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The Role of Volatile Substances and Food Preservatives in Controlling the Production of Fumonisins (B1) by Fusarium Moniliforme

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Abstract: ***Efficacy of volatile compounds and food preservatives in the management of fumonisins B_1 production by Fusarium moniliforme was assessed in vitro. The toxin production was analyzed by thin layer chromatographic technique. Among different volatile compounds studied, formic acid and propionic acid inhibited the growth and fumonisins (B1) production by F. moniliforme. Aniline, benzaldehyde, and formaldehyde can suppress fumonisis in B1 production to maximum extent. Camphorata concentration of 15mg/ml could inhibit fumonisins B_1 production among different food preservatives studied. From the above results it is concluded that volatile compounds and food preservatives can be employed in the management of fumonisins (B1).***

Keywords: Food preservatives, volatile compounds, Fusarium moniliforme, fumonisins

1. Introduction

Fumonisins are a group of structurally related mycotoxins produced by the fungus *Fusarium moniliforme* and several other *Fusarium* species (Nelson et al., 1991, 1992), which occur in corn and corn-based foods and feeds (Shephard et al., 1996a). Fumonisins, primarily fumonisin B_1 (FB_1) are hepatotoxic and nephrotoxic in many animal species. They have been shown to cause farm animal diseases such as, equine leukoencephalomalacia (ELEM) and procine pulmonary oedema (PPE) (Norred, 1993). Fumonisin B_1 has been shown to be a liver and kidney carcinogen in rodents (Gelderblom et al., 1996; NTP 1999). Although the role of fumonisins in human health is unclear, consumption of *F. moniliforme* contaminated corn has been correlated with human oesophageal cancer in areas of southern Africa and China (Marasas, 1996). Galvano et al. (2001) discussed elegantlythe strategies to be adopted to control fumonisins contamination of food grains. Fumonisins are generally concentrated on the pericorplayer of cornkernels (Sydenham et al., 1993). Sydenham et al. (1994) could reduce the fumonisins level very significantly by sorting out fine particles of less than 3 mm size. Sydenham et al. (1993) reduced the fumonisins level in corn by treating with 0.1m calcium hydroxide for 24 h at 25°C. Norred et al. (1991) could reduce the fumonisins level in corn by ammoniation. Detoxification of fumonisins in maize by ammonia could reduce to 30% (Chourasia, 2001). Bhat et al. (1997) recorded variation in fumonisins B_1 level in hybrids and hydroids of maize. Yamgish et al. (1997) could produce mutant of *F. proliferatum* which produced little or no fumonisins and can be used as a competitor for fumonisins producing strains and thus minimize fumonisins contamination. Beekrun et al. (2003) indicated that naturally occurring phenolics of plants such as chloro-phorin, iroka, benzoic acid, caffeic acid, ferulic acid and vanillic acid are effective inhibitors of fumonisins production by *F. verticilliodes.* Ono et al. (2002) postulated that the decreased seed moisture of freshly harvested maize through pre-drying of storage grains can control fungal growth and fumonisins contamination (Figure 1).

