Preservation of seeds by antifungal volatile compounds

Balen Nandi

Professor of Botany
Burdwan University
Burdwan - 713104
INDIA

INTRODUCTION

Fairly successful preservation of feed grains with volatile fatty acids was reported earlier (Jones, 1970; Larsen et al., 1972; Christensen, 1973; Herting and Drury, 1974). Frankenfeld et al. (1975) reported some alcohol (1,3 diols) and their esters to be not only more efficient than acids as seed preservatives but also to increase nutritive value of the treated grains. Nandi and Fries (1976) tested a number of compounds (aldehydes, ketones, esters and terpenoids) of which some proved to be very effective preservative of seeds to be used as animal feed. Binary mixtures of some volatile compounds were reported to show increased preservative efficacy (Nandi and Fries, 1976; Nandi, 1977; Nandi, 1978). Unfortunately, in most cases of such treatments with volatile compounds, either singly or in combinations, the treatments showed increased phytotoxicity, particularly in combinations of compounds.

In the present study, fairly low level of treatments with propionic acid (Pa), Citral (Ci), Allyl isothiocyanate (Ac) and Furfural (Fu), which are known to possess fungicidal property, were used either singly or in binary combinations to evaluate their possible use as seed preservative against fungal deterioration in storage while retaining germinability intact.

MATERIALS AND METHODS

'Sonalika' cultivar of wheat grains were collected from local market immediately after harvest. Grain moisture was determined by oven drying at 130°C to constant weights and expressed on a wet weight basis following Roberts and Roberts (1972). The grains were reconstituted to 15% moisture following Lutey and Christensen (1963) by adding requisite quantity of sterile distilled water to the grains in tightly stoppered bottles and storing them for 3 days at 4°C with occasional shaking to facilitate uniform distribution of moisture.

Grains (200 gms) were treated separately in closed containers with the different volatile antifungal compounds (0.05 µl/g grain). Binary mixture of the compounds (0.05 + 0.05 µl/g grain) were also tested. Replicate sets together with untreated control were kept for 3 days with periodic shaking. The containers were kept open for 24 hours to allow excess of volatile compounds to escape and then the grains

were transferred to perforated polythene bags and stored separately for 180 days at room temperature exposed to natural fluctuations of atmospheric conditions. Synergy factor (Sf) of the binary mixtures of the compounds was calculated following Samoucha and Cohen (1984) from the formula --

$$Sf = \frac{C_M}{(C_a + C_b) - 0.01\ C_a C_b}$$

where C_a, C_b and C_M are the percentage of 'infection control' by the compounds a, b and their mixture respectively.

Preservative efficacy of the treatments were determined by taking out grains at intervals of 30 days, by surface sterilizing (using 2% sodium hypochlorite), placing them on sterilized salt malt agar (10% NaCl in 2% malt agar) at pH 6.5 in Petridishes and incubating at 30 ± 1°C for 10 days. Fungi that grew out of the grains were isolated, identified and the extent of grain infection was recorded(**Table 1**).

Germinability of the grains was determined by blotter method using randomly selected grains (100) and incubating them at 28 ± 1°C. Germination was counted from third day onwards until no further germination occured(**Table 2**).

RESULTS

Germinability :

Control grains which exhibited 82% germinability at harvest, showed an abrupt decrease to 20% after 60 days and complete loss

Table 1. Wheat grain infection by storage fungi after treatment with different volatile compounds and their binary combinations and stored for different periods.

Treatment (µl/g grain)	Storage (Days)	Grain infection* (%)						
		0	30	60	90	120	150	180
Control		67	100	100	100	100	100	100
Pa (0.05)		61	68	70	72	72	71	71
Ac (0.05)		47	45	45	45	46	46	45
Ci (0.05)		64	70	68	72	72	73	72
Fu (0.05)		68	78	80	95	100	100	100
Pa (0.05) + Ci (0.05)		0	0	0	0	0	0	0
Pa (0.05) + Ac (0.05)		0	0	0	0	0	0	0
Pa (0.05) + Fu (0.05)		40	42	36	37	36	36	36
Ci (0.05) + Ac (0.05)		21	17	15	15	17	17	16
Ci (0.05) + Fu (0.05)		50	52	42	42	42	43	42
Ac (0.05) + Fu (0.05)		34	33	33	34	34	35	34

* Mean of three replicates of 100 grains each.

