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# CHEMICAL CONTROL OF *ASPERGILLUS FLAVUS* AND *PENICILLIUM CYCLOPIUM* IN STORAGE

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Effect of chemicals to inhibit storage fungi was investigaged. Grains inoculated separately with *Aspergillus flavus, Penicillium cyclopium* and a mixture of the two species were treated with 1% Luprosil (99.9% propionic acid). After three months, storage, gains yielded almost no inoculated fungi when surface disinfected and plated on media. Grains inoculated but not treated yielded the inoculated fungi in more than 90% of cases. One percent acetic acid when applied to the inoculated grains was relatively less effective than Luprosil in inhibiting the fungi.

## INTRODUCTION

The use of fungicides and chemicals to control microorganisms in stored grains and mixed feeds has met with limited success so far. Food grains cannot be treated with compounds leaving residue or for that matter any other kind of effect which may make the grain unfit for human or animal consumption. This, of course, eliminates many fungicides and chemical compounds which otherwise could have been extremely effective and useful in controlling microorganisms.

Simon [1] treated corn with different concentrations of propionic acid, acetic acid and sorbic acid. He found that corn treated with 0.8-1% of acetic acid and 0.4 – 1% propionic acid remained free of mold growth for a period of about 112 days. Sorbic acid at a concentration of 1% did not prevent mold growth for more than 22-24 days.

Vandegraft and Christensen [2] treated corn with either 2% ammonia or 1% propionic acid and found that both compounds significantly reduced mold growth and subsequent mycotoxin formation. Growth was inhibited more by propionic acid than by ammonia.

Al-Heti [3] reported that treatment of corn with Luprosil and Tecto [2-(4-Thiazylol)- benzamidozole] protected stored corn from fungi for five months.

The present investigation describes the results based on the treatment of different inoculated variety types of wheat, barley and rice with *A. flavus, P cyclopium* and a mixture of the two with and without subsequent treatment with Luprosil and acetic acid.

## MATERIALS AND METHODS

Experiments were designed to determine the effectiveness of Luprosil and acetic acid in inhibiting *Aspergillus flavus* and *Penicillium cyclopium* present in stored grains. These chemicals have no residual effect and also do not decrease the food value of the grains [4].

Mexipak and Saberbeg varieties of wheat, local white and local black barley, milled and rough amber rice were used as test grains. These grains were almost free of storage fungi. Luprosil, a product developed by BASF Co. containing 99.9% propionic acid and acetic acid (at a conc. of 1%), were the two chemicals used. The moisture content of the grain was adjusted to the desired level of 18.5%, according to the techniques outlined by Smith [5], which is in equilibrium with an RH of 85% in starchy cereal grains.

*A. flavus, P. cyclopium* and a mixture of the two were used for inoculation purposes. Sufficient amounts of grains of each variety/type were taken and moisture conditioned. Each sample of grain was divided into six lots, two each inoculated with *A. flavus, P. cyclopium* and their mixture separately. One out of the two (inoculated with test fungi) was sprayed by using an atomizer, with an even amount of 1% Luprosil enough to cover the seed surface. The remaining three lots were not treated with the chemical and left as controls. Each inoculation was replicated four times containing 20 g of grains per replicate. Similar procedures were adopted for acetic acid.

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Each replicate was placed in small plastic cups, covered with perforated polyethylene. All the cups were finally placed in a wooden cabinet covered with a double layer of polyethylene. Saturated potassium chloride solution was placed in the cabinet to maintain the required RH. The experiment was carried out at room temperature (25° – 28° approx.) and results recorded after 3 months. Percent prevalence of inoculated fungi appearing in treated and non-treated surface-disinfected seeds was used as criterion for determining the effectiveness of chemicals used.

## RESULTS AND DISCUSSION

Grains inoculated with *A. flavus, P. cyclopium* and their mixture and treated with 1% Luprosil, after three months of storage, show almost no organisms when the surface-disinfected seeds were plated on PDA. The grains inoculated but not treated invariably show a very high percentage of inoculated fungi on plating (Fig. 1).

