

Combined effects of aflatoxin B₁ and citrinin on maize seedlings

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Abstract

Effect of two important mycotoxins, aflatoxin B₁ and citrinin (concentration 2 g m⁻³) at various combinations (*i.e.*, 1:1, 1:2, 2:1, 1:3 and 3:1, v/v) on seed germination, seedling growth, chlorophyll, carotenoid, starch, sugar, protein and nucleic acid contents, α -amylase activity, and respiration quotient was studied in maize cv. Suwan composite. The maximum and minimum inhibitions were recorded in most of the above parameters (except starch) at 3:1 and 1:3 combination ratios of these toxins, respectively. However, the inhibition rates varied with the treatments.

Additional key words: α -amylase, carotenoids, chlorophyll, DNA, RNA, starch, sugars, *Zea mays*.

Introduction

Aflatoxin B₁ and citrinin are common mycotoxins produced by *Aspergillus flavus* Link ex Fries and *Penicillium citrinum* Thom., respectively. In nature *A. flavus* and other fungi co-exist and share individual substrates, like maize kernel (Bilgrami and Choudhary 1990, Sinha 1990). Wicklow and Hesseltine (1979) also found the co-existence of *A. flavus* with other microbial inhabitants which determined the production of aflatoxins in the maize kernels. Different fungi may influence aflatoxin production by *A. flavus* group of fungi (Wicklow *et al.* 1987, Choudhary 1992).

Individually, aflatoxin B₁ and citrinin inhibit important physiological and biochemical processes of maize seeds (Prasad *et al.* 1994, Sinha and Prasad 1997, Prasad 1995). Since these mycotoxins co-exist in nature, the possibility of the combined effects can not be ruled out. Synergistic effect of these toxins on animal systems is known (Kanisama 1984, Hoerr *et al.* 1981) but there is no such report available for plant system. An attempt has, therefore, been made in the present investigation to record the combined effect of aflatoxin B₁ and citrinin in various combinations on physiological and biochemical processes in maize seedlings.

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Materials and methods

Seeds of *Zea mays* L. cv. Suwan composite were obtained from the Cereal Division, Rejendra Agricultural University, Sabour Campus, India. Stock solutions of aflatoxin B₁ and citrinin (*Sigma*, St. Louis, USA) were initially prepared each in 1 cm³ ethanol from which the dilutions (2 g m⁻³) were made in sterilized distilled water. Solutions of these toxins were mixed in different combination ratios, like 1:1, 1:2, 2:1, 1:3, 3:1 (v/v) in order to record their combined effects. The seeds were steeped initially in distilled water for 1 h and subsequently in different combinations of these toxins for 20 h. For each treatment 100 seeds were taken in triplicate. The steeped seeds were subsequently placed on moist blotting paper and kept in a seed germinator at 28 ± 2° C.

Seed germination index (GI) was calculated after 5 d by the following formula:

$$GI = \frac{\text{number of germinated seeds}}{\text{number of seeds observed}} \times 100$$

Seedling growth was recorded after 7 d by measuring the lengths of radical and plumule. Chlorophyll (Chl) *a* and *b* and carotenoid (Car) contents of the newly emerged seedlings were estimated following the methods of Arnon (1949) and Davis (1976), respectively. The quantitative estimations of starch and total sugars, were done by the methods of Dubois *et al.* (1956) and Snell *et al.* (1961), respectively. Reducing sugar was determined by Nelson-Somogy's method (Plummer 1971) and the amount of non-reducing sugars was calculated by subtracting the value of reducing sugars from total sugars.

The α -amylase activity was estimated by the method of Bernfeld (1955) and was expressed as the amount of reducing sugars released per kg⁻¹(f.m.) of the tissue. Quantitative estimation of the protein of the seed samples was done by the method of Lowry *et al.* (1951), the qualitative analysis of proteins was done by disc electrophoresis (Ornstein and Davis 1964). Gels were scanned by *LKB Ultrascan-XL-Enhanced Laser Densitometer* (*LKB*, Sweden). The nucleic acid contents of the control and treated seeds were estimated by the method of Gottlieb and Tripathi (1968). Respiratory quotients (R.Q.) of germinating seeds was estimated by Warburg respirometer.

The values were subjected to one way analysis of variance. The least significant differences at 5 % confidence levels (LSD₀₅) were subsequently determined.

