Pakistan J. Sci. Ind. Res. Vol. 27, No. 6, December 1984.

PATHOGENICITY AND EFFECT ON GERMINATION CAUSED BY *ASPERGILLUS* AND *PENICILLIUM* SPECIES ON WHEAT RICE, BARLEY AND CORN

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(Received July 29, 1984)

Pathogenicity of several molds known to cause damage in storage was determined on selected varieties/types of stored food grains. Seeds almost free from fungi were inoculated separately with *Aspergillus flavus, A. amstelodami* and *Penicillium cyclopium* and a mixture of two *Aspergillus* species and then stored for three months. Seeds yielded the inoculated organisms in 90% of cases when surface disinfected and plated on PDA. Germinability of the grains was also reduced considerably. Control seeds remained free of fungi and had a high germination percentage.

INTRODUCTION

Studies on the invasion of stored grains by various microorganisms under different sets of conditions has been a favourite subject for investigation by storage pathologists. Papavizas and Christensen [1] surface disinfected wheat samples then inoculated with *Aspergillus amstelodami, A. ruber, A. restrictus* and *A. candidus* alone and in combinations and stored at 25° and at relative humidity (RH) 75, 80 and 85% for 1-5 months. It was observed that inoculations with mixtures of species did not result in greater loss of germination or development of more germ damage than did inoculation with the most pathogenic species alone. The non-inoculated controls retained a high germination percentage and developed little germ damage. Harman and Pfleger [2] inoculated wheat and some other seed with seven different *Aspergillus* isolates. They found that wheat seeds were infected by all isolates and the percentage of germination was also reduced by all isolates. The embryos of seeds were readily invaded and the infected embryos were dark.

Results reported here are a continuation of the work done by the authors (under publication) on a comprehensive survey carried out to determine fungi associated with stored food grains in silos in Iraq. An effort has been made to establish invasion of selected grains by the most important molds isolated during the survey and are known to cause deterioration in storage under favourable conditions.

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MATERIALS AND METHODS

Saberbeg wheat, local white barley, local black barley, milled amber rice, rough amber rice and yellow corn were selected for this investigation. The grains were so selected that either they carried no storage molds or very little amount was present after surface disinfection as has been shown from the results obtained in the survey. However, extra care was taken to select those grains which on visual examination appeared to be healthy and sound.

Quantities of grains sufficient for each test were immersed and shaken for 3 min. in 1% sodium hypochlorite solution. The seeds were then rinsed in sterile water, put in sterile paper bags, and dried to approximately 12% m.c. in an oven at 32° . Tuite and Christensen, [3] indicated that this treatment did not measurably affect germinability or susceptibility of the grain to subsequent invasion by storage fungi. The m.c. of the grain was adjusted to the desired level of 18.5% by adding sterile water to the controls and a suspension of spores in water to those inoculated. The spore suspension was so adjusted that the amount added to each gram of grain contained approximately 100 spores.

The grains were conditioned according to the techniques outlined by Smith [4]. Quantities of grains to be conditioned were placed separately in glass stoppered bottles, and sterile water was added in small portions from a burette. The quantity of sterile water added to each 100 g of grains was calculated according to the following formula:

$$\text{Quantity of sterile water, ml/100 g of grains} = \frac{100\,(M_2 - M_1)}{100 - M_2}$$

where M_1 = Initial grain moisture content (%)

M_2 = required grain moisture content (%)

The original moisture content of the grain was determined by the air draft oven method specified in the AOAC [5] using a Brabender semi-automatic moisture tester. The bottles containing the grains were shaken thoroughly after each addition of sterile water to mix the amount of grain until there were no wet grains left sticking to the inner surface of the bottles. The grains were kept in bottles for 5 days at 4° and shaken periodically to permit even distribution of water.

The inoculum consisted of spores of two species of *Aspergillus* separately and in mixture and a single species of *Penicillium*. The mixture contained the same total number of spores, evenly divided between the species making up the mixture. Grains inoculated with *A. flavus* Link ex Fr., *A. amstelodami* Thom & Church, *Penicillium cyclopium* Westling and *A. flavus* + *A. amstelodami* in mixture along with grains not inoculated with any organism (control) were used in pathogenicity studies. Four replicates were used for each inoculation as well as for controls. For each replicate, 20 g of the above treated seeds were placed in sterile plastic cups; the open ends of the cups were covered with perforated polyethylene, and the cups were placed in a cabinet covered with airtight polyethylene. Saturated potassium chloride solution, which maintained an RH of 85% in equilibrium with the moisture content, was placed in the cabinet [6]. The samples were stored for 3 months at room temperature. Percent germinability (based on 100 seeds) and percent surface disinfected seeds yielding inoculated fungi were the two criteria used for evaluating the results.

