INTERFERENCE IN TOXIN PRODUCTION AMONG
TOXIGENIC ASPERGILLUS SPECIES

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Abstract—Interference in toxin production among three toxigenic Aspergillus 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 B1 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 B1. 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 Aspergillus (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 10⁴ conidia of each particular species, and in mixed
cultures of two or three species, 10⁵ conidia of each species was used as the inoculum. After the
required period of incubation (Table 1) at 28 ± 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 Setz 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 B1 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 B1 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

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

<table>
<thead>
<tr>
<th>Fungi grown in combination</th>
<th>Aflatoxin B1</th>
<th>Ochratoxin A</th>
<th>Sterigmatocystin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liquid medium</td>
<td>Maize</td>
<td>Liquid medium</td>
</tr>
<tr>
<td>A. flavus (Control)</td>
<td>8</td>
<td>174</td>
<td>47</td>
</tr>
<tr>
<td>A. ochraceus (Control)</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A. versicolor (Control)</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A. flavus &amp; A. ochraceus</td>
<td>10</td>
<td>148</td>
<td>42</td>
</tr>
<tr>
<td>A. flavus &amp; A. versicolor</td>
<td>14</td>
<td>148</td>
<td>42</td>
</tr>
<tr>
<td>A. ochraceus &amp; A. versicolor</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A. flavus, A. ochraceus, and A. versicolor</td>
<td>14</td>
<td>120</td>
<td>32</td>
</tr>
<tr>
<td>A. flavus &amp; A. ochraceus (1.5 x 10^6)</td>
<td>10</td>
<td>120</td>
<td>42</td>
</tr>
<tr>
<td>A. flavus &amp; A. versicolor (1.5 x 10^6)</td>
<td>14</td>
<td>128</td>
<td>37</td>
</tr>
<tr>
<td>A. ochraceus &amp; A. versicolor (1.5 x 10^6)</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*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.

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 B1, 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.

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**REFERENCES**


