimated[16].

Results and Discussion

Altogether 16 spp of fungi were isolated from stored maize, 27 spp from gram and 22 spp from mustard seeds. Of these spp Deuteromycotina outnumbered the other. The frequency of *Aspergillus flavus. A. niger* and *Fusarium moniliforme* was maximum with all the three crop seeds. The germination of the seeds of all the crops was found to be significantly suppressed (Table 1) due to the extract of all the noted fungi. *A. flavus* and *A. niger* behaved alike. Considerable per cent of seeds produced curved and distinctly smaller radicles (Table 2 and Fig. 1). In this regard, *A. niger* proved most detrimental for maize and *A. flavus* for gram. The effect of the two fungi was most adverse on mustard. *A. flavus* inflicted maximum decrease in mitotic index followed in succession by *A. niger* and *F. moniliforme* (Table 3). The diameter of the nucleus in the cells of gram radicle tip was more due to *A. niger* and less due to *A. flavus* than the control. The diameter of nuclei was minimum due to *F. moniliforme* (Table 3).

The activity of amylase, protease and lipase of the seeds were found to be suppressed due to the metabolite of the fungi (Table 4). The untreated control seeds possessed highest activity of these enzymes. The length of the root, shoot and dry weight of the seedlings of the crops (Table 5) was suppressed to the maximum due to the metabolite of *A. flavus* followed in succession due to *A. niger* and *F. moniliforme*. Similarly, the concentration of TC, TSS and TFAA in the first leaf of the seedlings of crop plants were found to be the minimum due to *A. flavus* followed in succession by *A. niger* and *F. moniliforme* (Table 6). The concentration of Ca, Mg and Zn followed the similar trend in maize (Table 6).

The number of spp of fungi in association with stored seeds may depend upon the variety of seeds, storage condition, agricultural operations and the biochemical constitiuent of the seeds serving themselves as ecological niche[17] and many other factor. The suppression of seeds germination due to storage fungi has been established due to their toxic effect[18] and ultrastructural change of the embryo[19] disturbing the entire biochemistry of the seed. Curvature of the radicle and dwarfing of the seedlings might also be due to toxic principle secreted by fungi as reported in paddy[6]. Less mitotic index indicates the adverse effect of the said principle on the cell division including synthesis of proteins and DNA. The smaller

Table 1. *Germination of normal seeds of maize, gram and mustard after soaking them in acetone extract of fungus stored seeds.

Fungus spp	Crop Seeds		
	Maize	Gram	Mustard
A. flavus	52	68	52
A. niger	60	76	64
F. maniliforme	68	88	84
Control	92	94	92

CD at 5% level for seed - 9.03, CD at 5% level for fungus- 10.43
*Fractional figures were rounded to their whole number.

Table 2. Abnormalities in the radicle of maize, gram and mustard after germination of the seeds soaked in acetone extract of stored seedlot (Fractional figures were rounded to their whole number).

Crop Seed	Fungus spp.	Abnormalities(%)	
		Curved radicles	Distinctly small radicles
Maize	*A. flavus*	66	22
	A. niger	64	40
	F. moniliforme	22	64
	Control	00	00
Gram	*A. flavus*	48	60
	A. niger	32	72
	F. moniliforme	24	60
	Control	00	00
Mustard	*A. flavus*	72	28
	A. niger	86	16
	F. moniliforme	32	16
	Control	00	00

Table 3. Mitotic index and nuclear size in the cells of root tip of the seedlings of gram due to influence of acetone extract of the stored seeds.

Particulars	Crop seed	Fungus spp			
		A. flavus	*A. niger*	*F. maniliforme*	Control
Mitotic index	Maize	37	46	55	76
	Gram	41	50	59	87
	Mustard	59	48	58	89
Nuclear Size (in μm)		7.62	9.78	5.40	8.81

Table 4. *Amylase, protease** and lipase*** activities in the seeds of maize, gram and mustard after soaking them in the metabolite.

