#-22-

J. Agric. Res. & Dev. 11(1). Copy@2012. Faculty of Agriculture, University of Ilorin

The Inhibition of aflatoxin production from Aspergillus parasiticus NRRL 2999 by ethanol extract of Aframommon danielli flower.

M.O. AFOLABI[1], G.O. ADEGOKE[2], B.A. OLUNLADE[1], A.A. ADEOLA[3] and W.O. IBITOYE[1]

[1]*Food Science and Technology Department, Bowen University, Iwo*
[2]*Food Technology Department, University of Ibadan, Ibadan.*
[3]*Food Technology Department, Federal University of Agriculture, Abeokuta.*

ABSTRACT

The inhibition of Aflatoxin production from Aspergillus parasiticus strain NRRL 2999 was investigated using ethanol extracts of Aframommon danielli flower at concentrations of 250µg/g, 500µg/g, 750µg/g and 1000µg/g with whole wheat bread as a substrate. Aspergillus parasiticus grew abundantly on whole wheat bread; growth on samples containing 250µg/g and 500µg/g extracts were scanty and those of 750µg/g and 1000µg/g were not visible. The percentages aflatoxin (B1 + G1) inhibitions of the flower extract were 25.2% (250µg/g), 43.5 (500µg/g), 65.2% (750µg/g) and 70.2% (1000µg/g). The percentage inhibition for Tioconazole (100µg/g) was 88%. The result showed that Aframommon danielli flower ethanol extract can prevent mould growth and aflatoxin production in foods.

Key words: Aflatoxin, *Aspergillus parasiticus, danielli*

INTRODUCTION

Under favourable conditions, during harvesting, processing and storage of food commodities, moulds produce mycotoxins (Bullerman, 1986). Most mycotoxins are relatively heat stable, non-volatile compounds capable of producing diseases of acu e or chronic nature when ingested with food by affecting many target organs such as liver, kidney, nervous, endocrine and immune systems (Crocker *et al.*, 1984). Mycotoxins have also been implicated in animal feeds (Abarca *et al.*, 1994; Gourama and Bullerman, 1995). Favourable weather conditions for aflatoxin production are temperatures of 28-300C and relative humidity above 90% (Arun *et al.*, 1987; Pitt and Miscamble, 1994). Aflatoxins are secondary metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* which have been shown to be both toxic and carcinogenic in test animals (Bullerman, 1986). It was established that whole wheat bread provided a good substrate for aflatoxin production (Bullerman, 1974).

It has been discovered that essential oils from spices such as *A. danielli* seeds possess anti fungal activities and inhibit aflatoxin production in cereals, nuts and cocoa products (Adegoke *et al.*, 1994, 1998, 2000). However, effect of *A. danielli* flower extracts in the inhibition of mycotoxins has not been elucidated. The objective of this study is to determine the effects of *A. danielli* flower extracts on growth and aflatoxin production by known toxinogenic strain of *A. parasiticus.*

MATERIALS AND METHODS

Freshly plucked, matured flower of *A .danielli* flowers were obtained from a local farm in Ibadan, Oyo state. All chemicals, cultures and reagents were obtained from Waco chemicals, Osaka, Japan.

Preparation of *A. danielli* flower, leaves, powder and solvent extractions

These were carried out as described by Adegoke and Skura (1994) and Christine *et al.* (1998).

The inhibition of aflatoxin production by purified fractions of *A. danielli* leaf and flower

The inhibition of aflatoxin production by *Aspergillus parasiticus* NRRL 2999 was done using purified fractions of solvent extracts of *A. danielli* flower at concentrations of 250, 500, 750 and 1000µg/g with whole wheat bread as a substrate.

Baking of whole wheat bread

Whole wheat bread was baked using AOAC method (2005) and this was done in 3 batches as follows: one set was baked with *A. danielli* fractions (250, 500, 750 and 1000µg/g);the second set was baked with Tioconazole (100µg/g) and a control was set up without any preservative. The recipes used were as follows:

Preparation of innoculum and inoculation of bread slices

Innoculum preparation was carried out using the method of Bullerman (1974) while inoculation was made by slicing of bread which slices were aseptically removed from the packaged loaves and placed on sterile towels inside a bacteriological glove box (previously sanitized with a 50% solution of liquid

household bleach and exposed to UV light for 1 hour). The bread slices were exposed to UV light for 15mins per side prior to inoculation. The slices were inoculated using a sterile 1ml tuberculum syringe. The innoculum was distributed over the surface of the bread as much as possible by brushing with a flamed inoculating loop. The inoculated slices were individually packaged in polythene pouches (PL 540) and sealed. The inoculated bread slices in duplicates were stored for 10 days at 250C.

