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DETERIORATION OF STORED WHEAT GRAINS BY SOME FUNGI

By

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B.Sc. Agric. (Ain Shams University) 1968

THESIS

Submitted in Partial fulfilment of the
Requirements for the Degree of

MASTER OF SCIENCE
in
PLANT PATHOLOGY

Ain Shams University
Faculty of Agriculture
Plant Pathology Dept.

1972

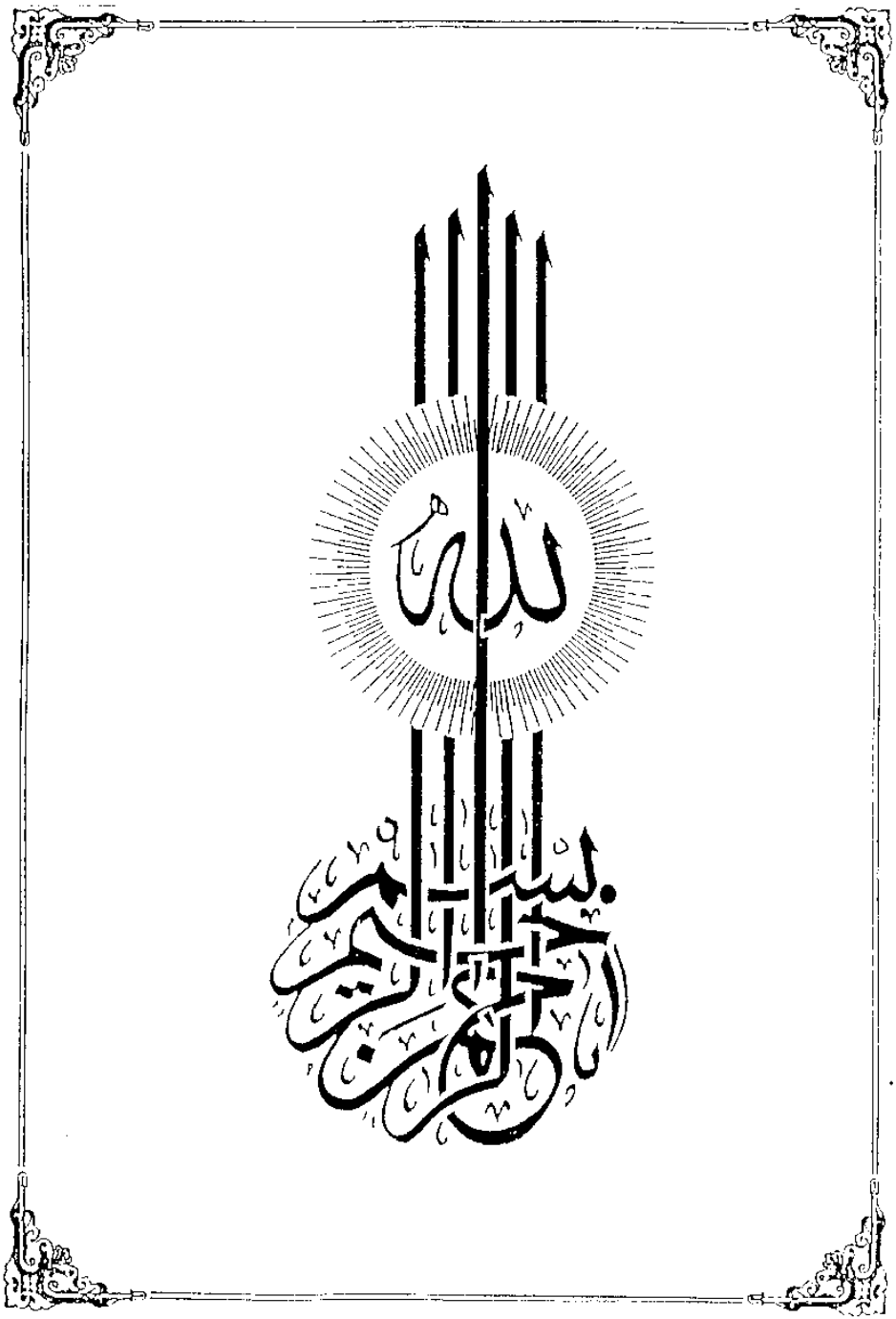
A C K N O W L E D G M E N T

This work was carried out under the supervision and direction of Prof. Dr. A. R. Serri, Dean of Faculty of Agriculture, Ain Shams University, Zagazig and Dr. M. F. Hegazi Lecturer of Plant Pathology, Faculty of Agriculture, Ain Shams University, Cairo.

The writer wishes to express his deepest gratitude and indebendness to them for suggesting the problem, supervision, progressive criticism, encouragement and direction throughout the whole work.

The writer also wishes to express his sincer thanks to Dr. S. M. F. Ahmed, Researcher in the N.C.R. and Mr. S. A. Habib, Assistant Lecturer, in Plant Pathology Department, for thier help and suggestion.

