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Effect of Substrate and Temperature on Growth of Aspergillus flavus in Peanuts from Georgia

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EFFECTS OF SUBSTRATE AND TEMPERATURE ON GROWTH OF *ASPERGILLUS FLAVUS* IN PEANUTS FROM GEORGIA

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ABSTRACT

We investigated the effects of different substrates (potato dextrose agar [PDA], nutrient agar [NA], and corn meal agar [CMA]) and temperatures on growth and aflatoxin production in *Aspergillus flavus*. Contaminated peanuts from retailers at different locations in Georgia were used throughout the experiment and 200 seeds were selected from each sample. PDA, NA, and CMA served as the substrate to express the mold from the contaminated seeds. Seeds were plated equidistantly on each of the media and incubated at 10° C, 27° C, 30° C, and 37° C, respectively. Seeds incubated on moist filter paper served as control. Neither growth of *A. flavus* nor detectable levels of aflatoxin was observed at the temperature of 10° C on any of the media. While no growth of this mold was observed at a temperature of 37° C in most media, some growth was seen in PDA media at this temperature. However, maximum growth of *A. flavus*, along with detectable levels of aflatoxin, was attained at the temperatures of 27° C and 30° C. Of the three media tested, PDA supported vigorous growth of *A. flavus* at the temperatures of 27° C and 30° C.

INTRODUCTION

Aspergillus flavus and *A. parasiticus* can invade peanuts in the field before harvest, during post harvest drying and curing, and in storage and transportation. Aflatoxins are polyketide secondary metabolites produced by these two important food contaminating *Aspergillus* species. The four main aflatoxins produced, aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2), are furanocoumarin derivatives and potent liver carcinogens for a wide variety of animal species including humans (Smith et al., 1994). Isolates of *A. parasiticus* produce both B and G aflatoxins, while most North American *A. flavus* isolates produce only B aflatoxins (Cotty and Cardwell, 1999). These mycotoxins endanger human health to such a degree that the Food and Drug Administration currently bans the sale and transport of peanuts when the level of aflatoxin contamination exceeds 20 parts per billion (Bilbo et al., 1983). The most important factors determining growth and

aflatoxin production by these two molds are substrate and temperature (Park and Bullerman 1983; Northolt et al. 1976). Under optimum conditions for growth, *A. flavus* can produce some aflatoxin within 24 hours and a biologically significant amount in a few days (Jacobsen et al., 1993).

In Georgia, aflatoxin is one of the most expensive problems the peanut industry faces. In Florida, Georgia, and Alabama, aflatoxin outbreaks from 1993 to 1996 caused losses averaging $26 million annually (Lamb and Sternitzke, 2001). Soil populations of *A. flavus* are predominantly aflatoxigenic in the southern United States, with over 95% of isolates producing aflatoxins in the peanut-growing region of Southern Alabama and Georgia (Horn and Dorner, 1999).

In spite of all the precautionary measures taken during harvest, storage, and transportation of peanuts and despite all the regulatory measures, both *Aspergillus* species exist at large in commercial outlets. It is also evident from previous studies that damaged peanut kernels contain higher levels of aflatoxin (Blankenship et al., 1984). Cracked kernels are easier for fungus to penetrate and may become dehydrated, thus leading to a more favorable environment for *A. flavus* (Payne, 1988). Although several research projects are underway in Georgia and other peanut-growing States to minimize loss due to pre-harvest infection of peanuts by *A. flavus* and *A. parasiticus*, very little is known about post-infection during storage at retail outlets and farmers markets. Initial screening of peanuts from several retail outlets at different locations in Georgia revealed a significant growth of *A. flavus* compared to *A. parasiticus*.

Hence the present investigation attempted to detect *A. flavus* in commercial peanuts during storage at retail outlets in Georgia. More specifically, the aim of this study was to test the following hypotheses for the growth and aflatoxin production of *A. flavus*: i) Variation in temperature affects the growth of *A. flavus* and there exists a temperature range that allows for the maximal growth of this mold; ii) Aflatoxin synthesis by *A. flavus* is affected by temperature and there is a temperature range that is supportive of maximum aflatoxin production by *A. flavus*; and iii) Substrate affects the growth of *A. flavus* and a particular type of substrate supports maximal growth of this mold.

