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Effect of Salt Concentrations on Aflatoxin Production in Peanut by *Aspergillus flavus*

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ABSTRACT

The use of various salt concentrations for controlling *Aspergillus flavus* growth and aflatoxin production in peanut was investigated. When *A. flavus* was grown in peanut alone, aflatoxin was produced with the yields of 11.25 and 3.75 μg/g at day 7 and 14 subsequently. Aflatoxin production was enhanced when the culture was added with 20 mg/g of salt and the yields were 16.68 and 6.90 μg/g at day 7 and 14 respectively. The concentration of salt added to the culture at 40 mg/g could reduce the growth and aflatoxin production. Aflatoxin was produced only in day 14 with the yield of 3.40 μg/g. However, no growth of *A. flavus* and aflatoxin production were detected when the cultures were added with salt at concentrations of 80, 120 and 160 mg/g. The result indicated that the higher concentration of salt inhibited the growth and aflatoxin production whereas the lower concentration had the reverse effect.

Key words : aflatoxin, *Aspergillus flavus*, peanut

INTRODUCTION

Aflatoxins, the toxic secondary metabolites of *Aspergillus flavus*, *A. parsasiticus* and *A. nomius* are the most hepatocarcinogens related to cancer incidence in human and animals (Kurtzman *et al.*, 1987; Abbas *et al.*, 1988). Various foodstuffs are susceptible to the fungal growth and aflatoxin formation. Peanuts, peanut products, grains, cereals and nuts have frequently been found to be contaminated with aflatoxins (Northolt and Bullerman, 1982: Diener *et al.*,1987). The incidence of liver cancer in human showed to be correlated with the consumption of aflatoxin contaminated foods. The severe outbreak of human hepatitis that resulted in the deaths of more than 100 people in Western India was traced to consumption of maize heavily contaminated with aflatoxins (Krishnamachari *et al.*,1975).

Numerous strategies have been proposed for the detoxification of aflatoxins in foods and feeds. However, preventing the contamination of food by the toxigenic fungi is the most rational and economic approach to avoid the potential hazards (Masood and Ranjan,1990; Samarajeewa *et al.*,1990). Previous study showed that the use of sodium chloride, the cheap and nontoxic salt, completely prevented the growth of *A. flavus* and aflatoxin production in corn (Thanaboripat *et al.*,1992). In this study the experiment was undertaken to observe the effect of such salt on growth of *A. flavus* and aflatoxin production in peanut.

MATERIALS AND METHODS

Source of organism and cultivation methods

Aspergillus flavus 102566 obtained from Commonwealth Mycological Institute, England, was grown at 30 °C for 7 days on malt extract agar. Spores were harvested in sterile distilled water plus 0.1% Tween 80. The spore suspension was pooled in a sterile bottle and the number of spores counted using an improved Neubrauer Haemacytometer.

Source of substrate and inoculation

Peanut (*Arachis hypogaea* cultivar Khonkaen-60), highly susceptible to *A. flavus*, was obtained from Department of Agriculture, Bangkok, Thailand. Peanut shell were cracked and kernels used as substrate for aflatoxin production. The initial moisture content of the substrate was adjusted to 23% by adding sterile water. Flasks containing peanut kernels to which water had been added were sealed with rubber stoppers

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and equilibrium was reached after 7 days of incubation at 4 °C.

Fifty grams of peanut were placed in a plastic bag plugged with cotton wool. Locally purchased sodium chloride salt at various concentrations was added to the substrate and mixed thoroughly before spores of *A. flavus* (10^6 spores/g) were inoculated.

Fungal growth observation

Fungal growth was visually assessed every 7 days using a semiquantitative scale, *viz.* (1) very little growth; (2) 25% of the grain covered; (3) 50% of the grain covered; (4) 75% of the grain covered; (5) all of the grain covered.

Aflatoxin analysis

The sample was extracted for aflatoxin by the Sep Pak method (Matsubara, 1985). 50 g sample was extracted with 250 ml chloroform and 25 ml distilled water in a shaker at 250 rpm for 30 min. The extract was then filtered through Whatman No.1 filter paper with the aid of Celite and the chloroform layer was separated from the filtrate. The filtrate was washed once again with 50 ml chloroform and the chloroform extracts were pooled together and evaporated to dryness. The extract was dissolved with 10 ml chloroform-hexane (3:7) and then added to the Sep Pak silica gel cartridge column. The column was drained and eluted with 10 ml hexane followed by 10 ml benzene-acetic acid (95.5:4.5) and 10 ml ethyl ether-hexane (60:40). The eluate was discarded and aflatoxin was eluted with 15 ml methylene chloride-acetone (9:1) and evaporated to dryness. The eluate was dissolved with 1 ml methanol , filtered through 0.45 µm membrane filter (Millipore) and 10 µl injected to the HPLC (Shimadzu) using the following condition: flow rate of 1 ml/min., UV spectrophotometric detector at 365 nm, reverse phase column C18 and mobile phase solvent (methanol:water:acetic acid = 6:2:2). The amount of aflatoxin was calculated from the chromatogram by comparison to the standard aflatoxin chromatogram.