Seeds	Fungus spp	Enzymes		
		Amylase	Protease	Lipase
Maize	*A. flavus*	23		
	A. niger	21		
	F. moniliforme	18		
	Control	09		
Gram	*A. flavus*	32	147	
	A. niger	26	162	
	F. moniliforme	19	182	
	Control	11	195	
Mustard	*A. flavus*			$16x10^{-4}$
	A. niger			$10x10^{-4}$
	F. moniliforme			$8x10^{-4}$
	Control			$30x10^{-4}$

* Expressed as concentration of unreacted starch
**Expressed as concentration of amino acid released
***Expressed in milliequivalent/min/g sample.

Table 5. Length of the root and shot (in cm) and dry weight (in mg) of the seedlings of maize, gram and mustard raised from the normal seeds soaked in the metabolite

Seeds	Fungus spp	Root length	Shoot length	Dry weight
Maize	*A. flavus*	5.91	19.47	11.47
	A. niger	7.46	21.29	14.52
	F. moniliforme	7.85	25.88	18.62
	Control	10.01	29.77	20.45
Gram	*A. flavus*	3.86	10.88	11.49
	A. niger	4.33	13.34	13.68
	F. moniliforme	5.96	14.23	16.61
	Control	6.76	16.16	20.62
Mustard	*A. flavus*	4.06	13.34	4.78
	A. niger	4.50	15.89	5.84
	F. moniliforme	6.40	17.66	7.54
	Control	7.40	20.77	9.43
C.D. at 1% level for seed		0.701	2.399	2.141
C.D. at 5% level for fungus		0.810	2.770	2.472

Table 6. Total chlorophyll (TC), Total soluble sugar (TSS), total free amino acid (TFAA) and Ca, Mg and Zn in the seedlings of maize, gram and mustard raised from the seeds soaked in the metabolite. (All items expressed as concentration)

Seeds	Fungus spp	TC	TSS	TFAA	Ca	Mg	Zn
Maize	*A. flavus*	0.84	68	7	0.005	0.032	0.006
	A. niger	0.89	72	10	0.011	0.069	0.013
	F. moniliforme	0.90	90	13	0.031	0.050	0.031
	Control	0.93	121	16	0.066	0.064	0.038
Gram	*A. flavus*	0.90	83	7			
	A. niger	0.92	107	8			
	F. moniliforme	0.95	126	12			
	Control	10.99	138	17			
Mustard	*A. flavus*	0.86	90	7			
	A. niger	0.89	103	9			
	F. moniliforme	0.92	120	13			
	Control	0.95	136	19			
C.D. at 1% level for seed		0.022	10.44	1.643			
C.D. at 5% level for fungus		0.025	12.02	1.887			

