Synergism of aflatoxin B₁ toxicity with the co-occurring fungal metabolite kojic acid to two caterpillars

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Introduction

Kojic acid is a secondary fungal metabolite that is commonly produced by many species of Aspergillus and Penicillium (Manabe et al., 1984). These fungi also produce mycotoxins that occur as components of distinctive secondary metabolite profiles, which can serve as reliable chemotaxonomic characters at the species level (Dorner, 1983; Frisvad & Filtenborg, 1983; Wicklow, 1984a). The mycotoxin aflatoxin B₁, which is produced by Aspergillus flavus Link: Fr., is highly toxic (LD₅₀ of 7.2 mg/kg to rats), mutagenic, and carcinogenic (Cole & Cox, 1981). However, kojic acid, which is also produced by A. flavus, is relatively nontoxic (LD₅₀ of 1765 mg/kg to mice (Cole & Cox, 1981), and has not been implicated in any naturally occurring toxicoses to man or animals (Wilson, 1981). In fact, kojic acid is produced by most of the strains of the domesticated kojic mold, A. oryzae (Ahlburg) Cohn [=Aspergillus flavus var. oryzae (Kurtzman et al., 1987)] that are used in the preparation of Oriental fermented foods (Murakami, 1971).

Mycotoxins are considered to be of adaptive significance in the defense of fungal thalli from predation (Wicklow & Cole, 1982), or in preventing mammals or birds from consuming seeds infected by the fungi (Janzén, 1977). Invertebrates (i.e. insects) are a likely target for these mycotoxins, since they consume a variety of products that are also infested by mycotoxin-producing fungi (Wicklow, 1984b). For example, A. flavus may colonize the developing maize kernels that may also be fed on by the fall armyworm [Spodoptera frugiperda (J. E. Smith)] and the corn earworm [Heliothis zea (Boddie)] (Fortnum, 1985).

In higher plants, chemicals that are of low toxicity and co-occur with plant toxins may act as synergists of these toxins (e.g. myristicin and xanthotoxin), although actual demonstration of this phenomenon has been limited (Berenbaum, 1985). Co-occurring mycotoxins may also cause additive or synergistic toxicity to mammals (Ciegler, 1972). Kojic acid has been reported as a synergist of the plant toxin nicotine (Mayer et al., 1946). However, its native or ecological role when produced by A. flavus may be to act as a synergist of the co-occurring aflatoxins. This research investigates the toxicity of aflatoxin B₁ and kojic acid, alone and in combination, to S. frugiperda and H. zea.

Material and methods

Aflatoxin B₁ and kojic acid were obtained from Sigma Chemical Co. All other chemicals were reagent grade. Aflatoxin B₁ and kojic acid were added to the pinto bean-based diets (Dowd, 1987) of the insects at levels at which they may be found in naturally infested corn. These levels were 25 ppm for kojic acid (Wilson, 1971), and either 2.5 or 0.25 ppm for aflatoxin B₁ (Shotwell & Hesseltine, 1981). The
chemicals were incorporated by keeping 5 ml aliquots of the mixed diet at 60 °C (to prevent premature solidification of the agar-based diet), adding the chemicals in 125 μl of either acetone (aflatoxin B₁) or water (kojic acid), and blending with a vortex mixer at high speed for 20 s. Preliminary observations with water or acetone-soluble colored chemicals indicated uniform blending after this period of time. The blended diets were dispensed into petri plates and allowed to cool. The plates containing the blended diets were placed in a fume hood until slight darkening occurred (ca. 10 min) to remove the potentially toxic solvent (acetone). The blended diets were sectioned into 20 pieces, and placed individually into the wells of 24-well immunoassay plates. Neonate larvae, continuously reared from insects originally obtained from the University of Illionois, Urbana (H. zea) or USDA-ARS, NRRC (S. frugiperda), were individually placed with a section of diet. To prevent drying of the diet, a sheet of parafilm was placed over the top of the plate, followed by a sheet of cardboard, and the lid was held in place by rubber bands. The plates were then placed in two polyethylene bags, held closed by rubber bands. Two groups of 20 insects were used for duplicate tests of each chemical concentration/mixture. Controls consisted of solvent blanks. The insects were held at 27 ± 1°C, 40 ± 10% r.h., and at L14:D10 photoperiod. The mortality was examined after 2, 4, and 7 days, and weights of the larvae were recorded after 7 days.