MATERIALS & METHODS

We collected fresh peanuts from retailers located in Acworth, Kennesaw and Marietta in Georgia. Two hundred seeds from shelled peanuts were selected from each sample to conduct the experiment. Potato dextrose agar (PDA), nutrient agar (NA), and corn meal agar (CMA) served as the substrate to express the mold from the seeds. Six seeds were plated equidistantly on each media and incubated at temperatures of 10° C, 27° C, 30° C, and 37° C, respectively. Seeds incubated on moist filter paper at the different temperatures mentioned served as the control group. Yeast-extract sucrose (YES) media, which has been shown experimentally to favor the production of high

concentrations of aflatoxin (Gqaleni et al., 1997), served as the substrate for the production of aflatoxin from the *A. flavus* from the peanuts. Ultraviolet (UV) light was used to detect the presence of aflatoxin production from *A. flavus* incubated on YES medium. All experiments were repeated twice. All experimental data were analyzed using analysis of variance (ANOVA) and Student's t test ($p < 0.05$) to compare the means.

RESULTS

A. flavus was present in contaminated peanuts (10%) from our retailers at temperature of 37 °C on PDA. There was no significant growth of *A. flavus* at a temperature of 10° C (Figure 1). Temperatures of 27° C and 30° C supported growth of this mold, with maximum growth occurring at 27° C (Figure 1). Furthermore, it was found that potato dextrose agar (PDA) supported maximum growth of *A. flavus* at various temperatures tested (Figure 2). Of the three media tested, PDA supported vigorous growth of *A. flavus* at the temperatures of 27° C and 30° C.

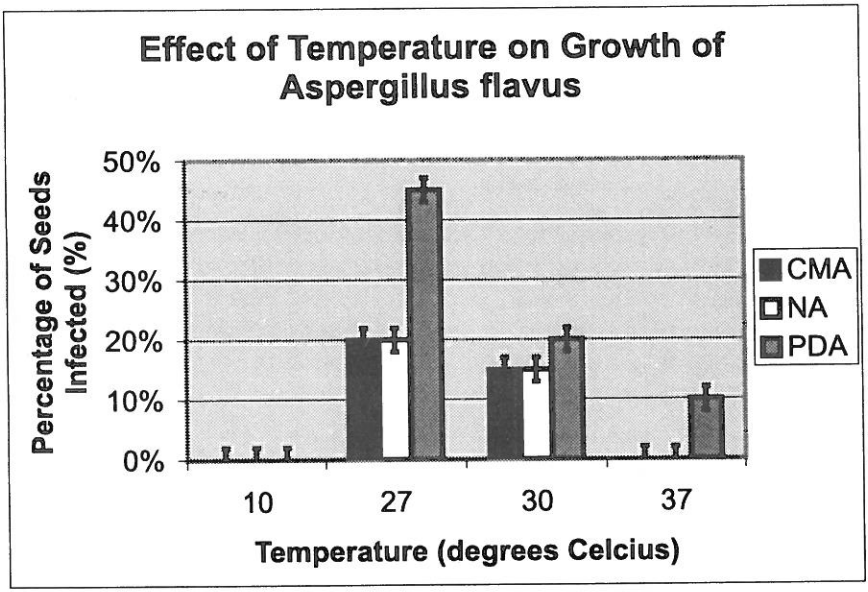

Figure 1. Effect of temperature on growth of *A. flavus* on the different media: CMA, NA, and PDA.

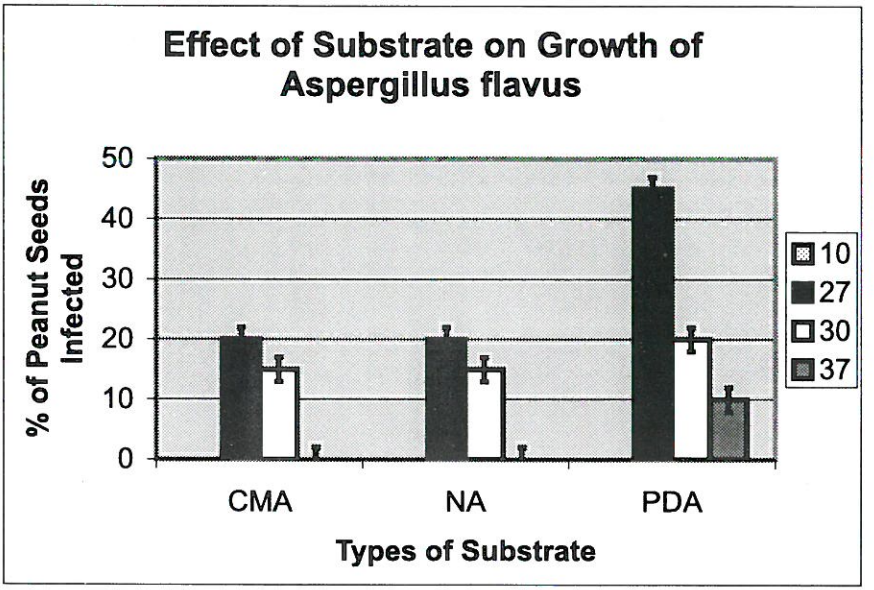

Figure 2. Effect of substrate on growth of *A. flavus* at different temperatures tested.

