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INHIBITORY EFFECT OF BLACK PEPPER ON GROWTH AND TOXIN PRODUCTION OF TOXIGENIC FUNGI

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The inhibitory effect of black pepper on the growth and toxin production by some common toxigenic fungi were observed. Of 14 different fungal species screened against black pepper for the efficacy in the control of mycotoxin production. Satratoxin D, Ochratoxin A and aflatoxin B_1 production were totally inhibited. Roridin and patulin production was also inbibited to the extent of 95%. The biomass of *Stachybotrys atra* was completely inbibited.

Key Words : Mycotoxin, biomass, black pepper, toxigenic fungi.

Spices, condiments which are ingredient of daily food are of great value in view of their varied biological activity. Spices are reported to inhibit the growth of some mycotoxigenic fungi (Bullerman *et al.*, 1977, Hitokto *et al.*, 1980). Scott and Kenney (1973), Hitokoto *et al.* (1977) and Mabrouk and El-Shayeb (1980) have also reported inhibition of fungal growth by black pepper seed extracts. Black pepper seed which is reported to be of great importance to flavour all kinds of savoury foods may have utility in checking the mould growth and mycotoxin contamination. Hence the present studies were aimed to study the effect of black pepper seed on the growth and mycotoxin production by some mycotoxigenic fungal strains isolated from different spices and dry fruits as part of a search for spices with antifungal nature.

MATERIALS AND METHODS

Black pepper seed powder was added to 50 ml of YES medium (for *Penicillium* sp.), SMKY medium (for *A. ochraceus*), rice flour medium (for *A. flavus*), minimal medium (for *A. terreus*), Raulin and Thom medium (for *A. ustus*) and Czapeks medium (for trichothecene mycotoxins producting fungi and patulin strain of *A. terreus*) contained in a 250 ml Erlenmayer conical flasks so as to get a final concentration of 5, 10, 15 and 20 mg/ml of black pepper before sterilization. Monosporic cultures of 7 days old *A. flavus* (isolated from *Brassica nigra* seeds), *A. fumigatus* and *A. ochraceus* (isolated from *Cocos nucifera*, copra), *A. terreus* (from rhizome of *Zinziber officinale*) and terreic acid (from corm of *Curcuma longa*), *A. ustus* (from *Prunus amygdalus, Batsch* seeds), *Fusarium moniliforme* (isolated from *Anacardium occidentalae* nuts), *Myrothecium roridum* (from cattle feeds), *Penicillium citrinum* (from seeds of *Brassica nigra*), *Penicillium crustosum* and *P. aurantiogriseum* (isolated from *Piper nigrum*), *P. purpurogenum* (from nuts of *Areca catechu*) *Stachybotrys atra* (from *cremocarps of Carum carvi*) and *Trichoderma viride* (from bulbs of *Alliumcepa*) cultured on PDA and CYA slants (Samson and Pitt, 1985) were inoculated to the respective media. Medium without black pepper seed powder served as controls. The flasks thus inoculated were incubated at 28±1°C for 10 days for *A. flavus*, 15 days for other species of *Aspergillus* and *Penicillium* and 25 days for trichothecene mycotoxins producing fungi. The fungal biomass was measured as described by Madhyastha and Bhat (1984). Different mycotoxins were extracted from culture filtrates by employing suitable solvents and analysed qualitatively by TLC (Scott *et al.*, 1970; Durackova *et al.*, 1976; Ueno, 1983; Rao *et al.*, 1985; Jarvis *et al.*, 1986) and quantitatively by colorimetric method (Hou *et al.*, 1970; Damodaran *et al.*, 1973; Subramaniyan *et al.*, 1978; Subramaniyan 1982 and Reddy and Girisham, 1993).

RESULTS

Perusal of Table 1 reveals that black pepper seed exhibited fungitoxicity on the growth and mycotoxin production by fungi under study. However, the degree of inhibition varied with the fungus. Aflatoxin B_1 production was totally inhibited at higher (10 mg/ml) concentration. However, it has low inhibitory effect on mycelial growth of *A. flavus* as there was only marginal decrease in biomass. The same was

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Table 1: Effect of Black Pepper seed (*P. nigrum*) on mycotoxin production by some mycotoxigenic fungi.