Pa - Propionic acid
Ac - Allyl iso thiocyanate
Ci - Citral
Fu - Furfural

Table 2. Germinability (%) of treated wheat grains for different periods (Initial germinability 82%)

Treatment (µl/g grain)	Storage (Days)	Germinability (%)		
		60	120	180
Control		15	0	0
Pa (0.05)		84	80	80
Ac (0.05)		85	78	78
Ci (0.05)		85	79	78
Fu (0.05)		75	74	74
Pa (0.05) + Ci (0.05)		80	76	79
Pa (0.05) + Ac (0.05)		80	79	79
Pa (0.05) + Fu (0.05)		70	72	72
Ci (0.05) + Ac (0.05)		80	79	79
Ci (0.05) + Fu (0.05)		72	72	72
Ac (0.05) + Fu (0.05)		70	72	73

* Mean of three replicates.

Pa - Propionic acid Ci - Citral

Ac - Allyl iso thiocyanate Fu - Furfural

from 120 days onwards. Grains treated with all the test compounds not only retained full germinability after 60 days but also prevented any loss after longer storage. Some treatments even showed marginal improvement in germinability over the initial control. Although some treatments with binary combinations of the volatile compounds showed slight decrease in germinability other treatments retained 80% germinability. Although the seedling from treated grains showed no apparent abnormality in any case, root growth was found to be slightly slower than the shoot growth.

Grain Infection :

Control grains, 67% of which were initially found to be heavily infected with fungi, became all infected within 30 days. When treated individually with the four volatile compounds, infection was considerably reduced in case of Ac but not with the other three compounds even when tested immediately after treatment. Ac showed maximum control of grain infection while Fu showed minimum preservative effect. No improvement in grain preservation was recorded from residual effect of the treatment even after longer storage.

Treatments with binary combinations of the compounds, however, exhibited considerably increased preservative efficacy which was evident even immediately after the treatment. Moreover, the treatments retained their preservative effect even after 180 days as no increase in grain infection through reinfection

of grains were recorded in any of the treatments. The mixtures controlled grain infection more efficiently than the individual compounds evidently through synergistic action and exhibited synergy factors of 1.03 to 1.91. Most effective control was recorded in binary mixtures of Pa + Ac and Pa + Ci where 100% infection control were recorded. Since, Sf value greater than 1 indicates synergistic action between the two compounds in a mixture, while Sf value of 1 indicates additive action, mixtures of Pa + Ci and Pa + Ac, in the present study, exhibited fairly strong synergistic action while mixtures of Ci + Fu and Ac + Fu showed more or less additive action (**Table 3**).

DISCUSSIONS

Germinability of the control grains fell sharply from the initial value, ultimately to zero. This was evidently due to the reconstituted high grain moisture used in the study that proved to be favourable for fungal growth and grain deterioration. The selected volatile compounds are known to destroy germinability (Ghosh *et al.*, 1982; Ghosh and Nandi, 1985) and hence the lower level of treatments were suitably worked out which affected germinability only to a limited extent.

Treatments with the volatile compounds, however, inhibited fungal growth in grains in all cases. Among the compounds tested, Ac proved to be the most effective to decrease grain infection.

Table 3. Grain preservative efficacy of some volatile compounds and their binary mixtures after 30 days of storage.

Treatment (µl/g grain)				Infection control* (%)	Synergy factor
Pa	Ac	Ci	Fu		
0.05	0	0	0	32	
0	0.05	0	0	55	
0	0	0.05	0	30	
0	0	0	0.05	22	
0.05	0	0	0.05	58	1.23
0	0.05	0	0.05	67	1.03
0	0	0.05	0.05	48	1.05
0	0.05	0.05	0	83	1.21
0.05	0.05	0	0	100	1.44
0.05	0	0.05	0	100	1.91

* Mean of three replicates of 100 grains each.

Pa - Propionic acid
Ac - Allyl iso thiocyanate
Ci - Citral
Fu - Furfural

Moreover, residual effect of the compounds prevented any reinfection of the grains as there was evidently no increase in the percentage of grain infection in longer storage. Furfural proved to be not only the least effective of the compounds but also that its preservative efficacy decreased as reinfection of grains was recorded in longer storage.

