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EFFECT OF CULTURE FILTRATE OF *ASPERGILLUS FLAVUS* ON THE VIGOUR AND VIABILITY OF STORED WHEAT SEED

BY

RENUKA SAHA AND R. N. BASU

University College of Agriculture, Calcutta University,
35, Ballygunge Circular Road,
Calcutta-700 019.

Wheat seeds placed for germination in contact with high concentrations of crude culture filtrate of the storage fungus, *Aspergillus flavus*, showed reduction in germinability but lower concentrations of culture filtrate did not affect germination. The undiluted culture filtrate when used in soaking-drying treatment for viability maintenance, adversely affected germinability of wheat seed stored under high humidity and high temperature conditions. The adverse effect of the soaking-drying treatment with culture filtrate on the storability of seeds was associated with significant increase in membrane permeability of the treated seeds and substantial reduction in dehydrogenase activity.

INTRODUCTION

In eastern parts of India, the loss of vigour and viability of stored wheat seed may be attributed to physiological deterioration coupled with pathological damage mainly due to invasion by storage fungi under the ambient warm-humid storage conditions. The mechanisms of physiological and pathological seed deterioration by which seeds lose vigour and viability are yet to be fully elucidated. An important consideration in this context is whether fungal invasion, through production of mycotoxins, would accelerate the pace of physiological deterioration.

The effect of fungal culture filtrate on seed germinability has been studied by several investigators. Gupta and Rana (1981) noted reduced germination of tomato, brinzal and chilli by culture filtrates of all fungi tested. Mathur and Sinha (1978) recorded reduction in germination and vigour of bajra seeds by using culture filtrates of fungi. However, in most studies with fungal culture filtrates, the immediate germinability of seeds rather than storability has been considered. The question of deterioration of seeds in storage, following treatment with fungal toxins, remains to be studied. In the present investigation, an attempt was therefore made to determine the role of fungal culture filtrate on immediate and post—storage germinability (storability) of wheat seeds.

MATERIALS AND METHODS

Seeds of 'Sonalika' wheat, collected immediately after harvest, were dried in the sun to 10% moisture content and stored in rubber-stoppered glass bottles until treatment. The storage fungus, *Aspergillus flavus*, was isolated from the stored wheat seeds. The fungus was purified by dilution plate method and then grown in potato-dextrose agar medium. This isolated fungus was finally grown in 50 ml of sterilized liquid medium (glucose casein hydrolysate basal medium) in 250 ml flasks. Ten such inoculated flasks were incubated at 30±1°C for 7 days. The culture medium was filtered through Whatman No. 42 filter paper. The filtrate was then used for the treatment of seeds to study the effect of fungal culture filtrate on germination and storability of seeds.

(i) *Effect of fungal culture filtrate on seed germination*

Five-month-old seeds of wheat (cv. Sonalika) were soaked in the same amount of sterile water, uninoculated medium and 7-day-old culture filtrate of *Aspergillus flovus* and in its different dilutions for 2 hr. After treatment, seeds were immediately placed for germination in petri dishes. One half of seeds of each treatment was placed in continuous contact with the respective solutions and the other half of the treated seeds was placed on blotters moistened with distilled water.

(ii) *Effect of soaking-drying with fungal culture filtrate*

Stored medium-vigour seeds of wheat (cv. Sonalika) were soaked in the same amount of sterile water, uninoculated medium, 7-day-old culture filtrate of *Aspergillus flavus* for 2, 4, 6 and 8 hr, followed by drying back to the original weight at 35±1°C in a current of air in a drying cabinet. The control seeds were not soaked but dried along with the other treatments. The seeds of all treatments were kept in a desiccator over fused calcium chloride for one week to stabilize the moisture content to a uniform level of 8.7%. Thereafter the seeds were subjected to accelerated ageing at 100% RH and 40±1°C for 10 days.

To study the effect of culture filtrate on seed vigour and viability, germination tests were carried out immediately after soaking (before drying), after drying and after accelerated ageing following the inclined glass plate technique of Dasgupta *et al.* (1976) as modified by Punjabi and Basu (1982). Before placing for germination, seeds were sterilized by soaking for 30 minutes in 0.05% mercuric chloride solution and thoroughly washed.