Yeast (dry)	-	0.30%
Lukewarm water(30°C)	-	20.82%1000µg/g
Vegetable fat	-	5.76%
Milk (non-fat)	-	2.88%
Sugar	-	4.32%
Salt	-	0.28%
Flour (all purpose 75%+ whole wheat 25%)	-	57.64%

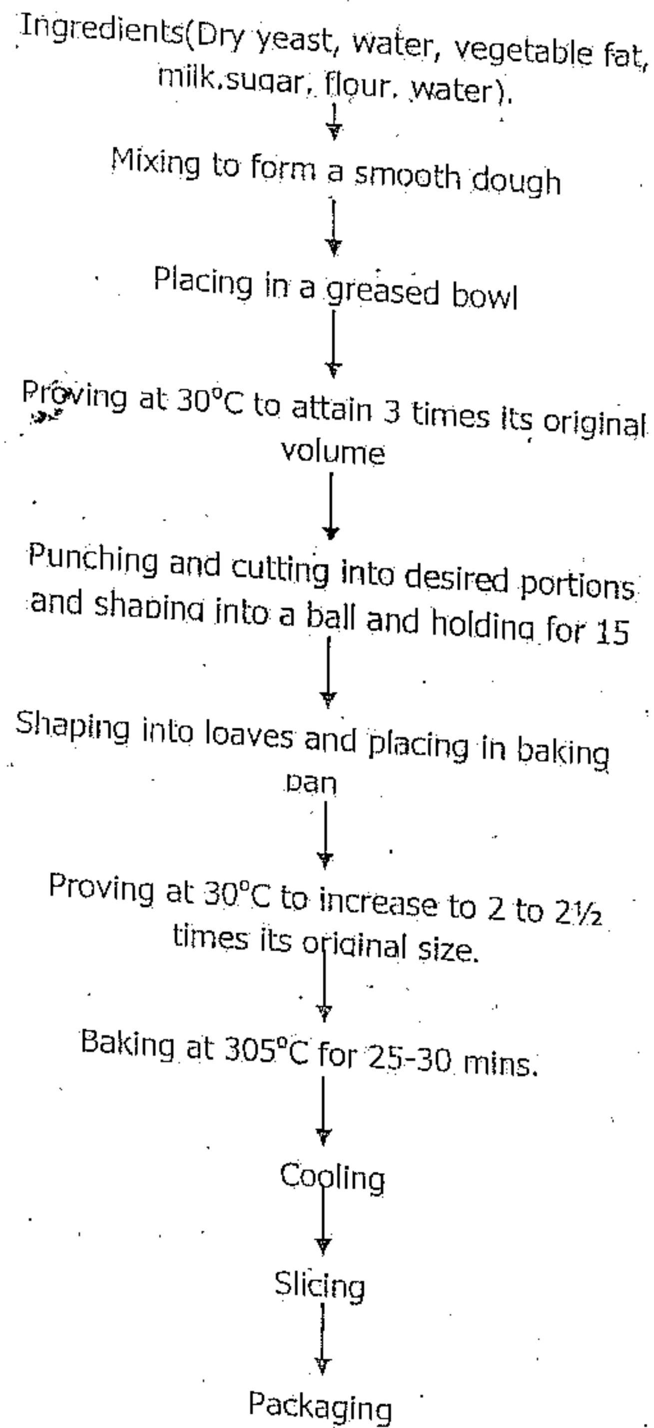
The straight dough method was used for mixing. The flow chart describes the method used for the production of whole wheat bread.

Analysis of bread slices for aflatoxin contamination

The extent of mould growth was assessed visually for each sample throughout the storage period. Following storage all duplicate samples were dry-milled using a sterile Osterizer blender. The milled samples were analyzed for aflatoxin content using 100% ethanol as extracting solvent (Bullerman, 1974). Extracts were separated on thin layer chromatography (TLC) plates (20x2.0cm, 0.2mm thick silica Gel G-HR). The TLC plates were developed in toluene – ethylacetate – 90% formic acid (60: 30: 10) mixture (Scott *et al.*, 1970). Aflatoxin concentrations in the extracts were estimated by visual comparison of the fluorescence of the respective aflatoxins of the bread samples to known standards on exposure to long wave UV light at 635nm (AOAC, 1995), using FUNA UV light SL 800G. The results were expressed as total aflatoxins B + G, and percentage inhibition at different levels of addition of *A. danielli* flower and leaf fractions to bread slices were calculated.

RESULTS AND DISCUSSIONS

Tables 1 and 2 show the effect of *A. danielli* fractions on growth and total aflatoxin (B1+G1) production by *Aspergillus parasiticus* (strain NRRL 2999). Strains of *A. parasiticus* grew abundantly on whole wheat bread (Table 1).



Flow chart for baking of whole wheat bread.