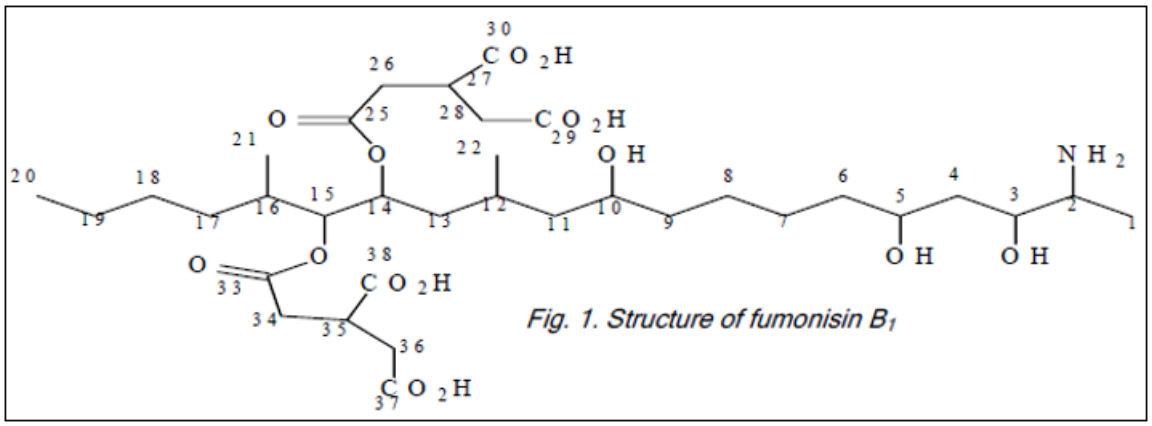

Fig. 1. Structure of fumonisin B_1

2. Materials and Methods

Chemicals and microorganism

Fumonisins (B1) was purchased from Sigma-Aldrich, Mumbai, India. Yeast extract, $NaNO_3$, KH_2PO_4, $MgSO_4.7H_2O$, KCl and all other chemicals of highest available purity were obtained from Himedia, Mumbai, India. F. moniliforme MRC 826 was purchased from Fusarium research center collection (Pennsylvania State University, University Park, Pa.). Stock culture was maintained on potatodex troseagarslants at 4°C and sub cultured for every 3 months

Influence of volatile compounds

50 ml of Czapeck's medium (Yeast extract 10 g; $NaNO_3$2 g, KH2PO41 g; $MgSO_4$.7H2O 0.5 g and distilled water 1000 ml) was taken in 250 ml Erlenmeyer conical flaks containing glass vial hanged from mouth of conical flask (5 ml capacity) and sterilized. Volatile compound viz. propanol, butanol-1, isoamyl alcohol, ethyl alcohol, chloroform, formaldehyde, benzaldehyde, formic acid, acetic acid, ethyl acetate, acetone, benzene, toluene, hexane, acetonitrile, kerosene, petrol, propionic acid, xylene and aniline, control are poured into glass vial separately after sterilization and flasks were inoculated with F. moniliforme and incubated at 26 ±2°C for 20 days.

Influence of food preservatives

Food preservatives viz boric acid, eucalyptus oil, camphor and naphthalene were added individually to 50 ml of sterilize dCzapeck's medium contained in 250 ml conical flasks to get 2.5,5.0, 10.0, 15.0 mg/ml and inoculated with *F. moniliforme*. All the flasks thus prepared were incubated at 26 ±2°C for 20 days.

Qualitative and quantitative analysis of fumonisinB1

At the end of incubation period cultures were harvested and the mycelial mat was dried and extracted with ethyl acetate in soxhelet apparatus for 24 h. Ethyl acetate was evaporated to near dryness and redissolved in methanol. A portion of methanol solution was spotted on TLC plates and developed in water: methanol (1:3) mixture. The plates thus developed were sprayed with p-anisaldehyde (0.2% in ethanol) and heated at 120°C until colour developed. Fumonisins appeared as brown colour spot. Fumonisin was estimated quantitatively as suggested by Sydenham (1989). The biomass attained by the fungus was estimated by drying the mycelium to complete dryness at 65-70°C for 48 h and weighed after cooling to room temperature.

3. Results and Discussion

Influence of different volatile compounds

Efficacy of different volatile compounds was studied on the growth and fumonisin B1 production by *F. monlilforme* and the results are presented inTable1. Propionic acid and formic acid were similarly; different volatile inhibited the growth of *F* aniline, formaldehyde and benzaldehyde were also effective as they were responsible for inhibition of fumonisins production by *F. moniliforme* to maximum extent. On the other hand, vapors of propane failed to inhibit the growth and fumonisins production. Acetic acid, acetone, butanol and benzene were also not much effective in the control of growth and fumonisins production by *F. moniliforme.* The rest of the volatile compounds were mild in their toxicity as they were responsible for decreased amount of fumonisins production.

Similarly, different volatiles inhibited the growth.