For studying membrane permeability, electrical conductivity of seed-steep water was recorded on a Toshniwal conductivity bridge (cell constant 0.656) at 25°C. The seeds were first sterilized with mercuric chloride (0.05%) then thoroughly

washed and soaked for 2 hr. The leachate was discarded and the seeds were resoaked in distilled water for 1 hr for studying the electrical conductivity of the leachate and 30 min and 7 hr for sugar and amino acid study respectively. The amount of sugar leached out of the seed was determined colorimetrically following in principle the method of McCready *et al.* (1950). Estimation of amino acid in the seed leachate was done following the method of Moore and Stein (1948). Dehydrogenase activity was studied employing the method of Kittock and Law (1968).

RESULTS

(i) *Effect of culture filtrate on immediate germinability*

Effect of culture filtrate of the storage fungus *Aspergillus flavus* on the germinability of wheat seed was studied by placing them in contact with the respective solutions and in distilled water (after presoaking for 2 hr in respective culture filtrate solutions or water). When the seeds were kept in continuous contact

TABLE 1. *Effect of culture filtrate of storage fungus,* Aspergillus flavus, *on germinability of wheat seed placed in contact with the solutions (after presoaking for 2 hr in respective culture filtrate solution)*

Treatments	Placed in continuous contact with culture filtrate				Placed in distilled water			
	Germination (%)	Germination Angle	Mean root length (mm)	Mean shoot length (mm)	Germination (%)	Germination Angle	Mean root length (mm)	Mean shoot length (mm)
Control	92	73.6	261	33	93	74.7	251	33
Sterile water	97	80.0	315	37	93	74.7	291	38
Culture Filtrate (% V/V)								
1	99	84.3	295	35	93	74.7	292	38
2	98	81.9	289	36	93	74.7	289	39
5	99	84.3	281	35	90	71.6	280	39
10	98	81.9	279	34	90	71.6	282	37
20	97	80.0	268	35	89	70.6	248	38
30	97	80.0	221	34	87	68.9	223	35
50	57	49.0	53	34	73	58.7	168	37
100	30	33.2	42	32	50	45.0	49	36
Uninoculated Medium	97	80.0	182	37	90	71.6	192	34
L.S.D. at 0.05 P	—	7.4	7.2	NS	—	2.6	6.7	NS
L.S.D. at 0.01 P	—	10.1	10.9	NS	—	3.6	9.2	NS

Germination tests were done with 5-month-old seed.
Seeds were soaked for 2 hr in sterile water and different concentrations of 7-day-old culture filtrate of *Aspergillus flavus*. Seeds were also soaked in uninoculated medium for 2 hr.
Germination percentage was recorded after 5 days of germination at 22±1°C.

with crude culture filtrate, inhibition was found both in germination percentage and seedling growth. But the inhibition was much less when the seeds were placed in distilled water following soaking in the solutions of culture filtrate. Undiluted culture filtrate showed significant inhibitory effect but with decrease in concentration of culture filtrate, a gradual increase in germination percentage and seedling vigour was observed (Table 1).

(ii) *Effect of soaking-drying treatment employing culture filtrate on vigour and viability :*

Effect of soaking drying with culture filtrate of storage fungus, *Aspergillus flavus*, on germinability of wheat (cv. Sonalika) was studied by soaking the seeds in the same amount of water, uninoculated medium and 7-day-old culture filtrate for 2, 4, 6 and 8 hr respectively followed in each case by drying back.

TABLE 2. *Effect of hydration-dehydration treatments with culture filtrate of storage fungus,* Aspergillus flavus, *on germinability of wheat (cv. Sonalika) immediately after drying (before ageing)*

Treatments	Germination		Mean root length (mm)	Mean shoot length (mm)
	(%)	Angle (arc-sin)		
Control	98	81.9	358	34
2 hr Sterile Water	100	90.0	420	38
2 hr Medium	96	78.5	416	33
2 hr Culture Filtrate	100	90.0	406	32
4 hr Sterile Water	98	81.9	392	33
4 hr Medium	100	90.0	382	30
4 hr Culture Filtrate	91	72.5	373	31
6 hr Sterile Water	96	78.5	381	34
6 hr Medium	100	90.0	374	31
6 hr Culture Filtrate	88	69.7	369	30
8 hr Sterile Water	95	77.1	376	34
8 hr Medium	100	90.0	368	31
8 hr Culture Filtrate	85	67.2	357	26
L.S.D. at 0.05 P.	—	5.8	8.2	5.2
L.S.D. at 0.01 P.	—	7.8	10.9	6.1

Medium = Uninoculated basal medium.
Other details same as in Table 1.