This is in agreement with the findings of Bullerman (1974) that whole wheat bread could serve as a good substrate for growth of *Aspergillus parasiticus*. Growth on samples containing 250µg/g and 500µg/g. *A. danielli* flower extracts were scanty while those of 750µg/g and 1000µg/g were not visible. The percentage aflatoxin (B1 + G1) inhibitions of the flower extract (Table 2) were 25.2% (250µg/g), 43.5 (500µg/g), 65.2% (750µg/g) and 70.2% (1000µg/g). The percentage inhibition for Tioconazole (100µg/g) was 88%. *Aframomon danielli* seed extracts have been reported to reduce aflatoxin production in maize (Adegoke *et al.*, 2000). Aroyeun *et al.*, (2009) reported 94.3% reduction efficiency of *A. danielli* seed extract on cocoa beans infected with Aspergillus. Inhibition of aflatoxin by the extract could be due to the presence of monoterpenes and alkaloids in *A. danielli* flower (Adegoke *et al.*, 1999; Afolabi *et al.*, 2011 and Aroyeun *et al.*, 2009).

Table 1: Growth of *Aspergillus parasiticus* on whole wheat flour bread at various concentrations of *A danielli* flower extracts.

	Concentration of *A danielli* Flower extract (µg/g)	Tioconazole (100 µg/g)
Growth	+++ ++ + -	-

- No growth + scanty growth ++ Moderate Growth +++ Extensive Growth

Table 2: Effect of *A. danielli* flower extract on total aflatoxin ($B_1 + G_1$) production by *Aspergillus parasiticus* NRRL 2999 at various concentrations.

	Concentration of *A. danielli* flower extract (µg/g)					Tioconazole (100 µg/g)
	0	250	500	750	1000	
Counts of aflatoxin ($B_1 + G_1$)	110	83	52	28	23	0
% inhibition	0	25.2	43.5	65.2	70.2	88%

CONCLUSION

It has been established in this work that *A. danielli* flower extract could be used to inhibit growth of mould and aflatoxin production in foods.

REFERENCE

Abarca, M.L., Bragulat, M.R., Castell, G. and Kabanes F.J. (1994). Mycoflora and aflatoxins producing strains in animal mixed feed. Journal of Food Production 57(3): 256-258.

Adegoke, G.O. and Gopala Krishna A.G. (1998). Extraction and identification of Antioxidants from the spice Aframomum. danielli (Family: zingiberaceae) Journal of Arne Oil Chem. Soc. 75, 104-105.

Adegoke, G.O., Fasoyiro, S.B., and Skura B. (2000a). Control of Microbial Gowth, Browning and Lipid oxidation by the spice Aframomum. danielli Food Res. Technology, 211, 342-345.

Adegoke, G.O.; Iwahashi, H.; Komatsu, Y., Obuchi, K and Iwahashi Y. (2000b). Inhibition of Food Spoilage Yeast and Anti-toxigenic Mould by Monoterpenes of the spice Aframomum danielli. Flavour and Fragrance Journal, 5(3) 147-150.

Afolabi,M.O.;Adegoke,G.O. and Mathooko,F.M.(2011) Phytochemical characterization of the extracts of Aframomum danielli flower, leaf, stem and root.African journal of Agricultural research vol 6(1) pp97-101

AOAC (2005) Official methods of Analysis of Association of Officcial Analytical Chemists international, Washington (D.C.).

Aroyeun S.O., Adegoke G.O, Varga J., Teran J. (2009) Reduction of aflatoxin B, and Ochratoxin A in Cocoa beans infected with Aspergillus via Ergosterol value. World review of science, Technology and sustainable Development. 6(1/2009).

Arun S.; Desai, P.S.R. and Nadkarni, G.B. (1987). A new method for aflatoxin free storage of agricultural commodities. Jounal of Food Science. 52(2): 497.

Bullerman, L. (1986). Mycotoxins and food safety. Food Technology. May 1980, pp. 59-66.

Bullerman, L. B. (1974). Inhibition of Aflatoxin production by Cinnamon. Journal of Food Science. 39(1163).

Christine E.L; John R.L; Walker, J. E; and Kelvin H. S. (1998). Determination of Anthocyannins, Flavonoids and Phenolic acids in Potatoes, coloured cultivars of Solanum tuberrosum L. Journal of Science, Food and Agriculture; 77, 45-47.

Cocker, R.D.; Jones, B.D.; Gilman G.A.; Wallbridge, A.J. and Penigrahi S. (1984), Mycotoxin training manual. Tropical development and Research Institute, London.

Gourauna, H. and Bullerman, L.B. (1995). Aspergillus flavus and Aspergillus parasiticus: aflatoxigenic fungi of concern in foods and feeds. J. Food Protection 58(2): 1395-1394.