Results

Combined treatments with aflatoxin B₁ and citrinin inhibited seed germination, shoot and root lengths and chlorophyll, carotenoid, protein and nucleic acid contents: maximum inhibition was at aflatoxin B₁:citrinin ratio 3:1, minimum at 1:3 (Tables 1, 2 and 5). There was a change in saccharide contents: all combinations of mycotoxin increased starch content, but lowered contents of both reducing and non-reducing sugars (Table 3); extreme differences were again at mycotoxin ratios 1:3 and 3:1. Mycotoxin application increased the activity of α -amylase (Table 4).

Table 1. Effects of aflatoxin B₁ and citrinin in different combinations (0:0, 1:1, 1:2, 2:1, 1:3 and 3:1; v/v) on germination index, GI, of maize seeds and root and shoot length of 7-d-old maize seedlings. Means \pm S.E.; $n = 3$. The differences from control were significant at 5 % level.

	0:0	1:1	1:2	2:1	1:3	3:1
GI	95.0 \pm 1.3	42.0 \pm 2.8	52.0 \pm 2.9	31.0 \pm 2.7	76.0 \pm 2.5	27.0 \pm 2.6
Root length [cm]	5.62 \pm 0.07	3.42 \pm 0.05	3.93 \pm 0.04	2.94 \pm 0.03	4.75 \pm 0.02	2.31 \pm 0.05
Shoot length [cm]	1.70 \pm 0.06	1.00 \pm 0.02	1.08 \pm 0.04	0.88 \pm 0.03	1.20 \pm 0.05	0.78 \pm 0.03

Table 2. Combined effects of aflatoxin B₁ and citrinin on pigment contents [g kg⁻¹(d.m.)] in 5-d-old maize seedlings. Means \pm S.E.; $n = 3$; the differences from control were significant at 5 % levels

Aflatoxin/citrinin	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a+b</i>	Car
0:0	8.86 \pm 0.00	3.37 \pm 0.00	12.24 \pm 0.00	0.16 \pm 0.00
1:1	4.48 \pm 0.02	1.53 \pm 0.05	6.02 \pm 0.02	0.08 \pm 0.00
1:2	5.04 \pm 0.04	1.86 \pm 0.00	6.90 \pm 0.00	0.09 \pm 0.00
2:1	3.56 \pm 0.04	1.13 \pm 0.03	4.68 \pm 0.01	0.06 \pm 0.00
1:3	7.21 \pm 0.05	2.75 \pm 0.07	9.95 \pm 0.06	0.13 \pm 0.00
3:1	2.96 \pm 0.03	1.03 \pm 0.06	3.98 \pm 0.08	0.05 \pm 0.00

Table 3. Combined effect of aflatoxin B₁ and citrinin on starch, total sugar, reducing sugar and non-reducing sugar contents [g kg⁻¹ (d.m.)] in 5-d-old maize seedlings. Means \pm S.E.; $n = 3$.

Aflatoxin/citrinin	Starch	Total sugars	Reducing sugars	Nonreducing sugars
0:0	51.23 \pm 0.52	45.20 \pm 0.07	14.64 \pm 0.04	30.56 \pm 0.11
1:1	511.36 \pm 1.24	0.68 \pm 0.04	0.21 \pm 0.02	0.47 \pm 0.06
1:2	420.40 \pm 1.12	0.82 \pm 0.02	0.25 \pm 0.01	0.57 \pm 0.03
2:1	572.60 \pm 1.58	0.57 \pm 0.03	0.17 \pm 0.01	0.40 \pm 0.04
1:3	229.23 \pm 1.38	5.51 \pm 0.04	1.63 \pm 0.03	3.88 \pm 0.06
3:1	607.66 \pm 1.47	0.52 \pm 0.03	0.15 \pm 0.01	0.37 \pm 0.02

Table 4. Combined effect of aflatoxin B₁ and citrinin on α -amylase activity measured as release of reducing sugars [g kg⁻¹(d.m.)] in 5-d-old maize seedlings.

