Vet. Med. J., Giza 40, No. 1, 21-29 (1992)

INFLUENCE OF MONOMETHYLAMINE: CITRIC ACID AND AFLAGIN ON AFLATOXINS PRODUCTION BY ASPERGILLUS PARASITICUS

By

EL-SAYED A.M. ABD ALLA, A. BADAWY, M.M. SAAD and KH. NAGUIB

Mycotoxins Central Lab., National Research Centre, Dokki, Cairo, Egypt

Received: 29.10.1991

INTRODUCTION

Decontamination of foods containing aflatoxins is a problem of current concern. Experimental data gathered during the last three decades on the loss of productivity in farm animals consuming contaminated feeds, and the carcinogenicity in experimental animals provide sufficient evidence regarding the hazardous nature of aflatoxin(4). The positive correlation between the consumption of aflatoxin contaminated foods and the increased incidence of liver cancer in several southeast Asian and African populations further suggest the threat posed to human health by aflatoxins. 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 aflatoxin(7). So this study was designed to monitor aflatoxins production and accumulation by *A. parasiticus*, where corn treated with monomethylamine: citric acid and Aflagen.

MATERIAL AND METHODS

Culture:

A. parasiticus was used throughout this study. The organism was grown at 28°C for 7 days on potato

dextrose agar (PDA), and spores were harvested and suspended in a sterile 0.1 % solution of tween 80 in distilled water. The number of conidia in the suspension was approx 10^6 conidia/ml[8].

Solution of Inhibitories

Monmethylamine . citric acid (T-1) analytical grade 20 %) was dissolved in sterilized distilled water (1.0 gm/L). And Aflagin (T-2) an antimycotic drug, manufactured by virbac, Co. was dissolved in sterilized distilled water (0.5 gm/L).

Preparation of Contaminated Corn

Yellow ground corn was dispensed in 100 gm quantities into 1000 ml Erlenmeyer flasks and sterilized by autoclaving at 121°C for 15 min and 20 ml of the inhibitory solution were added. Each flask was inoculated with 1.0 ml Spore suspensions. The infected corn and controls were incubated at 25°C with moisture content 17% for five weeks.

Aflatoxins Solution

Crystalline aflatoxins (B_1, B_2, G_1 and G_2) were purchased from Sigma Co. The toxins were dissolved in methanol and quantified spectrophotometrically at 361 mm[3]. The concentration of working solution was 0.5 ug/ml B_1 G_1 and 1/5 of this concentration for B_2 and G_2 in Benzene: acetonitrile (9:2, v/v).

Aflatoxin Analysis

The aflatoxins were analyzed by the CB method[3]. The amount of aflatoxins in the extracts were determined by thin layer chromatography using 20x20 cm plates coated wth 0.25 mm silica gel (E. Merck). After development in chloroform: acetone (9:1, v/v), aflatoxins spots on the plates were quantified using densitometer (TLD 100 Vitatron, Holland) developed by Dickens et al. [1]. For confirmatory test, the extracts were

respotted on plates and treated with trifluoroacetic acid to aid detection of possible traces of aflatoxin B_1 by converting it to B_{2a} [3]

RESULTS AND DISCUSSION

A. parasiticus FRR2752 showed mycelial growth within the end of the first week on ground yellow corn supplied with monomethylamine/citric acid (T-1) and aflagin (T-2); so the toxin production was inhibited during the first week.

For monomethylamine/citric acid treatment, production of aflatoxin B_1 was inhibited by 17.88 and 19.03 % during the second and fourth week, but the lower inhibitory effect was observed during the fifth and third week (5.59 and 9.34 %) respectively, as shown in Table (2) and Fig. (1). While aflatoxin B_2 was inhibited by 25.26 % then the inhibitory effect was decreased to 9.43 % during the third week as in Table (1) adn Fig. (2); During the third and fifth week the treatment stimulated, aflatoxin B_2 production, as described in Table (1). Whereas aflatoxin G_1 production was stimulated during the second, third and fourth weeks as shown in Table (1). During the fifth week aflatoxin G_1 was decreased by 11.68 % [(Table (2) and Fig. (3)[1]]. On the other hand, the maximum decrease for aflatoxin G_2 production observed during the fourth week (36.01 %), but the minimum inhibition effect was found during the fifth week(1.51%). Whereas the aflatoxin G_2 production was increased during the second and third weeks over than the control (Tables 1 and 2).

Early reports were published on the chemical inactivation of aflatoxin in cottonseed and peanuts meals with monomethylamine. Park et al. [5] reported that, when naturally contaminated peanut meal (5500 ug total aflatoxin/kg) was exposed to monomethylamine (0.5 %) and lime (2.0 %) at elevated temperature

Table (2) Inhibition of aflatoxins production (%) by monomethylamine/citric acid (T-I) and aflagin (T-II).

Treatment	T-I					T-II				
Period/ week	1*	2	3	4	5	1*	2	3	4	5
B_1	100.0	17.88	9.34	19.03	5.59	100.0	-	8.78	12.57	-
B_2	100.0	25.26	-	9.43	-	100.0	9.49	-	14.27	19.64
G_1	100.0	-	-	-	11.68	100.0	-	-	5.25	1.41
G_2	100.0	-	-	36.01	1.51	100.0	-	-	-	2.91

* Fungal growth was delayed during the first week.