Thanks also due to all members of Plant Pathology Dept. for assistance.



C O N T E N T S

I N T R O D U C T I O N

Wheat (Triticum vulgare) is considered as one of the most important crops in A.R.E. The annual yield was 10.109 million Ardab in 1970 (Anon. 1971). The local yield dose not cover the consumption. The shortage is compensated by importation. Both local and imported amounts are almost kept in storage a period which may exceed to one year.

In A.R.E. wheat is commercially stores in "shonas" or in elevators. Deterioration and decrease in quality occur in wheat grains when stored under unfavourable conditions i.e. high moisture content and high temperature.

As far as the writer is awar , no attempt was tried for studying the problem of deterioration of wheat grains by fungi in A.R.E. The present work was carried out to investigate the followings:

1 - The mold flora associated with stored grains.
2 - The Pathoginic capabilities and some physiological studies on these fungi.
3 - Storage studies
4 - The effect of some preservatives on reducing the damage.
5 - Histopathological studies on the infected grains.

continue to grow in grains or to reinvade after harvest. They might die within few months of storage and thus did not survive much longer. Their damage was established by length of time after harvest, and no further damage could occur during storage. The storage fungi were those which developed on and within seeds at moisture contents often encountered in storage, principally Aspergillus and Penicillium. Aspergillus glaucus group, is one of the major fungi that invade stored seeds. It invaded wheat at moisture content of 13.2 to 15 % wet weight basis, with increasing moisture content above 15 %, Aspergillus candidus Link, Aspergillus ochraceus Wilhelm, Aspergillus flavus Link, Aspergillus versicolor (Vuillemin) Triaboschi, Aspergillus tamarii Kita and perhaps few other species of Aspergillus and Penicillium appear. Christensen and Kaufmann(1965)

Christensen and Gordon (1948) found that the principal organisms on all wheat samples tested were the same species of Aspergillus such as Aspergillus glaucus group, Aspergillus candidus Link and Aspergillus ochraceus Wilhelm. Penicillium sp. and Aspergillus flavus Link was found occasionally in small numbers.

Christensen (1955) found that Aspergillus restrictus invaded the seed of different wheats stored at moisture content 13.5 - 14.5 for 16 months, Strains of Aspergillus glaucus mainly Aspergillus repens (Corda) de Bary and Aspergillus ruber (Spieckermann and Bennner) Thom and Church invaded seeds stored at moisture content above 15%.

Assowah and Elarosi (1961) found that Aspergillus niger van Tieghem, Aspergillus nidulans (Eidam) Wint, Penicillium rubrum Stoll, Rhizopus nigricans Ehremb, and Mucor sp. were recorded at high frequency among the total fungi isolated from wheat grains stored 9 - 12 months when plated on PDA. The frequency of Curvularia sp., Aspergillus flavus Link, Alternaria sp., Cladosporium sp. Clindrocarpon sp., Chaetomium sp. Fusarium oxysporum Schlecht and Fusarium sp. was quite low.

Types of Changes Occurring in Storage:

A. Germination:

Christensen and Drescher (1954) found that the viability of the contaminated stored grains decreased by the increase of moisture content.

Hummel *et al* (1954) and Golubchuk *et al* (1956) found that percentage of germination decreased by the increase of temperature and time of storage in the mold contaminated grains.

Papavizas and Christensen (1960) found that wheat inoculated with *Aspergillus candidus* and kept for three months at 25°C and 16 - 16.4% moisture content showed germination of 6%, whereas the non-inoculating under the same conditions and in the same period germinated 95%.

B. Seed invasion:

Christensen and Drescher (1954), Golubchuk *et al* (1956), Tuite and Christensen (1957) and Saur and Christensen (1966) investigated the percentage of kernel invasion of wheat as affected

by each of storage conditions such as moisture, temperature and time. Their investigations proved that this criterion was correlated increasingly with unfavourable conditions.

C. Fat changes:

Zeleny and Colmen (1938) reported that fat acidity value "FAV" could be used as an index for grain deterioration. This was later agreed by Anon (1960 and 1962).

Hummel et al (1954), Sorger-Domenigg et al (1955) and Golubchuk et al (1956) investigated FAV as an index for the deteriorative action of commercially stored wheat. Their results proved that FAV was correlated increasingly with either high moisture content or temperature in addition to prolonged time of storage.

D. Carbohydrate changes:

Hummel et al (1954) found that nonreducing sugars decreased in moldy wheat stored at high moisture content.

Zeleny (1954) found that nonreducing sugars content of grains, may be better index for moldiness degree and perhaps for over all deterioration, than fat acidity value.

Christensen (1957) reported that the increase in reducing sugars and the decrease in nonreducing sugars in moldy stored grains were attributed mainly to fungi.

