EFFECT OF TEMPERATURE ON PRODUCTION OF AFLATOXIN ON RICE BY ASPERGILLUS FLAVUS

by

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(with 1 fig.)

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INTRODUCTION

During the past 5 years, production of toxic metabolites by fungi has become widely recognized as a potentially serious problem. A group of compounds produced mainly by species of Aspergillus, and referred to as aflatoxins, have received special attention initially because of the so-called Turkey "X" disease which was traced to these compounds (for a review see SPENSLEY, 1963).

Aspergillus flavus LINK ex FRIES has long been known to be one of the most abundant of the Aspergilli, being cosmopolitan in distribution and occurring on a wide variety of substrates (RAPER & FENNELL, 1965). Although not in such abundance in the atmosphere as such other genera as Cladosporium, Alternaria, Fusarium and Penicillium, spores of Aspergillus species constitute a considerable part of the airborne fungus biota (KRAMER et al., 1963). According to SEMENIUK (1954), A. flavus has been found on various cereal grains and grain products and is common on corn. Its cardinal growth temperatures have been reported by PANASSENKO (1941).

Our study was undertaken to determine the effect of temperature on aflatoxin production by A. flavus when grown on a cereal grain substrate. Previous work at the Northern Laboratory showed that rice is an excellent substrate for aflatoxin production (HESSELTINE et al., 1966; SHOTWELL et al., 1966). RABIE (1965) studied the influence of temperature on aflatoxin production in a semisynthetic medium, but he presented no quantitative data.

1) This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.
MATERIALS AND METHODS

Culture and inoculum

Aspergillus flavus NRRL 2999 was used throughout these experiments. Inoculum was grown on potato dextrose agar (Haynes et al., 1955) and incubated at 28°C for at least 7 days before use. Cultures older than 21 days were not used. Spore suspensions were prepared by adding 3 ml of 0.005 % Triton X-100 (2) per slant, gently scraping the surface of the agar with a sterile loop and then thoroughly shaking the slant. Several such suspensions were pooled to make a common one for any given series of flasks. The detergent aided in wetting the spores.

Fermentation

The substrate for these experiments was polished long-grain rice (Sunnyfield Brand) used at the rate of 50 g per 300 ml Erlenmeyer flask. The rice was allowed to stand 2 hours with 25 ml tap water and autoclaved for 15 minutes at 15 psi. After cooling, the rice was inoculated with 0.5 ml of spore suspensions per flask. The flasks, except for the controls, were incubated in a New Brunswick Psychrotherm shaker-incubator for various periods of time at temperatures ranging from 8—37°C. The shaker has a 2-inch orbit and was operated at 188 rpm. Sterile tap water (5 ml per flask) was added at 48 hours. An equal amount of water was added to a flask at 24 hours only if the substrate appeared excessively dry. Each flask was vigorously shaken by hand daily to prevent clumping. After incubation, the flasks were autoclaved for 1 to 2 minutes at 15 psi and stored in a Deepfreeze until assayed for aflatoxin content.

Assay procedure

Aflatoxins were extracted by mixing the fermented substrate (50 g) with methanol:water (55:45 v/v) (250 ml) in a Waring Blender 2 minutes (Nesheim et al., 1964). The resulting slurry was centrifuged 15 minutes at 5,000 rpm and filtered. The residue was washed with 50 ml methanol:water (55:45 v/v) and the filtrate plus wash was extracted with 100 ml hexane in a separatory funnel to remove lipids. The hexane layer was washed once with the methanol:water mixture (50 ml). The methanol layer and wash were combined and concentrated in vacuo to remove methanol. The concentrated aqueous solution was extracted once with 75 ml of chloroform and twice with 50-ml portions of chloroform. Combined extracts were concentrated almost to dryness in vacuo and transferred quantitatively with chloroform to 10-ml volumetric flasks in which the volume was made up to 10 ml with chloroform for thin-

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