RESULTS AND DISCUSSION

Test fungi rapidly invaded the seeds of all varieties/types of the grains inoculated-kept at moisture content in equilibrium with a RH of 85%. Seeds yielded between 80-100% inoculated organisms in almost all cases. This invasion was accompanied by moderate to drastic reduction in germination percentage of seeds as compared with non-inoculated controls. Detailed results are given in Fig. 1.

In the case of wheat variety Saberbeg the germination in the control was 82%. In inoculated grains it ranged between 32-45%. Seeds inoculated separately with *A. flavus*, *A. amstelodami* and *P. cyclopium*, and with the mixture of two *Aspergillus* species, when plated on PDA, produced more than 90% of respective fungi in all cases (Fig. 2) Control seeds showed 5% infection (apparently from

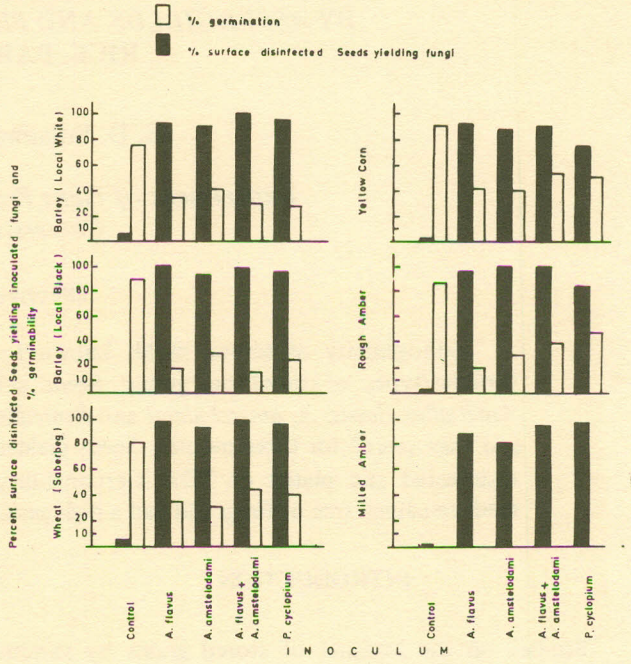

Fig. 1. Germination and surface disinfected seeds yielding fungi on different grains not inoculated and inoculated with spores of individual species and mixture of species of Aspergillus and P. *cyclopium*

Fig. 2. Inoculated and non-inoculated seeds of wheat var. Saberbeg. Note heavy infection of *P. cyclopium* (left) control (right).

inoculum that was not eliminated by the surface disinfection treatment at the beginning of the tests). These results are in conformity with Harman and Pfleger [2] who working with 7 isolates on wheat reported that wheat seeds were infected and percentage germination was reduced by all isolates.

Germination percentage of local white barley inoculated with three fungi separately ranged in the ratio of 28-

41%. The germination percentage of kernels inoculated with the mixture of two *Aspergillus* spp. was of the order of 30%. Surface disinfected kernels inoculated with fungi separately and with mixture of two *Aspergillus* species, yielded them between 90-100% on plating (Fig. 3).

Plated kernels of local black barley, inoculated with selected species of *Aspergillus* and *Penicillium* separately, yielded these fungi in the range of 93-100%. Kernels inoculated with a mixture of two *Aspergillus* species on plating yielded the fungi in 98% of cases (Fig. 4). Rough and milled amber rice inoculated with test fungi separately yielded them in more than 80% cases, whereas the mixture of two *Aspergillus* species on plating yielded the organism in more than 95% of cases (Fig. 5 and 6).