Table 1: Effect of volatile compounds on growth and fumonisins production by *F. moniliforme*

Name of the compound	Final pH	Dry weight (mg/ml)	Fumonisins B1(µg/ml)
Propanol	7.0	14.23	0.90
Butanol-1	5.8	13.89	0.64
Isoamyl alcohol	5.3	8.43	0.23
Ethylalcohol	6.8	10.49	0.33
Chloroform	5.4	1.23	0.34
Formaldehyde	5.2	2.09	0.15
Benzaldehyde	4.8	4.80	0.18
Formic acid	3.9	--	--
Acetic acid	3.4	0.18	0.72
Ethylacetate	6.6	8.42	0.28
Acetone	7.3	4.20	0.71
Benzene	6.1	12.01	0.63
Toluene	5.4	8.20	0.33
Hexane	6.8	11.80	0.31
Acetonitrile	6.3	10.18	0.30
Kerosene	5.4	7.14	0.21
Petrol	5.1	8.30	0.24
Propionicacid	3.4	--	--
Xylene	5.6	6.22	0.21
Aniline	5.8	5.81	0.19
Control	6.5	14.20	1.09

moniliforme to varying level. Acetic acid and chloroform were responsible for maximum inhibition of growth of *F. moniliforme* while, propanol, butanol, benzene, hexane, ethylalcohol and acetonitrile failed to inhibit the growth of *F. moniliforme.* The rest of the volatile compounds were responsible for intermediate degree of inhibition of growth of *F. moniliforme.* The present observations are in agreement with those of Surekha and Reddy (1992), Laxma and Reddy (1992) and Al-Hilli et al. (1992) who also recorded the control of mould infestation and mycotoxin contamination by the volatiles tested by them. The final pH recorded was highly variable. The final pH in media containing propanol, ethylalcohol, ethylacetate, acetone and hexane were near neutral, while medium containing propionic acid and acetic acid and formic acid was highly acidic.

Influence of food preservatives

Influence of different food preservatives were studied on the growth and fumonisins B1 production by *F. moniliforme* and the results were recorded in Table 2. From the Table 2 it is evident that different food preservatives exerted significant in fluence on fumonisins production by *F. moniliforme.* Boric acid at 2.5 mg/ml of concentration stimulated the fumonisins (B1) production, while higher concentrations it inhibits fumonisins (B1) production. The degree of inhibition increased with the increase in boric acid concentration. Eucalyptus oil was toxic and its degree of inhibition increased with the increase in eucalyptus oil concentration. Vegetative growth of the fungus, which was very good at 2.5

mg/ml concentration, decree sed with increase in concentration of eucalyptus oil up to 15 mg/ml. Similarly, camphor inhibited the fumonisins (B1) production by *F. moniliforme* at 15 mg/ml. Naphthalene was also responsible for increased inhibition of fumonisins production and vegetative growth of *F. moniliforme* with increased concentration.

4. Conclusion

Based on the above results, it can be concluded that food preservatives tried in the present investigations were effective in checking *F. moniliforme* infestation and fumonisins (B1) production and can be exploited in protection of food grains from infestation of *F. moniliforme* and fumonisins contamination.

Table 2: Effect of some food preservatives on growth and fumonisins production by *F. moniliforme.*

Compound	Conc. (mg/ml)	Dry weight (mg/ml)	Fumonisins B_1(µg/ml)
Boricacid	2.5	18.3	1.00
	5.0	15.6	0.74
	10.0	9.8	0.65
	15.0	4.3	0.43
	2.5	17.6	0.73
Eucalyptus oil	5.0	16.4	0.67
	10.0	8.0	0.42
	15.0	6.3	0.32
	2.5	8.6	0.66
Camphor	5.0	7.2	0.46
	10.0	4.8	0.41
	15.0	2.9	0.10
	2.5	13.4	0.72
Napthalene	5.0	12.8	0.71
	10.0	8.3	0.40
	15.0	4.3	0.42
Control	--	14.9	0.91

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