Binary combinations of the compounds exhibited much higher fungitoxicity and preservative efficacy thus exhibiting considerable potentiality for practical seed protection work. In most cases, the increased fungicidal effect was coupled with increased phytotoxicity. Moreover, in some of the combinations synergism in the antifungal property, as reflected in decreased grain infection, was demonstrated. This was in accordance with earlier synergistic effects observed in some binary and ternary combinations of active compounds by Nandi and Fries (1976) and Nandi (1978).

The efficacy of a volatile compound is known to depend on the penetrability of the compound into the seed. The amount of the volatiles that penetrate the grains during treatment varied from compound to compound. Quantitative measurement of the compounds supposed to have penetrated into the grains suggested that penetration probably had some relation to the lipophilic nature of the compounds on one hand and to the size of the molecule on the other (Nandi, 1978).

In the present study the level of treatment of the preservatives deliberately used was much lower than the minimum inhibitory concentration (MIC) used in earlier studies in order to retain germinability as far as possible, which was otherwise lost at higher level of treatments. While results of treatments with individual compound as grain preservative were met with limited success particularly in respect of infection control, some of the binary combinations proved highly promising. Synergy factor of Pa + Ac and Pa + Li was found to be highest and this corresponded with 100% control of the infection. On the other-hand, combinations of Ac + Fu and Ci + Fu, which exhibited some additive effects, exhibited only marginal improvement in infection control. The results show great promise of future use of suitable volatile antifungal compounds and their mixtures as seed preservatives, which will reduce future infection to a great extent while keeping the germinability intact.

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SUMMARY

Preservation of seeds by effective antifungal volatile compounds to prevent fungal deterioration in storage has proved a cheap and advantageous alternative to conventional methods of seed drying, hermetic or low temperature storage which many poor countries can hardly afford. Several volatile fatty acids, aldehydes, esters, possessing fungitoxicity proved quite effective as seed preservatives. Most such treatments not only reduce seed infection considerably but also prevent them from being reinfected in storage for different length of time. Treated seeds also show much less fluctuation of seed moisture than untreated seeds even when stored under fluctuating temperature and RH in natural storage. However, germinability of the treated seeds is often hampered due to phytotoxicity of the compounds particularly at higher levels. Search for an ideal volatile preservative possessing strong fungitoxicity but very low or no phytotoxicity is still on.

Binary and ternary mixtures of some of the compounds show synergism in their fungicidal property, thus lowering the required effective dosage and sometimes decreasing the phytotoxicity. A number of essential oils of plant origin, in binary combinations with fatty acid or ester proved effective in controlling fungi in seeds while retaining germinability to a great extent.

Treated seeds show no appreciable loss in nutritive quality and often show increased palatability as feed to animals.

PRESERVATION DES GRAINS PAR LES SUBSTANCES VOLATILES ANTIFONGIQUES

Balen NANDI

Burdwan University
Botany Department, Golapbag, Burdwan
713 104 India

RESUME

La préservation des grains par des substances volatiles antifongiques efficaces afin d'éviter la détérioration fongique des stocks s'est avérée une alternative avantageuse et économique vis-à-vis des méthodes conventionnelles de séchage et de stockage hermétique à basses températures que peu de pays pauvres peuvent se permettre d'utiliser. Plusieurs acides gras volatils, aldéhydes et esters possédant une activité antifongique se sont avérés très efficaces pour préserver le grain. La plupart de ces traitements ne réduisent pas seulement considérablement l'infestation des grains mais les empêchent aussi d'être réinfestés lorsqu'ils sont stockés pendant des longues périodes. Les grains traités présentent également beaucoup moins de fluctuations de leurs taux d'humidité que ceux qui ne le sont pas, même si la température et l'HR varient dans les conditions naturelles de stockage. Cependant, la capacité germinative des grains traités est souvent réduite par la phytotoxicité des substances de protection utilisées, particulièrement au doses élevées. La recherche d'un conservateur volatil possédant une fongitoxicité forte mais à faible phytotoxicité, se poursuit.

Les mélanges binaires et ternaires de certaines substances montrent une synergie de leurs propriétés fongicides en réduisant, par conséquent, la dose efficace nécessaire et parfois la phytotoxicité. Un certain nombre d'huiles essentielles d'origine végétale, en combinaison binaire avec un acide gras ou un ester, se sont montrées efficaces pour éliminer les champignons dans les grains en conservant convenablement la germinabilité.

Les grains traités ne montraient pas de perte appréciable de leurs qualités nutritives et conduisaient souvent à une amélioration de la palatabilité des tourteaux pour les animaux.