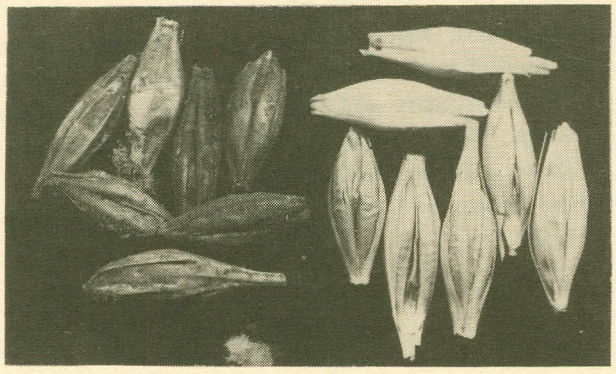

Fig. 3. Kernels of local white barley showing invasion of *A. amstelodami* (left) and control (right).

Fig. 4. Local black barley showing invasion of a mixture of *A. Flavus* and *A. amstelodami* (left) and (right) control.

Fig. 5. Rough amber rice invaded by *A. flavus* (left) and control (right).

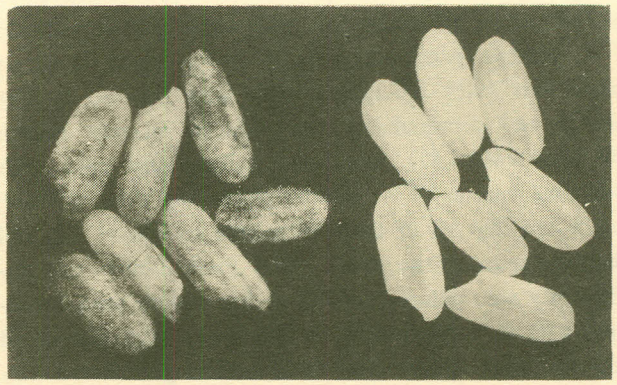

Fig. 6. Milled amber rice seeds inoculated with *P. cyclopium* showing heavy invasion (left) and control (right).

Corn inoculated separately with *A. flavus* and *A. amstelodami* produced the two fungi in the ratio of 93 and 89% respectively when the seeds were surface sterilized and subsequently plated. 90% of the seeds yielded *A. flavus* (Fig. 7) and *A. amstelodami* (mixture). 75% seeds produced *P. cyclopium* on plating.

Germination of local black barley (19%) and rough amber rice (21%) was severely reduced by *A. flavus*, but not so much by others. On corn, the fungus relatively did not have

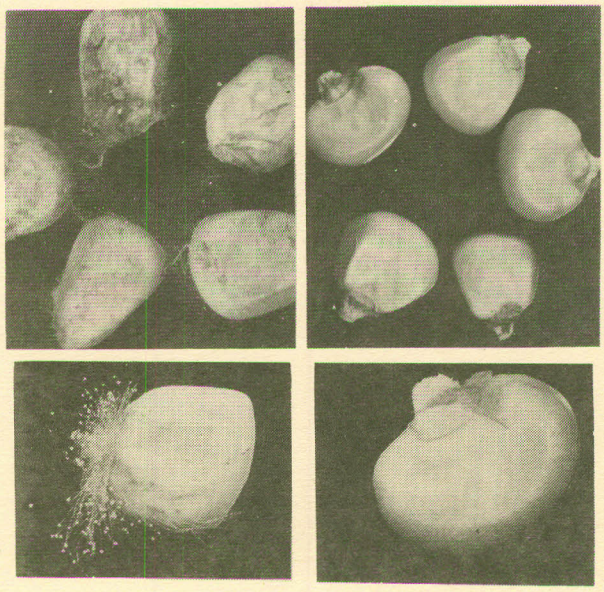

Fig. 7. (top) Yellow corn (left) deterioration due to invasion of A. flavus (right) control (bottom) and a close-up of above (left) infected with *A. flavus* (right) control.

the same effect. *A. amstelodami* and *P. cyclopium* reduced the germinability of all grains considerably. The mixture of two *Aspergillus* species was not appreciably more damaging than the individual member of the mixture when used alone. These results are in accordance with those obtained by Tuite and Christensen [3], who working with 4 species of *A. glaucus* group including *A. amstelodami* on wheat found that 100% inoculated seeds were invaded in four months and germinability was reduced severely. Qasem and Christensen [7] inoculated yellow dent corn with individual species of *A. glaucus* group and their mixture, and noted that all of them were able to invade, kill and discolour germs of the grain. After three months the germinability was reduced to 45% in the case of seeds inoculated with *A. amstelodami*. In this study corn inoculated with the same species after three months germinated only in 40% of cases, a little less percent germinability than what they have reported.

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