



RESEARCH ARTICLE

MICROBIOLOGY

VOLATILE COMPOUNDS IN THE MANAGEMENT OF ASPERGILLUS FLAVUS INFESTATION AND AFLATOXIN CONTAMINATION

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ABSTRACT

Efficacy of volatile compounds in the management of aflatoxin contamination and infestation caused by *A. flavus* was assessed *in vitro*. Among different volatile compounds studied formaldehyde, acetic acid propionic acid and salicylaldehyde inhibited the growth and aflatoxin production by *A. flavus*. Formic acid and benzaldehyde inhibited toxin production to maximum extent. From the above results it is concluded that volatile compounds can be employed in the management of aflatoxin production.



KEY WORDS

Aspergillus flavus, Aflatoxins, volatile compounds.

INTRODUCTION

Decontamination includes the prevention of mould growth, removal and inactivation of toxins. Several chemicals such as use of ammonia¹, fungicides², pesticides³, calcium hydroxide and formaldehyde⁴ are reported to be effective inhibitors of aflatoxin production. Mallik and Nandhi⁵ have recommended the use of volatile compounds in the control of mould infection of rice during storage. Girisham and Reddy⁶ have advocated the use of volatile compounds in the management of patulin production by *Aspergillus terreus*. In the present investigations, volatile compounds which are inexpensive and free from health hazards, are evaluated for the management of aflatoxin problem.

MATERIALS AND METHODS

Monosporic culture of *Aspergillus flavus* isolated from maize were grown in 50 ml of sterilized rice flour medium (rice flour, 40 g 100 ml; 5.5) contained in 250 ml Erlen mayer conical flasks containing 5 ml glass vial, hanged with the help of thread and incubated. One ml of different organic volatile compound was poured into glass vials aseptically. One ml

of water in glass vial in place of volatile compound served as control. At the end of 15 days incubation, the cultures were harvested on previously dried and weighed Whatmann No. 42 filter paper for determining fungal biomass by the dry weight method. The mycelium was repeatedly washed with sterile water to remove adhering rice flour particles as described earlier. The pH of culture filtrate was also recorded. Aflatoxin was extracted and estimated by the method suggested by Nabney and Nesbitt⁷.

Fifty ml of culture filtrate and 25 ml of chloroform were shaken thoroughly for 15 min. in a separating funnel. The organic phase was concentrated to dryness. To this 1 ml of methanol was added and 0.2 ml was placed on activated TLC and run in a solvent (chloroform : methanol; 97 : 3) mixture. The spot thus developed was eluted in 5 ml methanol. The intensity of fluorescence thus developed was read 360 nm with the help of spectrophotometer and the amount of aflatoxin produced was calculated by the following formula.

$$\text{Amount of aflatoxin (in } \mu\text{g/ml)} = \frac{D \times M \times 10^6}{E \times 400 \times 1000}$$

D = Optical density (at 365 nm)

M = Molecular weight of aflatoxin (Aflatoxin B, 312)

E = Molar extinction coefficient (22,000)

l = Path length (2 x 200)

RESULTS AND DISCUSSION

Table 1 reveals that vapours of formaldehyde, acetic acid, propionic acid and

salicylaldehyde caused total inhibition of aflatoxin production by *A. flavus*. Formic acid, benzaldehyde and acetic anhydride were also responsible for checking the



aflatoxin production to a significant level. Similarly formaldehyde, acetic acid and propionic acid were reported to be inhibitory to satrotoxin production by *Stachybotrys atra*⁸. Toluene, 1,4-dioxids, iso-amyl alcohol and 1-butanol were next in their efficacy in inhibiting

aflatoxin production by *A. flavus*. Ethyl acetate and acetonitrile failed to check the aflatoxin production. Rest of the compounds inhibited aflatoxin production to an intermediate level.

TABLE – 1
EFFECT OF VOLATILE COMPOUNDS ON GROWTH AND AFLATOXIN PRODUCTION BY A. FLAVUS

Name of the compound	Final pH	Dry weight (in mg/ml)	Aflatoxin (in ppb)
Propanol	7.1	12.68	50.71
Isoamyl alcohol	5.2	6.73	33.10
1-Butanol	5.6	10.67	36.02
2-Butanol	5.7	12.20	42.61
Ethyl alcohol	6.3	11.92	39.31
Chloroform	5.3	10.23	38.71
Formaldehyde	5.0	1.01	--
Dichloromethane	6.5	12.26	37.61
Benzaldehyde	4.3	2.70	6.20
Formic acid	4.1	1.26	4.52
Acetic acid	3.9	2.03	--
Propionic acid	3.5	1.19	--
Aniline	5.2	10.41	36.20
Ethylacetate	6.3	9.72	49.36
Acetone	7.2	13.16	45.21
Benzene	6.2	11.90	37.61
Acetic anhydride	4.2	1.60	3.13
1,4-dioxane	5.4	9.26	22.60
Toluene	5.3	10.12	21.17
Acetonitrile	6.2	11.21	44.62
Hexane	6.7	12.42	38.31
Salicylaldehyde	4.1	1.50	--
Control	6.5	13.62	55.11

Biomass production by *A. flavus* was inhibited significantly in the gaseous atmosphere of acetic acid, propionic acid, salicylaldehyde and formaldehyde. Other volatile compounds were moderate in their toxicity towards the growth of *A. flavus*. Acetone, propanol, dichloromethane and hexane were ineffective in checking the mycelial growth of *A. flavus*. With a few exoptions a positive correlation could be observed between mycelial growth and aflatoxin production.

From the present investigations it is clear that formaldehyde, acetic acid, propionic acid and salicylaldehyde can be exploited for checking *A. flavus* infestation and aflatoxin contamination of agricultural commodities.

ACKNOWLEDGEMENT

Thanks are due to Head, Department of Microbiology for providing necessary facilities. Financial assistance of ICMR



(S.No. 5/3/8/1/93 HR) is also gratefully acknowledged.

REFERENCES

1. E.B. Bagley. *J. Amer. Oil Chem.*, **56** : 808:811 (1979).
2. Ahmad Masood. Proceedings Sympium on mycotoxins incidence and human health (eds. K.S. Bilgrami, T. Prasad and K.K. Sinha) Allied Press, Bhagalpur, pp. 191:200 (1991).
3. F.A. Draughan and D.C. Chrurchville. *Phytopathology*, **175** : 553:556 (1985).
4. G. Piva, A. Pietri and E. Carini, 1985, *Zotec Nutri. Anim.*, **11** : 303:310 (1985).
5. A.K. Mallik and B. Nandi. *Seed. Sci. Tech.*, **10** : 315:320 (1982).
6. S. Girisham and S.M. Reddy. *J. Indian Bot. Soc.*, **68** : 42:43 (1985).
7. J. Nabney and B.F. Nesbitt. *Analyst*, **90** : 155:160 (1965).
8. G. Laxma Reddy and S.M. Reddy, *Indian J. Microbiol.* **32** :401:403 (1989).