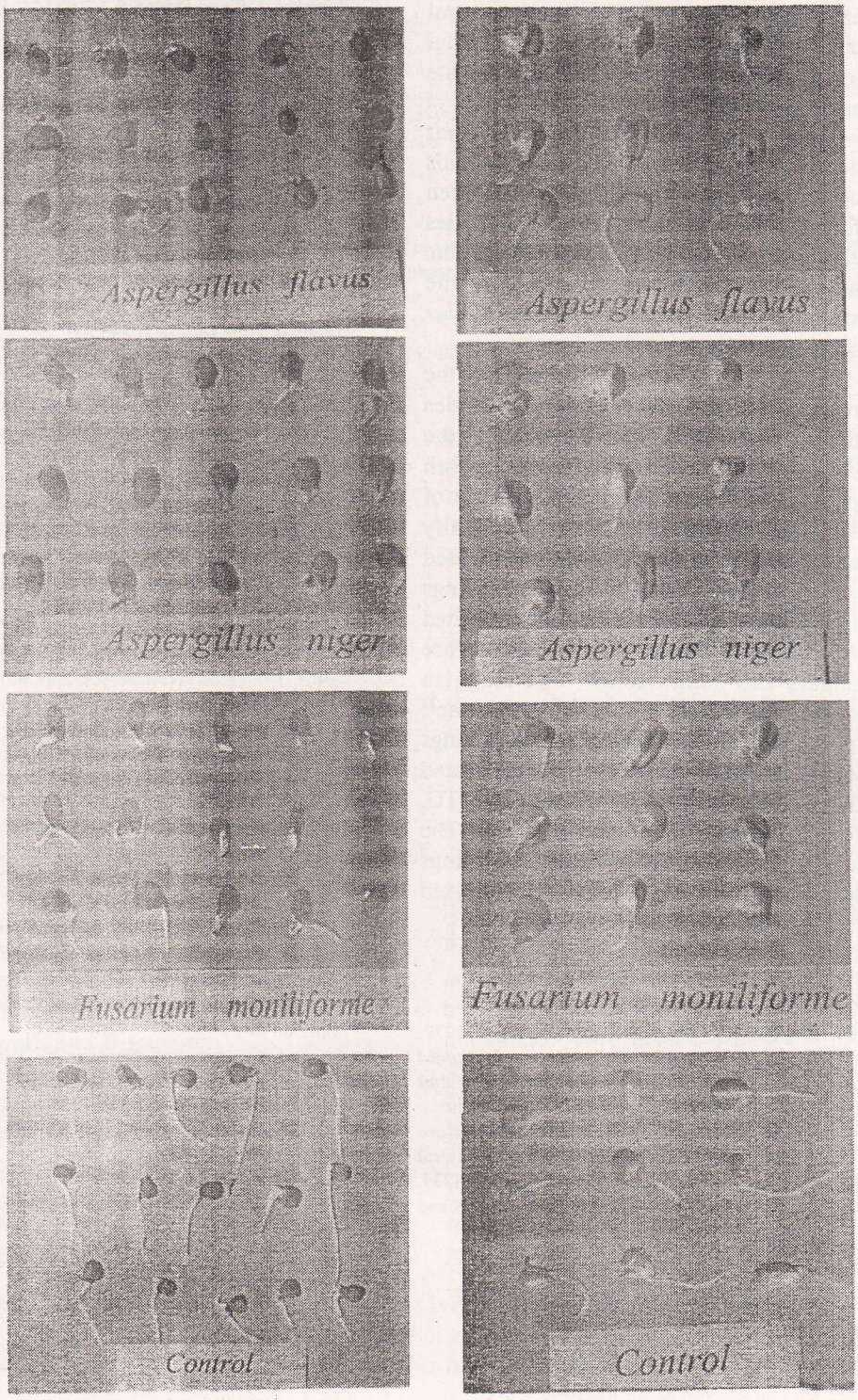

Fig. 1. Abnormality in the radicle of gram and maize due to storage fungi.

and bigger size of the nuclei than the control under the influence of the metabolite of fungi seems enigmatic till the time of elaborate and meticulous observation.

The hydrolytic enzymes that dissolve reserve starch, proteins and lipids of the seed during germination, have been found to be suppressed in their activities earlier[20]. This phenomenon again recalls the adverse effect of toxic principle of the storage fungi[21]. In this regard, *A. flavus* seemed to take the lead.

The shortening of the length of the root and shoot of the seedlings besides decrease in their dry weight point out the adverse effect of the storage fungi on growth physiology that includes the synthesis of food and other biochemicals essentially required for normal growth. Stimulated respiration and IAA oxidase of the seedlings as worked out earlier in radish[5, 22] excited exudation of cations, hexose and pentose sugars and amino acids[23] and sluggish activity of nitrate reductase and urease[24] might disarray the physiology. The seedlings of the crop plants undertaken have indicated impoverished growth and diminution in TC, TSS and TFAA due to the metabolite. The deficiency of Ca, Mg and Zn in the seedlings of maize might distrub the enzyme catalysed reaction as these serve as co-factor[25].

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