Name of the fungus	Conc. mg/ml Mycotoxin	Control			5.0			10.0			15.0			20.0		
		pH	DW (mg/ml)	Toxin[a] (µg/ml)	pH	DW (mg/ml)	Toxin[a] (µg/ml)	pH	DW (mg/ml)	Toxin[a] (µg/ml)	pH	DW (mg/ml)	Toxin[a] (µg/ml)	pH	DW (mg/ml)	Toxin[a] (µg/ml)
Aspergillus flavus	Aflatoxin B_1	6.0	11.8	+4	6.0	11.4	+1	6.0	11.0	trace	6.0	10.8	-	6.0	10.4	-
A. fumigatus	Gliotoxin	2.0	11.2	+	2.0	11.2	+	2.5	10.7	+	3.0	7.0	+	3.0	4.9	trace
A. terreus	Terreic acid*	6.0	18.4	13.4	4.0	13.6	9.2	3.5	11.6	8.5	3.5	10.2	8.0	3.5	9.6	7.7
A. terreus	Patulin*	7.0	15.6	12.0	7.0	14.1	8.2	7.0	12.5	6.4	7.0	11.1	5.3	7.0	10.9	4.1
A. ochraceus	Ochratoxin A	7.5	9.5	+2	7.5	6.9	+	7.5	7.0	+	7.5	6.7	+	7.5	6.4	trace
A. ustus	Ustic acid	5.5	18.4	+	6.0	16.8	+	6.0	1.4	+	6.0	12.0	+	6.0	11.1	+
Penicillium citrinum	Citrinin	7.5	13.6	38.0	7.5	15.8	50.0	7.5	16.5	62.5	7.5	18.0	67.0	7.5	19.9	76.0
P. purpurogenum	Secalonic acid D	7.5	12.3	+	7.0	9.5	+	7.0	8.1	+	7.0	6.2	+	7.0	6.0	+
P. aurantiogriseum	Penitrem B	7.5	11.0	9.6	7.0	11.2	10.5	7.0	11.8	12.8	7.0	12.4	16.0	7.0	12.8	19.2
P. crustosum	Penitrem A	7.5	11.4	11.7	7.0	11.6	12.0	7.0	11.8	13.7	7.0	12.0	14.4	7.0	12.2	15.0
Fusarium moniliforme	Zearelenone	7.0	9.2	+	7.0	8.9	+	7.0	8.0	+	7.0	7.8	+	7.0	7.0	+
Stachybotrys atra	Satratoxin	8.5	8.4	160	6.0	8.1	70.0	5.0	0.2	-	-	-	-	-	-	-
Myrothecium roridum	Roridin	8.5	11.1	152	6.0	10.2	140	5.5	9.7	93.0	5.5	9.1	17.5	5.5	8.0	6.0
Trichoderma viride	Trichodermin	7.5	5.8	16.0	7.5	5.7	10.0	7.5	5.3	7.5	7.5	2.9	4.5	7.5	1.4	-

[a] An average of two colorimetric analyses which the results (mycotoxins) agreed within ±1%.
+ The intensity of mycotoxins when compared with standard toxins (TLC)
* 0.01 O.D. = 10 ppb.

true with *A. ochraceus* for ochratoxin A production at 20 mg/ml concentration. Similarly the black pepper seed powder inhibited the production of gliotoxin by 56%, terreic acid by 43% and patulin by 66% at higher concentrations tried (Table 1). The biomass production was partially inhibited and inhibition was slight progressive with the increase in concentration of black pepper. *A. ustus* was least affected by the presence of black pepper, both for biomass and ustic acid production. Interestingly citrinin and penitrem A & B production by respective fungi was stimulated in the presence of black pepper, which increased with the increase in concentration of black pepper. The same was true with biomass of these fungi. The trichothecences producing fungi showed varied responses to the black pepper. Satratoxin D production by *S. atra* was completely inhibited by the black pepper even at 19 mg/ml concentration while trichodermin and roridin were inhibited by 100% and 95% respectively at 20 mg/ml concentration. Similarly mycelial growth was inhibited to a significant level. The rest of the fungi were marginally affected by black pepper seed.

DISCUSSION

From the present studies it is clear that the black pepper seed is fungitoxic to different mycotoxigenic fungi. However, species of *Penicillium* under study were stimulated by the presence of black pepper. The inhibition of aflatoxin B_1 and ochratoxin A production was well supported by some earlier reports (Hitokoto *et al.*, 1977; Mabrouk and El Shayeb, 1980). Hitokoto *et al.* (1978) also reported that some herbal drugs and commercial dry condiments to be antifungal and inhibited the growth and toxin production by some toxigenic fungi. Scott and Kenney (1973) pointed out that *Piper nigrum* prevented aflatoxin production by *A. flavus*. Fungitoxicity of black pepper may be due to pungant principle, pepper oil and piperine which readily diffuses into the liquid medium (Madhyastha and Bhat, 1984).

Present studies reveal that the black pepper seed may be useful in the management of infestation of trichothecene producing fungi and toxigenic *Aspergillus* sp. and mycotoxin contamination. Present observations are interesting as in many foods black pepper along with other spices like cloves and cinnamon are added may serve as possible alternative to the food additives and food preservatives in use at present. However, more detailed studies are desirable about their feasibility and safety.

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