Above 90% emergence of two fungi and their mixture was observed in controls from plated seeds of varieties types used in these studies. 100% inhibition of *A. flavus* was observed on wheat var. Saberbeg, local white and local

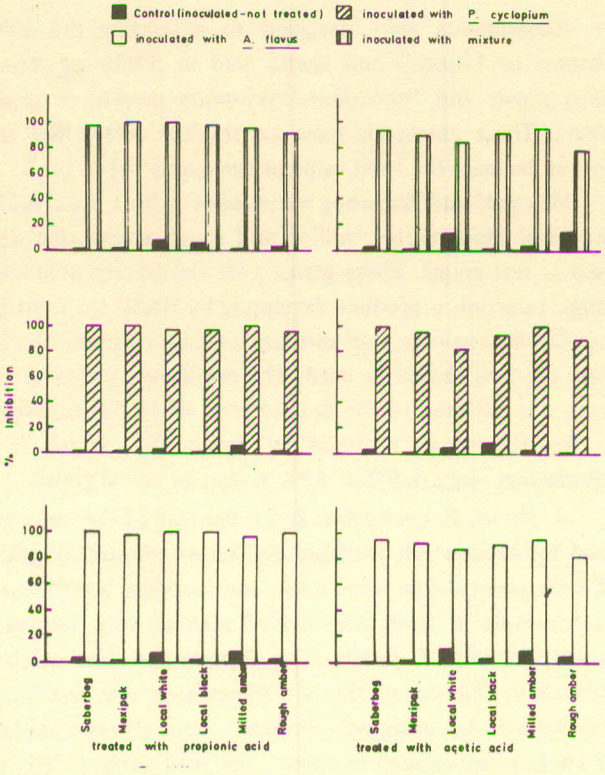


**Fig. 1. % Inhibition of fungi inoculated-not treated and inoculated-treated, on different grains not inoculated and inoculated with spores of individual species and mixture of species of *Aspergillus* and *P. cyclopium*.**

black barley and rough *amber* rice. *P. cyclopium* was completely inhibited by Luprosil as far as Saberbeg, Mexipak and milled amber rice were concerned. Neither *P. cyclopium* nor *A. flavus* when inoculated in the form of mixture could be isolated from Mexipak wheat and local white barley after treatment with the chemical. On the remaining treated seeds the presence of inoculated organisms was negligible. These results are in accordance with those of Simon [1] and Vandegraft and Christensen [2], who, working with corn mentioned that no mold growth was observed in 65 days storage on corn treated with 0.6% propionic acid, while Vandegraft *et al.* noted that treatment with 1% propionic acid significantly reduced the growth and subsequent aflatoxin and ochratoxin formation.

Acetic acid did not prove to be equally effective as compared to Luprosil in inhibiting the growth of fungi. Surface disinfected seeds of varieties/types employed in these studies demonstrate some emergence of inoculated fungi on plating. As much as 18% of *A. flavus* appeared on rough amber rice after treatment with the chemical when the results were taken at the end of the three months. Appearance of *A. flavus* on other varieties/types of grains ranged between 4 – 11%. *P. cyclopium* was isolated in 17% cases from local black barley, after the inoculated grains were treated with acetic acid and plated on PDA after 3 months storage although hundred percent inhibition was observed with Mexipak and milled amber rice. Mixture of the two fungi show a little more emergence than the individual organisms alone. 21% seeds of rough amber rice yielded the two fungi on plating while on local white barley, their appearance was in the order of 15%. On other varieties/types the prevalence was between 6 – 10%. Herting and Drury [6] reported on the antifungal activity of a number of volatile fatty acids on grains. They found that propionic, formic, acetic, butyric and isobutyric acid were effective against corn, sorghum, wheat, oats, barley and soybeans. These results are in line with the present investigation. Buchanan and Ayres [7] studied the comparative effect of propionic acid, sodium acetate and sodium propionate and noticed that propionic acid was more effective than the other two, which again supports the results reported in the present study regarding the effectiveness of Luprosil. An indirect support as to the efficacy of Luprosil obtained in the work reported here is provided by Richardson and Hallick [8]. They observed that low concentrations of propionic acid prevented heating in corn meals containing 15.2% m.c.

As to the effectiveness of acetic acid, Simon [1] and several others found it effective in inhibiting mold growth

equally well but for shorter periods. In the present studies, acetic acid was less effective than Luprosil. Propionic acid as a fungicide on cereal grains can be safely employed for the effective control of molds.

## REFERENCES

1. J. A. Simon, Ph.D. thesis, Kanasas State Univ. (1970).
2. E. E. Vandegraft and C.M. Christensen, Cer. Chem., **52**, 79 (1975).
3. A.A. Al-Heti, M.Sc. thesis, Univ. of Baghdad -59 (1977)
4. Bulletin of BASF on Luprosil, BASF Aktiengesellscchaft, 6700 Ludvigshafen, Federal Republic of Germany, 46 pp. (1974)
5. L. Smith, Flour milling technology, Northern Publishing Co. Ltd., Liverpool, England (1958).
6. D.C. Herting, and Drury, Cer. Chem. **51**, 74 (1974).
7. R.L. Buchanan and J.C. Ayres, J. Food Sc., **41**, 128 (1976).
8. L.R. Richardson, and J.V. Hallic, Texas Agr. Exp. Sta. Bull. 6 pp. (1975).