

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

Influence of monomethylamine: Citric acid and.....

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

Monomethylamine . 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 nm⁽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 with 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

EL-SAYED A.M. *et al.*

respotted on plates and treated with trifluoroacetic acid to aid detection of possible traces of aflatoxin B₁ 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 B₁ 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 B₂ was inhibited by 25.26 % then the inhibitory effect was decreased to 9.43 % during the third week as in Table (1) and Fig. (2); During the third and fifth week the treatment stimulated, aflatoxin B₂ production, as described in Table (1). Whereas aflatoxin G₁ production was stimulated during the second, third and fourth weeks as shown in Table (1). During the fifth week aflatoxin G₁ was decreased by 11.68 % [(Table (2) and Fig. (3))]. On the other hand, the maximum decrease for aflatoxin G₂ production observed during the fourth week (36.01 %), but the minimum inhibition effect was found during the fifth week (1.51%). Whereas the aflatoxin G₂ 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).

Period/ week	T-I					T-II				
	1*	2	3	4	5	1*	2	3	4	5
B1	100.0	17.88	9.34	19.03	5.59	100.0	-	8.70	12.57	-
B2	100.0	25.26	-	9.43	-	100.0	9.49	-	14.27	19.64
G1	100.0	-	-	-	11.68	100.0	-	-	5.25	1.41
G2	100.0	-	-	36.01	1.51	100.0	-	-	-	2.91

* Fungal growth was delayed during the first week.

Table (1) Production of aflatoxins ($\mu\text{g}/\text{kg}$) by *A. Parasiticus* on corn in the presence of monomethylamine/citric acid (T-I) and aflagin (T-II).

Treatment	Control					T-I					T-II				
	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.

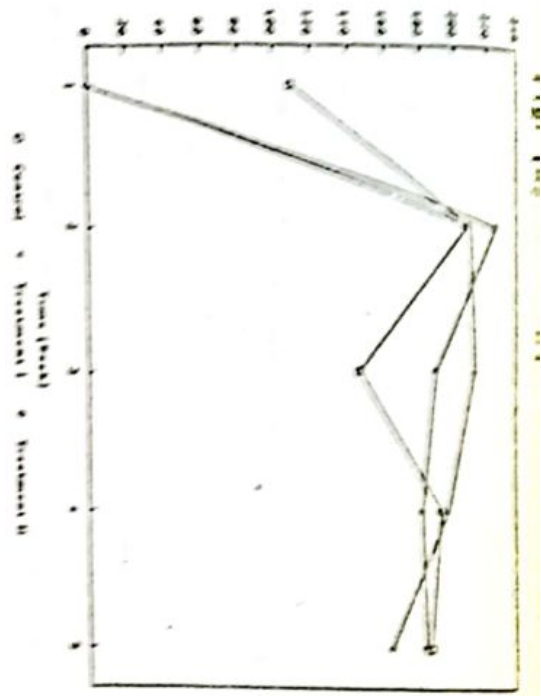


Fig. (4) G2

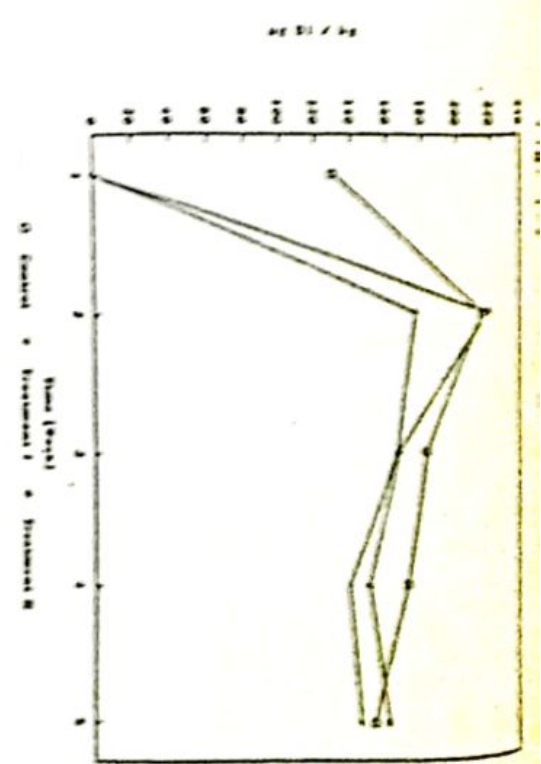


Fig. (2) B2

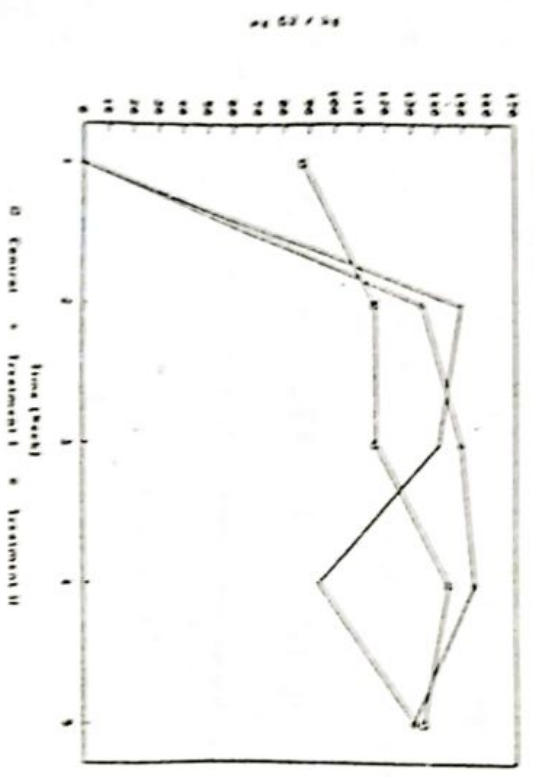


Fig. (1) Effect of monomethylamine, citric acid and aflagin on Aflatoxins production by *A. parasiticus*.

Influence of monomethylamine: Citric acid and.....

(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 B₂ production during the experiment, as described in Table (2) the reduction was increased with increasing the incubation period, (Fig. 2). But aflatoxin G₁ and G₂ 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 G₁ and also during the fourth week for aflatoxin G₂ as shown in Table (1). On the other hand, aflatoxin B₁ 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 aflatoxin production except aflatoxin B₂ 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 B₁ to other compounds (ii) to inhibit enzymes which process versicolorin A and averufin to sterigmatocystin and ultimately aflatoxin. (iii) or to conversion of aflatoxin B₁ to its hemiacetal by addition of water molecule to the vinyle ether double bond of the aflatoxin B₁ terminal ring in the acid⁽⁶⁾. And also acid treatment leads to hydration of aflatoxin B₁ at the 8,9- olefinic bond of the terminal furan ring to form aflatoxin B_{2a}; a similar reaction occurs' with aflatoxin G₁ 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 B₁ production much better than aflagin, while the highest reduction for aflatoxin B₂ 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.

Influence of Mohomethylamine: Citric acid and.....

6. Pohland, A.E., Cushmac, M.E. and Andrellos, P.J. (1968): "Aflatoxin B₁ hemiacetal". J. Ass. Off. Anal. Chem. 51: 907.¹
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* spores on aflatoxin production". Appl. Environ. Microbiol. 40: 989-993.