Statistical analysis

The analysis of variance (ANOVA) was calculated to indicate the effect of salt on aflatoxin production at significant difference (P) of 0.01. The statistical method was according to Montgomery (1984). All experiments were repeated three times.

RESULTS

The effects of various concentrations of salt on growth of *A. flavus* and aflatoxin production in peanut are given in Table 1 and 2. *A. flavus* grew most rapidly on peanut alone and peanut added with 20 mg/g of salt. Slow growth of *A. flavus* at salt concentration of 40 mg/g occurred at the beginning but continued rapidly after day 7. No growth was observed at concentrations of 80, 120 and 160 mg/g (Table 1).

Production of aflatoxin was completely inhibited at concentrations of 80, 120 and 160 mg/g. At concentration of 40 mg/g, aflatoxin production was inhibited on day 7 but could only reduce the toxin production from 3.75 to 3.40 µg/g on day 14. At low level (20 mg/g), salt appeared to stimulate aflatoxin production from 11.25 to 16.68 and 3.75 to 6.90 µg/g on day 7 and 14 respectively (Table 2). It is notable that the stimulation is predominantly on aflatoxin B1.

DISCUSSION

Both fungal growth and aflatoxin production were completely inhibited at salt concentrations of 80, 120 and 160 mg/g (8,12 and 16%). Aflatoxin production was reduced when 40 mg/g (4%) of salt was employed. However, at concentration of 20 mg/g (2%), aflatoxin production was stimulated. Mabrouk and El-Shayeb(1980) found that sodium chloride concentrations of 5-50 mg/l (0.0005-0.005%) enhanced aflatoxin formation in a liquid medium whereas afla-

Table 1 Effect of various concentrations of salt on growth of *A. flavus* 102566 in peanut.

Concentraion of salt added (mg/g)	Extent of fungal colonisation* Time (days)			
	7	14	21	28
0	5	5	5	5
20	5	5	5	5
40	4	5	5	5
80	NG	NG	NG	NG
120	NG	NG	NG	NG
160	NG	NG	NG	NG

* semiquantitative scale 1-5 (See Methods)
1 = very little growth
2 = 25% of the grain covered
3 = 50% of the grain covered
4 = 75% of the grain covered
5 = all of the grain covered
NG = No growth

Table 2 Effect of various concentrations of salt on aflatoxin production of *A. flavus* 102566 in peanut.

Day	Concentration of Salt (mg/g)	Aflatoxin (μg/g) B1	G1	Total B1 + G1
7	0	7.28	3.97	11.25
	20	16.68	0	16.68*
	40	0	0	0
	80	0	0	0
	120	0	0	0
	160	0	0	0
14	0	3.75	0	3.75
	20	6.90	0	6.90*
	40	3.40	0	3.40*
	80	0	0	0
	120	0	0	0
	160	0	0	0

* = significantly different at p = 0.01

toxin formation was reduced at concentrations of 40 and 80 g/l (4 and 8%) and both growth and aflatoxin formation were inhibited at concentration of 120 g/l (12%). Uraih and Chipley (1976) observed that growth and aflatoxin production in basal medium were inhibited at 12% sodium chloride while at 8% or less, growth and aflatoxin production were stimulated. Many investigators reported the presence of low levels of aflatoxin when either *A. flavus* or *A. parasiticus* grew on various types of cheese with salt content between 2-7% (Ingram and Kitchell, 1967: Kulik and Hanlin, 1968: Matches and Liston, 1972). Shih and Marth (1972) found that growth of *A. flavus* and *A. parasiticus* was completely inhibited when the medium contained 14% sodium chloride. Bullerman *et al.*(1969) also observed that sodium chloride inhibited aflatoxin production in a glucose-ammonium nitrate broth. Growth of *A. flavus* and aflatoxin production on corn were completely inhibited when sodium chloride at concentration of 120 mg/g (12%) was used (Thanaboripat *et al.*,1992). El-Gazzar *et al.* (1986) also found that increasing the concentration of sodium chloride accumulation of aflatoxin and induced a lag in growth of the culture. It has been suggested that high sodium chloride concentrations might have adversely affected the water activity required for growth and subsequently toxin production by *A. flavus* and stimulation of aflatoxin production by low levels of salt might be a function of sodium ion (Uraih and Chipley, 1976).

The results of this study indicated that sodium chloride at certain concentrations could be used for prevention of growth and aflatoxin production by *A. flavus* in peanut and could also by applied to control mould growth and aflatoxin production in other agricultural commodities.

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