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PHYSICO-CHEMICAL ALTERATIONS IN MUSTARD SEEDLINGS DUE TO SEED BORNE *ASPERGILLUS FLAVUS*

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As the RH level of storage increased, the germinability of seeds; dry weight of shoot and root, total chlorophyll, pentose and hexose and total free amino acid of the seedlings decreased. The value of the noted parameters is significantly inflated due to the involvement of *Aspergillus flavus.*

Keywords : *Aspergillus flavus;* Physico-chemical alterations; Mustard.

Introduction

The quality of seedlots deteriorates due to storage fungi. These adversely affects the seed germination and raises the electrical conductivity of the seed leachate[1-3]. Present paper deals with the storage of mustard seed with *Aspergillus flavus Link* ex Fries and observation of the seed germination and physico-chemical characteristics of the seedlings.

Material and Method

Fifty g of mustard (*Brassica campestris* L) varuna seedlot possessing 98% germinability and 6.53% moisture, was surface sterilized with 10 ml of 0.1% $HgCl_2$ solution for 1 min. The seedlot was washed thrice with sterilized distilled water and infested with 0.5 ml of spore suspension of *A. flavus* (10^5 spore approx/ml suspension). Adherent water was removed keeping the infested seedlot over fused $CaCl_2$ for 2 hr. The seedlot was stored in triplicate over glycerine solutions to maintain 50, 60, 70 and 80% RH at 30 ± 0.5°C[4] for 30 days. After the expiry of storage, the seedlots were set for germination 1 cm deep in garden soil sterilized at 20 lbs psi for 15 min on two consecutive days in earthern pots of the size 20 cm top diameter and 15 cm base diameter and 20 cm height. 20 seeds per pot were sown and as such five replicates were taken. Pots were maintained for 10 days at 25 ± 1.5°C, 75-80% RH and 12 hr light (10,000 lux) and 12 hr darkness in a germinator. Pots were lightly watered every alternate day. The shoot and root of randomly harvested 50 seedlings were separated by cutting with a razor blade and dried at 80°C for 24 hr and cooled over fused $CaCl_2$ for next 48 hr. The dry weight was taken and calculation was made as mg of shoot and root/seedlings. Total chlorophyll of cotyledonary leaves was estimated[5].

Pentose and hexose[6] and total free amino acid[7] were determined and extraction of the seedlings with 80% warm ethanol and decolorizing with activated charcol. Urease activity in the seedling was assayed by oxidation of NADPH[6] in a reaction mixture containing urea.

Results and Discussion

As the RH level of storage increased from 50 to 80%, the germination of the seeds gradually decreased. The value further decreased for the seedlot stored with *A.flavus*. The shoot

Table 1. Germination (%) of mustard seed, dry weight of shoot and root (mg/seedling), chlorophylls (mg/g cotyledonary leaf) and hexose and pentose sugars and total free amino acid in the seedlings (mg/g fresh weight) raised from the seeds stored with *Aspergillus flavus* at varying RH at 30 ± 0.5°C for 30 days.

Particulars		RH (%) of storage of the seeds				C.D. at 1%
		50	60	70	80	
Germination	C	94.36	89.67	72.23	62.56	6.53
	I	87.72	67.81	43.52	35.73	
Shoot	C	4.23	4.80	5.62	3.56	0.61
	I	3.08	3.23	2.65	2.07	
Root	C	1.86	1.98	2.53	1.16	0.34
	I	1.75	1.52	1.16	0.79	
Total Chlorophyll	C	0.63	0.75	0.95	0.74	0.05
	I	0.56	0.69	0.79	0.60	
Pentose sugar	C	2.25	1.89	1.12	0.93	0.17
	I	1.63	0.95	0.63	0.45	
Hexose sugar	C	22.82	18.20	14.23	9.07	1.07
	I	15.28	11.25	8.20	5.92	
Total amino acids	C	7.12	5.82	4.27	3.08	0.32
	I	5.65	3.58	1.82		

C = Control; I = Infested.

Table 2. Urease activity of the seedlings of mustard raised from the seeds stored with *A. flavus* at 80% RH at 30 ± 0.5°C for 30 days (expressed as change in O.D./min. due to oxidation of NADPH).

	Time in min									
	1	2	3	4	5	6	7	8	9	10
C	0.410	0.414	0.416	0.419	0.421	0.423	0.426	0.427	0.429	0.432
I	0.314	0.314	0.315	0.317	0.318	0.319	0.320	0.320	0.322	0.323

C = Control; I = Infested

and the root of the seedlings were found to be gradually deficient in dry weight. So was the condition of total chlorophylls, pentose, hexose and total free amino acid in the sedlings (Table 1). The rate of oxidation of NADPH was found sluggish in the seedlings raised from *A.flavus* infested seed stored at 80% RH (Table 2).

Low ambient RH of storage of seeds proved innocuous even on infestation with *A.flavus* while high RH alone proved injurious probably due to denaturation of proteins and plasmamembrane. This condition worsens due to involvement of *A.flavus*[2] and other seedborne fungi due to secretion of toxins[8]. The deterioration of seeds not only reduced their germinability but also produces physiologically crippled and morphologically weak seedlings. These have earlier been reported in jowar[9] and recently in radish[10].

The reduction in dry weight of the shoot and root of the seedlings appears due to degradation of chlorophylls or their restrained synthesis[11] resulting in availability of scanty amount of hexose and pentose. Meagre amount of free amino acid might result due to slow activity of urease.

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