

## NITRATE REDUCTASE AND UREASE ACTIVITY IN CUCUMBER SEEDLINGS DUE TO STORAGE OF SEEDS WITH FUNGUS

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Nitrate reductase and urease activity in the seedlings of cucumber were found to be moderate due to storage of seeds with *Aspergillus flavus* and *A. niger* and prolongation of the storage period. Total free amino acids also decreased in the seedlings in similar condition. *A. flavus* proved to be more deleterious than *A. niger* with respect to above determinations.

**Keywords :** *Aspergillus flavus*; *A. niger*; Cucumber seeds; Nitrate reductase; Seedling; Urease.

### Introduction

Physiology and biochemistry of the seedlings raised from fungus stored seeds of some crop plants have been worked out. Resultantly, slow growth of seedlings was observed besides scanty amount of chlorophylls, sugar, total free amino acids therein and stimulated activity of respiratory enzymes and those related with amino acid degradation<sup>1</sup>. The present paper deals with the activity of nitrate reductase and urease and magnitude of total free amino acid in the seedlings of cucumber after storage of seeds with fungi.

### Materials and Methods

Fifty g of cucumber (*Cucumis sativus* L.) var Pusa Sanyog seed was surface sterilized with 0.1% HgCl<sub>2</sub>, washed four times with sterilized distilled water and infested with the spore suspension<sup>1</sup> of *Aspergillus flavus* Link : Fr. and *A. niger* van Tieghum after growing them for 7 days on Czapevis Dox Agar medium at 25 ± 20°C. The number of spores per 50 g seed was adjusted to 5 × 10<sup>3</sup> with the help of dilution and counting it with haemocytometer. The seedlots so infested and uninfested control were stored in triplicate for each determination at 70, 80, and 90% RH maintained with glycerin<sup>2</sup> at 30 ± 10°C for 10, 20, and 30 days.

After expiry of the storage periods, 50 seeds were randomly taken from each lot, surface sterilized as described earlier and sown 1 cm deep at equidistance in garden soil sterilized at 20 psi for two consecutive days taking in earthen pot of the size 20 cm top diameter, 20 cm depth and 15 cm base diameter. Ten seedlings were raised in each pot for 21 days. Seedlings were watered lightly on 20th day with 1.0% aqueous KNO<sub>3</sub> and Urea for estimation of nitrate reductase and Urease respectively.

Nitrate reductase (NR) was estimated by conversion of KNO<sub>3</sub> to KNO<sub>2</sub>. Urease (UR) was estimated by oxidation of NADPH<sub>2</sub> to NADP<sup>+</sup> in the third leaf on 21st day randomly taking one seedling from each pot. Total free amino acids (TFAA) were estimated adopting Umbreit *et al.*<sup>5</sup>

### Results and Discussion

There seems (Table 1) highly significant -ve correlation between RH level, fungus storage and prolongation of storage period and NR activity ( $r = -0.827$ , HS,  $P < 0.01$ ). The rate of oxidation of NADPH<sub>2</sub> to NADP (Table 2) which is the measure of UR activity, was slower in the seedlings due to storage with fungi, prolongation of storage period and

**Table 1.** Nitrate reductase activity in the leaf of cucumber seedlings raised from the seeds stored with storage fungi at varying RH level for varying periods (Expressed as unit of enzyme/g fresh leaf).

Period of storage of seeds (in days)	Storage fungi vs. control	RH (%)			Correlation coefficient
		70	80	90	
10	<i>A. flavus</i>	0.098	0.087	0.075	-1.000, VHS
	<i>A. niger</i>	0.110	0.100	0.091	-0.999, VHS
	Control	0.129	0.125	0.120	-0.998, HS
20	<i>A. flavus</i>	0.082	0.065	0.048	-1.000, VHS
	<i>A. niger</i>	0.093	0.077	0.062	-0.999, VHS
	Control	0.125	0.116	0.105	-0.998, HS
30	<i>A. flavus</i>	0.071	0.040	0.011	-1.000, VHS
	<i>A. niger</i>	0.08	0.050	0.025	-0.999, VHS
	Control	0.120	0.107	0.096	-0.998, HS
'F' value for fungi vs. control		37.7 HS	28.4 HS	22.9 HS	-
'F' value for duration of storage		10.6S	14.0S	13.6S	-

**Table 2.** Urease activity\* in the leaf of cucumber seedlings raised from the seeds stored with storage fungi at varying RH for varying periods (expressed as O.D.).