When the seeds were kept in continuous contact with the crude culture filtrate, inhibition was found in respect of germination percentage and seedling growth. There was a reduction of root length of seedlings in the uninoculated medium itself in comparison to sterile water, attributable possibly to the osmotic effect of

TABLE 3. *Effect of hydration-dehydration treatments with culture filtrate of storage fungus* Aspergillus flavus, *on vigour and viability of seeds after accelerated ageing at 100% RH and 40°C for 8 days*

Treatments	Germination		Mean root length (mm)	Mean shoot length (mm)
	(%)	Angle (arc-sin)		
Control	51	45.6	206	25
2 hr Sterile Water	92	73.6	299	31
2 hr Medium	92	73.6	305	32
2 hr Culture Filtrate	69	56.2	246	30
4 hr Sterile Water	90	71.6	290	31
4 hr Medium	89	70.6	291	30
4 hr Culture Filtrate	45	42.1	173	26
6 hr Sterile Water	90	71.6	288	30
6 hr Medium	86	68.0	289	31
6 hr Culture Filtrate	39	38.6	162	19
8 hr Sterile Water	84	66.4	289	32
8 hr Medium	86	68.0	292	30
8 hr Culture Filtrate	35	36.3	160	19
L.S.D. at 0.05 P	—	3.2	8.4	5.1
L.S.D. at 0.01 P	—	4.2	11.2	6.8

Medium = Uninoculated basal medium.
Other details same as in Table 1.

the medium. Immediately after drying (before ageing), crude culture filtrate treatment showed no noticeable inhibition of germination percentage and vigour (Table 2). But after accelerated ageing for 8 days, the crude culture filtrate treatment showed significantly greater inhibition than water and uninoculated medium. The inhibitory effect was more when the duration of treatment with crude culture filtrate was longer. But shorter durations (2 hr) did not show any inhibitory effect (Table 3). Longer durations of soaking (followed by drying) with water, uninoculated medium or culture filtrate showed increased conductivity, leakage of sugars and amino acids than the shorter durations, giving maximum values in crude culture filtrate (Table 4). Dehydrogenase activity has been found to be correlated with germinability of seeds (Table 4). Lowest activity of the enzyme was observed in crude culture filtrate treated seeds.

TABLE 4. *Effect of hydration-dehydration treatments with culture filtrate of storage fungus* Aspergillus flavus, *on membrane permeability and dehydrogenase activity of wheat seeds after accelerated ageing at 100% RH* and *40°C for 5 days*

Treatments	Germination (%)	Germination Angle (arc-sin)	Electrical conductance (μmho)	Leakage of sugar (μg glucose equiv./ml)	Leakage of amino acid (μg glycine equiv /ml)	Dehydrogenase activity (OD 470)
Control	50	45 0±2.5	134.5±3.1	43.0±2.3	31.2±2.0	0.108±0.01
2 hr Sterile Water	68	55.5±2.2	75.4±4.6	28.2±1.8	10.2±1.7	0.208±0 01
2 hr Medium	70	56.8±3.3	75.4±3.6	24.2±1.9	18.0±2.2	0.180±0.02
2 hr Culture Filtrate	45	42.1±1.5	118.1±2 7	44.7±2.1	57.5±1.5	0.071±0.05
8 hr Sterile Water	42	40.4±2.0	137.8±4.5	54.0±1.6	35.5±1.2	0.108±0.02
8 hr Medium	48	43.8±2.1	127.9±3.3	49.7±1.4	22.5±1.4	0.119±0.01
8 hr Culture Filtrate	28	31.9±1.9	164.0±3.9	69.0±1.9	70.4±2.0	0.066±0.01

Medium = Uninoculated basal medium.
Twenty five seeds of wheat were soaked for 2 hr in 15 ml of water and then washed out, 15 ml of fresh water was again added to the seed and soaked for 30 min, 1 hr and 7 hr for studying the leakage of sugar, electrolytes and amino acid respectively.
To study the dehydrogenase activity, 8 uniformly sprouted embryos were taken.
Other details same as in Table 1.

