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ROLE OF TRACE ELEMENTS IN AFLATOXIN PRODUCTION BY ASPERGILLUS FLAVUS.

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SUMMARY

The treatment of *Aspergillus flavus* with Zn, Fe, Mn and Mo for the production of aflatoxin led to the following results: Both zinc and manganese are stimulatory for aflatoxins B_1 and B_2 at 50 and 100 mg/l.,while they are inhibitory at 200 mg/l. Ferrous is inhibitory for aflatoxin B_1 at all the concentrations used and is stimulatory for aflatoxin B_2 at 50 and 100 mg/l, where as it is inhibitory for aflatoxin B_2 at 200 mg/l. Molybdenum is inhibitory for aflatoxins B_1 and B_2 at all the concentrations used.

INTRODUCTION

It has been shown that trace elements, especially zinc, may influence quantitatively the production of aflatoxins, not only in synthetic media, but also on agricultural products such as corn, soybeans, coconuts, etc. (Davis *et al.*, 1965; Lee *et al.*, 1966; Lillehoj *et al.*, 1974; Maggon *et al.* 1973; Mateles and Adye 1965; Reddy *et al.* 1971 and Venkitasubramanian 1977).

Trace elements can exert an influence on the production of some of the known aflatoxin B_1 precursors (Bennett *et al.*, 1979).

There is conclusive evidence that zinc stimulates the biosynthesis of the aflatoxins (Venkitasubramanian 1977; Gupta *et al.*, 1976) but contradictory results have been reported for most of the other trace elements.

Considerable research has been directed at elucidating the aflatoxin biosynthetic pathway. Aflatoxins are acetate-derived decaketides in which the general steps of biosynthesis are acetate anthraquinones→xanthones→aflatoxin B_1 (Maggon *et al.*, 1977).

Many early workers investigated the ratio of different aflatoxins produced when physiological parameters were varied. Carbon source, nitrogen source, type of natural substrate, temperature and micro-nutrients were all found to be important parameters affecting the B:G ratio (Diener and Davis, 1969).

Taxonomnic surveys indicated that strains of *Aspergillus flavus* tend to produce only the B aflatoxins or be non-toxigenic while *A. Parasiticus* regularly produced the four major aflatoxins (Hesseltine *et al.*, 1970).

MATERIALS AND METHODS

Metals used:

The tested trace elements were used in the form of salts as zinc sulphate, ferrous sulphate, manganese chloride and ammonium molybdate.

Culture:

Aspergillus flavus was grown on Czapek's medium. This medium was divided into two parts. The first one is left without treatment and used as control. The second part was treated with the tested trace elements separately and each one at the three tested concentrations (50, 100 and 200 mg/l).

Growth determination

Growth was determined by mycelial dry weight, the mycelium was filtered off using dry, weighed filter paper, dried for 24 h at 80°C and weighed.

Assay of Aflatoxin:

The mycelium was moistened with 20 ml water and left to soften for 12 h. Maceration was achieved with a soniprobe after addition of 50 ml $CHCl_3$. The

sintered glass crucible used for filtration and drying was washed through with 200 ml $CHCl_3$ and the washing was added to the macerated solution. This solution was shaken for 1 h and filtered through phase - separation paper (Whatman 1 ps). Aflatoxin analysis was done directly on the filtrate by visual estimation on thin layer chromatographic plates, diluting or concentrating the solutions where necessary.

Merck silica gel 60 plates were used with chloroform : acetone (9:1) as mobile phase. Aflatoxins B_1 and B_2 (5 ug/ml) were used as standard, (Rabie *et al.*, 1981).

Estimation of aflatoxins:

The aflatoxins were eluted from silica gel with methanol. The amount of aflatoxin present was calculated by measuring the absorbance at 363 nm and using their extinction coefficient (Tyagi and Venkitasubramanian, 1981).

RESULTS

The results presented in (table 1) showed that both zinc sulphate and manganese chloride are stimulatory for aflatoxins (B_1 and B_2) yield at 50 and 100 mg/l and have an inhibitory effect on the mycelial dry weight. At 200 mg/l, both zinc sulphate and manganese chloride have an inhibitory effect on both mycelial dry weight and aflatoxins (B_1 and B_2) yield. Ferrous sulphate has an inhibitory effect on mycelial dry weight and aflatoxin B1 yield at all the concentrations used, while it has a stimulating effect on aflatoxin B2 yield at both 50 and 100 mg/l. However, it has an inhibitory effect on aflatoxin B2 yield at 200 mg/L. Malybdenum ion has no effect on mycelial dry weight at all the concentrations used, while it has an inhibitory effect on aflatoxins (B_1 and B_2) yield at all the concentrations used.

DISCUSSION

Aflatoxins are secondary metabolites of *Aspergillus flavus* and therefore, several physiological parameters such as, carbon and nitrogen supply and micronutrients especially zinc play an important role in their production.

From the results of the present study, it was shown that both zinc sulphate and manganese chloride have stimulatory effect on the production of aflatoxin at concentrations 50 and 100 mg/L, while molybdenum has an inhibitory effect on aflatoxin production. These results run parallel with that reported by Rabie et al. (1981), who found that both molybdenum and vanadium have inhibitory effect on aflatoxin production at 0.0012 mol, and with that reported by Lee et al. (1966) who found that the minimum concentratioon of zinc required for optimum growth and optimum aflatoxin production was 0.8 ppm.

The stimulatory effect of zinc on aflatoxin productioon may be due to its role in the glycolytic pathway that leads to the formation acetate-derived secondary metabolites, of which aflatoxins are a group. Zinc may play a role in the accumulation of phosphoenol pyruvate (PEP) and pyruvate (PYR). (both being precursoers of acetate and malonate). This result runs parallel with that indicated by Tyagi and Venkitasubramanian (1981).

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Table (1) : Effect of trace elements on growth and aflatoxin production by *Aspergillus flavus*.

Metalic salt added (mg/L)		Mycelial dry weight (g)	Aflatoxin B_1 yield (µg/g mycelium)	Aflatoxin B_2 yield (µg/g mycelium)
Non added (control)		0.98	21.5	18.4
$ZnSO_4.7H_2O$	50	0.96	30.2	24.0
	100	0.82	34.8	26.0
	200	0.58	12.4	9.6
$FeSO_4.7H_2O$	50	0.98	21.2	21.8
	100	0.92	16.1	25.2
	200	0.64	9.8	13.3
$MnCl_2.4H_2O$	50	0.98	38.2	20.8
	100	0.95	64.4	22.6
	200	0.84	14.2	11.2
$(NH_4)_6\ Mo_7O_{24}.4H_2O$				
	50	0.89	20.6	16.8
	100	0.96	15.4	13.4
	200	0.97	10.6	8.8