RH (%)	Storage fungi vs. control	PERIOD OF STORAGE (IN DAYS)											
		10				20				30			
		Time of reading (in min.)				Time of reading (in min.)				Time of reading (in min.)			
		1st	4th	7th	10th	1st	4th	7th	10th	1st	4th	7th	10
70	<i>A. flavus</i>	0.217	0.224	0.232	0.239	0.132	0.140	0.147	0.154	0.100	0.107	0.115	0.121
	<i>A. niger</i>	0.260	0.267	0.274	0.281	0.165	0.172	0.180	0.127	0.127	0.134	0.142	0.148
	Control	0.315	0.322	0.330	0.337	0.217	0.224	0.232	0.167	0.167	0.175	0.182	0.189
80	<i>A. flavus</i>	0.150	0.157	0.164	0.171	0.102	0.110	0.117	0.124	0.085	0.092	0.100	0.106
	<i>A. niger</i>	0.175	0.182	0.190	0.196	0.125	0.132	0.140	0.146	0.105	0.112	0.120	0.126
	Control	0.247	0.254	0.262	0.269	0.194	0.202	0.210	0.216	0.155	0.162	0.170	0.177
90	<i>A. flavus</i>	0.107	0.114	0.122	0.129	0.082	0.090	0.097	0.104	0.055	0.062	0.070	0.076
	<i>A. niger</i>	0.132	0.140	0.147	0.154	0.097	0.104	0.112	0.118	0.067	0.075	0.082	0.088
	Control	0.210	0.217	0.225	0.232	0.175	0.182	0.190	0.197	0.147	0.155	0.162	0.169

\*CD. was observed per minute but it was noted on the 1st, 4th, 7th and 10th minutes.

**Table 3.** Total free amino acid content in the leaf of cucumber seedlings raised from the seeds stored with storage fungi at varying RH level for varying periods. (Expressed as mg of amino acid/g fresh weight of leaf).

Period of storage of seeds (in days)	Storage fungi vs. control	RH (%)			Correlation coefficient
		70	80	90	
10	<i>A. flavus</i>	0.197	0.174	0.147	-0.998, VHS
	<i>A. niger</i>	0.254	0.241	0.217	-0.985, S
	Control	0.322	0.315	0.303	-0.988, S
20	<i>A. flavus</i>	0.150	0.125	0.093	-0.997, VHS
	<i>A. niger</i>	0.185	0.165	0.136	-0.994, HS
	Control	0.284	0.272	0.255	-0.995, VHS
30	<i>A. flavus</i>	0.094	0.064	0.025	-0.997, VHS
	<i>A. niger</i>	0.125	0.100	0.070	-0.998, VHS
	Control	0.232	0.212	0.187	-0.997, VHS
'F' value for fungi vs. control		124.3 VHS	144.7 VHS	149.6 VHS	-
'F' value for duration of storage		78.1 VHS	146.7 VHS	161.2 VHS	-

increase in the RH level of storage. The amount of TFAA in the seedlings responded in the same way (Table 3).

Slow activity of NR and UR resulting in scanty amount of TFAA accordingly suggests the deleterious effect of high RH and the activity of *A. flavus* and *A. niger* and reflecting more with prolongation of storage period. Similar effect was observed in radish<sup>1</sup>. Sao *et al.*<sup>1</sup> have interpreted this sort of change due to toxic effect of storage fungus *Memnoniella echinata*. Harman and Nash<sup>6</sup> proved the involvement of toxic principle in pea seed due to *Aspergillus ruber*. The reduction of TFAA in the seedlings might result due to accelerated deaminase, deaminase, decarboxylase and oxidase of particular amino acid<sup>1</sup>, exudation of cations and amino acids from the root of myco deteriorated mustard seedlings<sup>7</sup> besides sluggish rate of synthesis of amino nitrogen due to less availability of ammonia caused by

deleterious effect of storage of seeds on NR and UR.

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