DISCUSSION

Germination of wheat seeds placed in direct contact with the undiluted culture filtrate of *A. flavus* was strongly inhibited. This confirms the presence of germination inhibiting chemicals in the culture filtrate of storage fungi and lends support to the earlier reports on the adverse effects of mycotoxins on seed germination (Mathur and Sinha, 1978 ; Mishra and Tripathi, 1980).

A very important observation of the present study, which to our knowledge has not been reported so far is the influence of the mycotoxins in the culture filtrate on the storability of the dry-stored seed. Earlier researchers confined themselves to studying the effect of the toxins on immediate germination because the technique of soaking-drying for storability study, as done in the present laboratory, was not practised earlier. Soaking-drying of medium-vigour seed, empyloing water or dilute solutions of culture filtrate, showed a counteraction of ageing damage. The beneficial effects of soaking-drying on viability maintenance have been discussed earlier (Basu, 1976 ; Basu and Rudrapal, 1982). That the diluted culture filtrate showed similar effects as water indicates that to reverse the beneficial effect of water the concentration of mycotoxin within the seed should be sufficiently high.

The adverse effect of mycotoxins was less conspicuous immediately after treatment but showed up very convincingly after storage. This would mean that besides the direct effect of invasion by the storage fungi, the mycotoxins produced by the fungi would give latent harmful effects even after the fungus is killed by surface sterilization with fungicides (especially dry-dressing with fungicidal formulations). Perhaps, in such a situations, wet soak treatment for controlling storage fungi would be better as that would kill the fungi and may leach out, at least in part, the accumulated mycotoxins.

The exact mechanism of action of the mycotoxins on germinability is still unclear. Perhaps, they acted as metabolic inhibitors and the biochemical reactions leading to germination and growth were inhibited. Regarding the reduction in the storability of dry-stored seeds by mycotoxins, it was noted that thorough leaching of the inhibitors from the treated seed by placing under running tap water would increase post-aging germinability to a certain extent only. But considerable inhibition of germination would still be noticed implying firm binding of the toxins with biomolecules and organelles. As such, subsequent washing of the toxins did not enable the seed to recover fully. During dry storage, the mycotoxins caused damage to the cell organelles and reduced their functional activities.

There was significant loss of membrane permeability as shown by greater leaching of electrolytes, sugars and amino acids from the seeds treated with culture filtrate and then stored. That would show that the mycotoxins damaged the cell membranes and impaired membrane functions. The activity of enzyme was also lower in those seeds indicating derangement of metabolic functions essential for germination.

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REFERENCES

Basu, R. N. (1976). Physico-chemical control of seed deterioration. *Seed Res.*, **4**, 15-23.

Basu, R. N. and Rudrapal, A. B. (1982). Post-harvest seed physiology and seed invigoration treatments. *Proceeding of the Indian Statistical Institute Golden Jubilee International Conference on Frontiers of Research in Agriculture*, (ed. S. K. Roy), pp. 374-397.

Dasgupta, M., Basu, P. and Basu, R. N. (1976). Seed treatment for vigour, viability and productivity of wheat (*Triticum aestivum* L.). *Indian Agric.*, **20**., 265-273.

Gupta, P. C. and Rana, V. P. (1981). Effect of culture filtrate of some seed mycoflora on seed germination of solanaceous crops. *Seed Res.*, **9**, 192-193.

Kittock. D. L. and Law, A. G. (1968). Relationship of seedling vigour, respiration and tetrazolium chloride reduction by germinating wheat seeds. *Agron. J.*, **60**, 286-288.

Mathur, S. K. and Sinha, S. (1978). Effect of fungal metabolites on seed viability and seedling vigour of bajra *(Pennisetum typhoides)*. *Seed Res.*, **6**, 181-187.

McCready, R. M., Guggals, J., Silviera, V. and Owens, H. S. (1950). Determination of starch and amylose in vegetables. *Anal. Chem.*, **22**. 1156-1158.

Misra, R. S., and Tripathi, R. K. (1980). Effect of aflatoxin B_1 on germination, respiration and α-amylase in maize. *Zeitschrift fur pflanzen kran kheiten und pflanzen schu'z.* **87**, 155-160.

Moore, S. and Stein, W. H. (1948). Photometric ninhydrin method for use in the chromatography of amino acids. *J. Biol. Chem.*, **176**, 367-388.

Punjabi, B. and Basu, R. N. (1982). Testing germination and seedling growth by an inclined glass plate blotter method. *Indian J. Pl. Physiol.*, **25**, 289-295.