Yeast-extract sucrose (YES) media served as the substrate to trigger the production of aflatoxin from the *A. flavus* from collected samples. In order to detect the presence of aflatoxin, the YES plates, incubated with the *A. flavus*, were observed under UV light to see if the mold glowed. Presence of aflatoxin was detected at temperatures of 27° C and 30° C, while no aflatoxin was observed at temperatures of 10° C and 37° C (Table I).

Table I. Presence of aflatoxin at different temperatures from *Aspergillus flavus*

Temperature	Presence of Aflatoxin*
10° C	-
27° C	+
30° C	+
37° C	-

* + Present , -Absent

DISCUSSION

We conclude from the present investigation that peanuts from few retail outlets in Georgia were indeed contaminated with *A. flavus*. When seeds from these peanuts were exposed to low temperature neither growth of either mold nor detectable levels of aflatoxin was observed at 10° C. The most important factors for mycelial growth and sporulation of *A. flavus* from these peanuts were temperatures of 27° C and 30° C. While there are species of *Aspergillus* which grow at low temperatures, such as *A. malignus* and *A. sydowii*, our experiment showed that *A. flavus* from our sample do not tolerate such conditions. The only commonly encountered thermotolerant *Aspergillus* species are *A. fischerianus*, *A. fumigatus*, and *A. nidulans* (Morris et al, 1988). Furthermore, our investigation revealed that *A. flavus* was present in peanuts from our retailers at temperature 37 °C, however, maximum growth of this mold, along with detectable levels of aflatoxin was attained at the temperatures of both 27° C and 30° C. It appears that fresh peanuts in this region are available to consumers with a certain amount of seedborne mycelium of *A. flavus*. Peanuts exposed to variation in temperature during storage may become a public health hazard because of possible production of aflatoxin, a potential carcinogen.

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REFERENCES

1. Blankenship PD, Cole RJ, Sanders TH and Hill RA: Effect of geocarposphere temperature on pre-harvest colonization of drought stressed peanuts by *Aspergillus flavus* and subsequent aflatoxin contamination. Mycopathologia 85: 69-74, 1984.
2. Cotty PJ and Cardwell KF: Divergence of West African and North American Communities of *Aspergillus* section *Flavi*. Applied and Environmental Microbiology 65: 2264-2266, 1999.
3. Gqualeni N, Smith JE, Lacey J, and Gettinby, G: Effects of temperature, water activity, and incubation time on production of aflatoxins and cyclopiazonic acid by an isolate of *Aspergillus flavus* in surface agar culture. Applied and Environmental Microbiology 63 (3): 1048-1052, 1997.
4. Hill RA, Blankenship PD, Cole RJ and Sanders TH: Effects of soil moisture and temperature on preharvest invasion of Peanuts by the *Aspergillus flavus* group and subsequent Aflatoxin development. Applied and Environmental Microbiology 45 (2): 628-633, 1983.

5. Horn BW and Dorner JW: Regional differences in production of aflatoxin B1 and cyclopiazonic acid by soil isoplates of *Aspergillus flavus* along a transect within the United States. Applied Environmental Microbiology 65: 1444-1449, 1999.
6. Jacobsen BJ, Brown KI, Shelby RA, Diener UL, Kemppainen BW and Floyd J: Mycotoxins and mycotoxicoses. Alabama Cooperative Extension Service, Circular ANR 767, Auburn University Auburn Alabama, 1993.
7. Lamb MC and Sternitzke DA: Cost of Aflatoxin to the farmer, buying point, and sheller segments of the southeast United States peanut industry. Peanut Science 28: 59-63, 2001.
8. Mazur P: Cryobiology: The freezing of biological systems. Science 168: 939-949, 1970.
9. Morris GJ, Smith D, and Coulson GE: A comparative study of the morphology of hyphae during freezing with the viability upon thawing of 20 species of fungi. Journal of General Microbiology 134: 2897-2906, 1988.
10. Northolt MD, Verhulsdonk CH, Soentoro PS and Paulsch WE: Effect of water activity and temperature on aflatoxin production by *Aspergillus parasiticus*. Journal of Milk Food Technology39 (3): 170-174, 1976.
11. Park KY and Bullerman LB: Effects of substrate and temperature on Aflatoxin production by *Aspergillus parasiticus* and *Aspergillus flavus*. Journal of Food Protection 46 (3): 178-184, 1983.
12. Payne GA and Brown MP: Genetics and physiology of Aflatoxin biosynthesis. Annual Review of Phytopathology 36:329-362, 1998.
13. Smith JE, Lewis CW, Anderson JG, Solomons GL: Mycotoxins in human nutrition and health. IN Report EUR 16048 EN, European Commission, Directorate-General XII, Brussels, Belgium, 1994.