Table (1) Production of aflatoxins (µg/kg) by A. parasiticus on corn in the presence of monomethylamine/citric acid (T-I) and aflagin (T-II).

Treatment	Control					T-I					T-II				
Period/ week	1	2	3	4	5	1*	2	3	4	5	1*	2	3	4	5
B_1	130.00	215.68	182.75	172.05	154.22	0	177.11	165.68	139.31	145.60	0	218.52	166.70	150.42	162.09
B_2	65.20	111.97	53.86	79.14	84.22	0	83.69	71.47	71.68	89.14	0	101.34	70.85	67.85	67.68
G_1	109.72	208.15	146.55	192.54	187.03	0	209.49	212.47	196.96	165.18	0	226.34	189.73	182.44	184.40
G_2	87.37	115.21	114.81	143.89	133.83	0	148.95	139.79	92.07	131.81	0	134.35	148.92	153.78	130.61

* Fungal growth was delayed during the first week.

Fig. (1) Effect of monomethylamine; citric acid and aflagin on Aflatoxins production by A. parasiticus.

(100°C) and atmospheric pressure (24 % moisture content), residual aflatoxin levels of 310 ug/kg (94 %) reduction resulted. Mann et al.[2] found that aflatoxin contamination (334 ug/kg) in cottonseed meal was reduced to 7 ug/kg following treatment with monomethylamine (2%) and sodium hydroxide (1 %) at 100°C for 30 min. (15% moisture content).

Aflagin treatment (T-2) markedly inhibited aflatoxin B_2 production during the experiment, as described in Table (2) the reduction was increased with increasing the incubation period, (Fig. 2). But aflatoxin G_1 and G_2 was inhibited some whate during the fourth and fifth weeks (Table 2 and Figs. 3 and 4. Whereas; these toxins production was stimulated during the second and thrid for aflatoxin G_1 and also during the fourth week for aflatoxin G_2 as shown in Table (1). On the other hand, aflatoxin B_1 was inhibited only during the third and fourth weeks with 8.78 and 12.57 % respectively (Table 2). In our laboratory studies, aflagin (antimycetic drug) was not effective as inhibitor for the growth and alflatoxin production except aflatoxin B_2 by *A. parasiticus* on ground yellow corn (17 % moisture). While monomethylamine/citric acid reduced maximum aflatoxin production to about 19.03 % during the fourth week of the control.

Generally, these data suggest that the inhibitory effect may be due to one or more of these (i) the conversion of aflatoxin B_1 to other compounds (ii) to inhibit enzymes which process versicolorin A and averufin to sterigmatocystin and altimately aflatoxin. (iii) or to convertion of aflatoxin B_1 to its hemiacetal by addition of water molecule to the vinyle ether double bond of the aflatoxin B_1 terminal ring in the acid[6]. And also acid treatment leads to hydration of aflatoxin B_1 at the 8,9- olefinic bond of the terminal furan ring to form aflatoxin B_{2a}; a similar reaction occurs with aflatoxin G_1 to produce aflatoxin G_{2a}[7].

SUMMARY

During the first week the growth of *A. parasiticus* was delayed by monomethylamine/citric acid and aflagin treatments. On the other hand, both of these treatments were not effective for inhibition of aflatoxins production by *A. parasiticus* on corn. But monomethylamine/citric acid treatment reduced aflatoxin B_1 production much better than aflagin, while the highest reduction for aflatoxin B_2 production was obtained with aflagin treatment during the experimental period.

REFERENCES

1. Dickens, J.W., W.F. McClure and T.B. Whitaker (1980): "Densitometric equipment for rapid quantitation of aflatoxins on thin layers chromatography". J. Amer. Oil. Chem. Soc. 57: 205-208.

2. Mann, G.E., Gardoner, H.H., Jr., Booth, A.N. and Gumbamann, M.R. (1971): "Aflatoxin inactivation: chemical and biological properties of ammonia and methylamine treated cottonseed meal". J. Agric. Food Chem., 19, 1155-1158.

3. Official Methods of Analysis (1984): 14th Ed., AOAC, Washington Va, Chapter 26, "Natural Poisons".

4. Palmgren, M.S. and A.W. Hayes (1987): "Aflatoxins in food". pp. 65-96. In Mycotoxins in Food. Ed. Palle Krogh, Academic Press, London.

5. Park, D.L., Jemmali, M., Frayssinet, C. Frayssinet-La Farge, C. and Yvon, M. (1981): "Decontamination of aflatoxin-contaminated peanut meal using monomethylamine: Ca (OH) 2". J. Am. Oil Chem. Soc. 58, 995A-1002A.

6. Pohland, A.E., Cushmac, M.E. and Andrellos, P.J. (1968): "Aflatoxin B_1 hemiacetal". J. Ass. Off. Anal. Chem. 51: 907.

7. Samarajeewa, Y., Sen, A.C., Cohen, M.D. and Wei, C.I. (1990): "Detoxification of aflatoxins in foods and feeds by physical and chemical methods". J. Food Prot. 53: 489-501.

8. Sharma, A., Bahere, A.G., Padwal-Desai, S.R. and Nadkarni, G.B. (1980): "Influence of inoculum size of *Aspergillus parasiticus* spares on aflatoxin production". Appl. Environ. Microbiol. 40: 989-993.