Results and discussion
Since no change in mortality occurred after 48 h, only one set of values is reported. At 0.25 ppm, the aflatoxin caused 10% mortality of S. frugiperda, while at 2.5 ppm the mortality was approximately 4.6x greater (Table 1). Kojic acid at 25 ppm had essentially no adverse effect. However, in the presence of kojic acid, the mortality due to aflatoxin B₁ at 0.25 ppm increased to approximately the same level as that seen with aflatoxin B₁ alone at 2.5 ppm. Weights of larvae fed on the diet with both chemicals were somewhat less than the weights of larvae fed on diet containing aflatoxin B₁ at 2.5 ppm alone.

The mortality caused by all chemicals, alone and in combination, to H. zea was less than 10% (Table 1); much lower than the values found for S. frugiperda. Aflatoxin B₁ at 0.25 ppm caused a weight reduction in developing H. zea of ca. 3 x; and at 2.5 ppm, a weight reduction of ca. 20 x. Kojic acid at 25 ppm had essentially no effect on the larvae. However, when the kojic acid was combined with the aflatoxin B₁ at 0.25 ppm, the combination caused approximately the same reduction in weight as the aflatoxin alone at 10 x higher concentration. Thus, kojic acid synergized the toxicity of aflatoxin B₁ in both insect species.

Aflatoxin B₁ has previously been shown to be toxic to second and fifth instar larvae of both S. frugiperda and H. zea (McMillian et al., 1980).

Table 1. Toxicity of aflatoxin B₁ and kojic acid to Spodoptera frugiperda and Heliothis zea.

<table>
<thead>
<tr>
<th>Compound</th>
<th>S. frugiperda</th>
<th></th>
<th>H. zea</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mortality</td>
<td>7 day wt.</td>
<td>Mortality</td>
<td>7 day wt.</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>(mg)</td>
<td>(%)</td>
<td>(mg)</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
<td>43.6±2.9</td>
<td>0.0</td>
<td>37.5±1.5</td>
</tr>
<tr>
<td>Kojic acid (25 ppm)</td>
<td>0.0</td>
<td>55.8±4.7</td>
<td>0.0</td>
<td>43.9±2.5</td>
</tr>
<tr>
<td>Aflatoxin B₁ (0.25 ppm)</td>
<td>10.0</td>
<td>45.2±3.8</td>
<td>5.0</td>
<td>12.2±1.2</td>
</tr>
<tr>
<td>Aflatoxin B₁ (2.5 ppm)</td>
<td>45.9</td>
<td>36.6±3.5</td>
<td>7.5</td>
<td>2.2±0.2</td>
</tr>
<tr>
<td>Aflatoxin B₁ (0.25 ppm) + Kojic acid (25 ppm)</td>
<td>50.0*</td>
<td>24.1±3.3*</td>
<td>2.5</td>
<td>2.1±0.2*</td>
</tr>
</tbody>
</table>

Mortality values for two sets of 20 insects each: weights means ± s.e. for survivors of mortality tests. Control values: combinations of both water and acetone results. Mortality values followed by asterisks differ significantly at p < 0.05 from predicted additive values when analyzed by log-likelihood ratio tests with Yate’s correction for small sample size. Weights followed by asterisks: significant interaction between the toxin and kojic acid at p < 0.05 when analyzed by factorial analysis. Some larvae escaped; mortality values adjusted accordingly.
Aflatoxin was more toxic at lower concentrations to larvae in the present study than in the earlier study, perhaps because of their smaller size. Aflatoxin B$_1$ had a greater effect on the weight of H. zea than S. frugiperda, both in the present and in the earlier study (McMillian et al., 1980). However, the similar level of mortality for both insect species seen earlier (McMillian et al., 1980) was not demonstrated in the present study. The differences in mortality due to aflatoxin B$_1$ in the two studies may be due to the difference in the method used to incorporate the toxin into the diet. In the present study, the aflatoxin B$_1$ was uniformly incorporated into the diet, while the toxin was only applied to the diet surface in the earlier study (McMillian et al., 1980).

Kojic acid synergized the toxicity of nicotine in insects such as melonworm [Diaphania hyalinata (L.)], southern armyworm [Spodoptera eridania (Cramer)], and bean leafroller [Urbanus proteus (L.)] (Mayer et al., 1946). Nicotine is metabolized by oxidative enzymes in a variety of insects [Dowd et al., 1983 (review)]. Thus, kojic acid may be inhibiting the oxidative enzymes that could detoxify nicotine, or in case of the present study, aflatoxin B$_1$. Kojic acid could also enhance the effects of aflatoxin in mammals. Regardless of its mode of action, this study demonstrates that fungi, like higher plants, can produce secondary metabolites that synergize coexisting toxins.

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References