E. Respiration:

Bailey and Gurjar (1918) stated that several lines of evidence indicated that high respiratory rate observed after storage at high moisture levels and moderately high temperature were chiefly due to microorganisms.

Larmour et al (1935) concluded that there were two kinds of respiration in moist wheat grains, that due to the embryo of the seed, and the other due to molds. Thus if molds could be inhibited, wheat grains with 18 - 21% moisture content would not respire any faster than those at 12% moisture content.

sulfanilamide was confirmed by Milner (1956) who found that 500 ppm of this chemical, markedly inhibited both mold growth and respiration at high moisture levels, accompanied by little effect on viability of wheat.

Milner et al (1947b) treated wheat grains of 20 % moisture content, with each of calcium propionate, sulfanilamide and thiourea at the rate of 1000 ppm. They found that chemical treatments resulted in lower respiratory rate, fat acidity value and mold count, than those of untreated ones. This was accompanied by unaltered viability Calcium propionate, exceptionally caused a viability decrease, besides fat acidity value increased than the check.

Vayssiere (1948) in studies sponsored by the "Food and Agriculture Organization" recommended sulfa drugs as a preserving agent of stored grains against mold causing deterioration especially for those of high moisture content, as a post-harvest and pre-storage applications.

Jensen et al (1952) reported that 8-hydroxyquinoline was an effective compound that inhibit mold growth on cotton seeds of high moisture content.

content to 20 %. After 40 days of storage at 25°C both seed germination and Kernel invasion were determined.

Measurement of deterioration development and its progress:

A - Percentage of germination:

Samples of about 100 kernels were kept at room temperature between moist filter paper in Petri dishes for about 7 days. The number of seeds which produced normal sprouts were counted and the percentage of germination was calculated.

B - Percentage of kernel invasion:

Samples of about 100 kernels were surface disinfected, washed, placed on malt-salt-agar, then incubated at 28°C for 7 days. Kernels that exhibited growth of one or more mold fungi were counted and then the percentage of kernel invasion was calculated (Christensen and Drescher 1954).

Physiological Studies:

Growth measurement:

The linear method was used for measuring the radial growth of the tested fungi. Two diameters at right angle

Germination of spores:

Spore suspension was prepared by adding 10 - 20 ml sterilized water to 12 days old culture of the tested fungus. The spores were then separated with a camel's-hair brush. Two drops of this suspension were placed on a slide over two glass rings on the bottom of the Petri dish containing sterilized water to offer adequate humidity. A set of four dishes were then incubated for 24 hours for the different treatments. The percentage of germinating spores was calculated out from 100 - 200 spores from microscopic fields chosen at random.

Effect of relative humidity :-

The method devised by Solmon (1951) was used to obtain relative humidities 65, 70, 75, 80, 85, 90 and 100 % To study the effect of relative humidities on linear growth. Petri dishes containing Czapek's agar medium were inoculated with fungal discs and turned up-side down. Different concentrations of KOH to give different humidities, were poured in the lids of the dishes.

To determine the relative humidity required for germination of spores, fine films of Czqpek's agar medium were prepared on slides placed over two glass rings

Reducing and nonreducing sugars:

They were determined by the alkali ferricyanide method offered by Amon.(1962), which could be summarized as follows:

Sugars in 5.675 g of wheat flour were extracted using 5 ml ethanol and 50 ml acetic acid buffer solution (3 ml glacial acetic acid, 4.1 g anhydrous sodium acetate, 4.5 ml sulfuric acid and distilled water to complete litre). Other constituents rather than sugars were precipitated using sodium tungestate. 10 ml of potassium ferricyanide reagent (0.1N) were boiled with 5 ml of previous filtrates, in water bath. The reduced ferricyanide volume was calculated by substracting the ml volum of thiosulfate required from the ml volume of blank check. Reducing sugars were calculated as mg maltose per 10 g flour referring to the ferricyanide - maltose, sucrose conversion table (the same reference).

For [illegible] of the filtrate were acidically hydrolized in a water bath [illegible]

represented the total of reducing and nonreducing sugars was calculated. The previously calculated ml of ferricyanide reduced only by reducing sugars were substracted from this volume. The difference was expressed as mg sucrose per 10 g of flour referring to the same table.

Respiratory rate:

It was determined at 37.8°C (100°F), employing 100 g of each sample under controlled airiation in an apparatus devised by Bailey (1940). The incoming air passed through series of bottles containing sodium hydroxide and barium hydroxide to give carbon dioxide-free air, then passed through series of bottles containing sulfuric acid of specific gravity required to bring the air to relative humidity in equilibrium with moisture content of the grains, then passed through bottles containing the samples and at the end it passed through a gas absorbors containing standard barium hydroxide.

At the begining of the trial sufficient carbon dioxide-free air was drawn through the grains to replace completely all the air in the system. Then the rubber connections of the containers of the grains were closed.