J. stored Prod. Res. Vol. 23, No. 3, pp. 149–150, 1987
Printed in Great Britain.

0022-474X/87 $3.00 + 0.00

INTERFERENCE IN TOXIN PRODUCTION AMONG TOXIGENIC *ASPERGILLUS* SPECIES

G. Rama Devi and H. Polasa*
Department of Microbiology, Osmania University, Hyderabad-7 (A.P.), India

(*Received* 18 *March* 1987)

Abstract—Interference in toxin production among three toxigenic *Aspergilli* was found to exist when they were grown together on liquid medium or maize. When *Aspergillus flavus* was grown in combination either with *A. ochraceus* or with *A. versicolor*, only aflatoxin B_1 was produced, but not ochratoxin A or sterigmatocystin. Similarly, only ochratoxin A was produced when *A. ochraceus* and *A. versicolor* were grown together. Growth of all three of the above fungi together yielded only aflatoxin B_1. Increasing the inoculum size of a particular species did not encourage the production of more than one toxin in mixed cultures, suggesting interference in toxin production of one *Aspergillus* species by another.

INTRODUCTION

A regional survey of maize samples for fungal and mycotoxin contamination under field and storage conditions showed considerable variation in the occurrence of toxigenic fungal species and their mycotoxins (Rama Devi and Polasa, 1982). Although some of the maize samples were found infected with more than one toxigenic species of *Aspergillus*, viz: *A. flavus*, *A. ochraceus*, and *A. versicolor*, only one toxin (aflatoxin, ochratoxin, or sterigmatocystin) was detected in such samples, suggesting some sort of interference in toxin production among the *Aspergilli* (Rama Devi and Polasa, 1982). This phenomenon was studied under laboratory conditions to determine whether interference or antagonism among toxigenic fungi exists with reference to toxin production. The following experiments were carried out.

MATERIALS AND METHODS

Cultures of *Aspergillus flavus*, *A. ochraceus*, and *A. versicolor* were grown individually and in combinations of two or three species in liquid Czapek Dox medium and on maize, as shown in Table 1. In individual cultures, one 100 ml batch of liquid medium and a 50 g lot of sterilized maize (20% moisture content) were inoculated with 10^5 conidia of each particular species, and in mixed cultures of two or three species, 10^5 conidia of each species was used as the inoculum. After the required period of incubation (Table 1) at $28 \pm 1°C$, the toxins were extracted from the culture filtrates and maize (Roberts and Patterson, 1975). Aflatoxin, sterigmatocystin, and ochratoxin were identified and quantified using the thin layer chromatography methods of Seitz and Mohr (1977).

RESULTS

It was observed that when *Aspergillus flavus* was grown either with *A. ochraceus* or *A. versicolor* in liquid media or on maize, only aflatoxin B_1 was produced. No ochratoxin A or sterigmatocystin was detected after incubation for 10 or 14 days (Table 1). Similarly when *A. ochraceus* and *A. versicolor* were grown together, only ochratoxin A was detected on both the substrates after 14 days incubation. Further, only aflatoxin B_1 was produced when all three species of *Aspergillus* were grown together on either substrate after 14 days incubation.

The absence of toxin production by one of the fungi in mixed cultures was not due to the lack of adequate growth of that particular species as visual observation indicated considerable growth of each species in all the mixed cultures. Lack of adequate growth as a factor in producing the results obtained was ruled out by conducting experiments under similar conditions, in which the

*Author for correspondence.

Table 1. Antagonism in toxin production among three *Aspergillus* species[a]

Fungi grown in combination	Incubation period (days)	Toxin produced[b]					
		Aflatoxin B_1		Ochratoxin A		Sterigmatocystin	
		Liquid medium	Maize	Liquid medium	Maize	Liquid medium	Maize
A. flavus (Control)	8	174	47	0	0	0	0
A. ochraceus (Control)	10	0	0	100	40	0	0
A. versicolor (Control)	14	0	0	0	0	148	60
A. flavus & *A. ochraceus*	10	148	42	ND	ND	0	0
A. flavus & *A. versicolor*	14	148	42	0	0	ND	ND
A. ochraceus & *A. versicolor*	14	0	0	82	25	ND	ND
A. flavus, *A. ochraceus*, and *A. versicolor*	14	120	32	ND	ND	ND	ND
A. flavus & *A. ochraceus* [(1:5) × 10^5]	10	120	42	ND	25	0	0
A. flavus & *A. versicolor* [(1:5) × 10^5]	14	128	37	0	0	ND	ND
A. ochraceus & *A. versicolor* [(1:5) × 10^5]	14	0	0	82	25	ND	ND

[a]Inoculum size for each fungus in each experiment was 10^5 conidia except as indicated in the final three tests where the inoculum size for the second component was increased 5 times.
[b]Toxin production expressed in μg/100 ml liquid medium and 50 g maize.
ND: not detected.

spore inoculum of one of the species which did not produce toxin in mixed culture was increased five fold (Table 1). In several repeated experiments, a small quantity of ochratoxin A was detected in only one case in maize when the spore inoculum was increased. In the control experiments, in which the species were cultured individually, toxin was produced in all cases.

DISCUSSION

The results presented here indicated that in mixed culture *Aspergillus flavus* produced aflatoxin B_1 while suppressing the synthesis of ochratoxin A and sterigmatocystin by *A. ochraceus* and *A. versicolor* respectively. When grown with *A. versicolor*, *A. ochraceus* produced ochratoxin A and suppressed the synthesis of sterigmatocystin. Clearly an interference or antagonism existed among these toxigenic *Aspergillus* species in the production of toxins. Wicklow *et al.* (1980) also observed the phenomenon of interference in aflatoxin production in mixed cultures of *A. flavus*, *A. niger*, *A. candidus*, and *A. chevalieri*.

The mechanism of this phenomenon is not known. We suggest that when the fungi are grown together one species may be actively competing for nutrients essential to the other species for the production of toxin. Thus, it appears that microbial interaction is an important environmental factor in the production of fungal metabolites.

Acknowledgements—The authors thank Dr Michael E. Stack, Food and Drug Administration, Washington, D.C., U.S.A., for providing toxin standards.

REFERENCES

Rama Devi G. and Polasa H. (1982) Mycotoxins from fungi on maize. *Curr. Sci., Mysore* **51,** 571.
Roberts B. A. and Patterson D. S. P. (1975) Detection of twelve mycotoxins in mixed animal feed stuffs using a novel membrane cleanup procedure. *J. Assoc. Off. Anal. Chem.* **58,** 1178–1182.
Seitz L. M. and Mohr H. E. (1977) A new method for quantitation of aflatoxin in corn. *Cereal Chem.* **54,** 179–183.
Wicklow D. T., Heseltine C. W., Shotwell O. L. and Adams G. L. (1980) Interference competition and aflatoxin levels in corn. *Phytopathology* **17,** 761–764.