Aflatoxin/citrinin	0 min	30 min	60 min	90 min
0:0	14.64	5.69	3.25	0
1:1	0.21	0.12	0.13	0.02
1:2	0.25	0.14	0.06	0
2:1	0.17	0.09	0.04	0.02
1:3	1.63	0.93	0.45	0
3:1	0.15	0.08	0.04	0.01

Table 5. Combined effect of aflatoxin B₁ and citrinin on protein [g kg⁻¹(d.m.)], DNA and RNA [mg kg⁻¹(d.m.)] contents in 5-d-old maize seedlings. Means ± S.E.; n = 3; the differences from control were significant at 5 % level

Aflatoxin/citrinin	Protein	DNA	RNA
0:0	83.9 ± 2.6	114.7 ± 1.0	392.6 ± 4.8
1:1	48.8 ± 1.4	77.6 ± 1.9	287.3 ± 2.6
1:2	56.0 ± 2.8	84.8 ± 1.6	306.2 ± 7.9
2:1	38.9 ± 1.4	73.6 ± 1.0	271.3 ± 12.1
1:3	71.3 ± 1.5	94.6 ± 1.3	342.0 ± 8.0
3:1	32.0 ± 1.6	61.6 ± 0.9	244.5 ± 3.9

Besides reduction in the content of proteins, the toxins also altered their quality. Protein spectra in disc electrophoresis (Fig. 1) showed nine peaks in the control seeds, of which 5 significant peaks were designated I, III, V, VIII and IX. Peaks V and IX were lost at all the combination ratios except at 1:3 while the concentration of peak VIII was reduced almost at all the combination ratios. The minimum RQ values

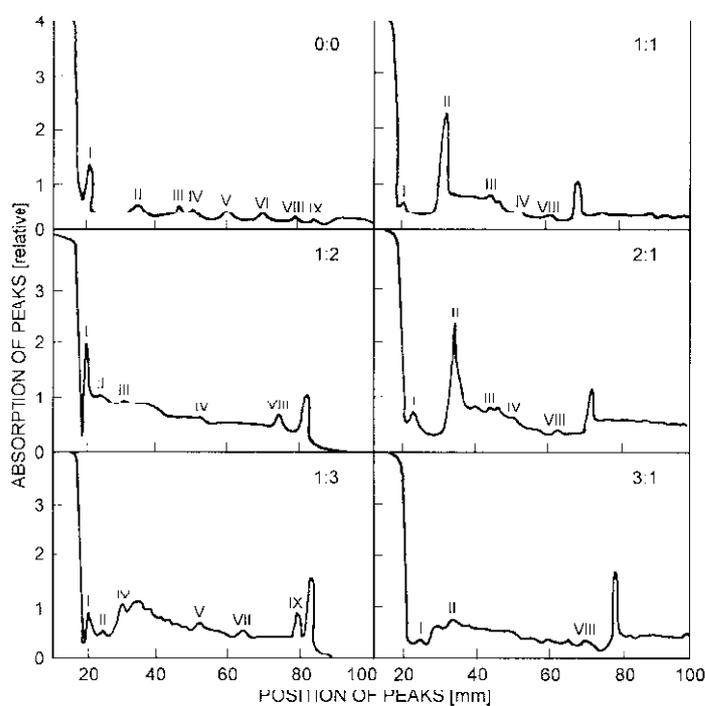


Fig. 1. Combined effect of aflatoxin B₁ and citrinin in different combinations on protein quality in 5-d-old maize seedlings according to gel electrophoresis.

of 0.91, 0.27 and 0.32 were recorded at 3:1 ratio of these toxins after 24, 48 and 72 h, respectively (Fig. 2).

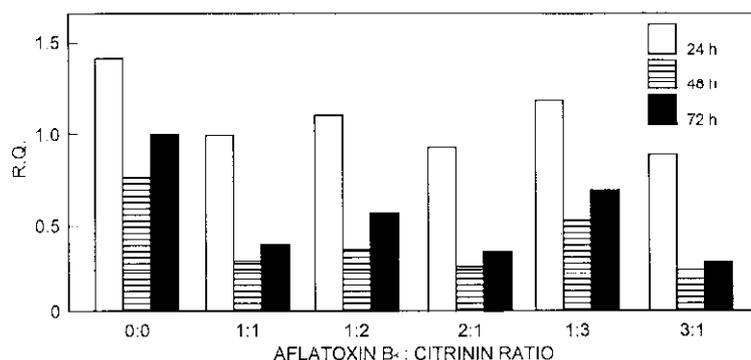


Fig. 2. Combined effect of aflatoxin B₁ and citrinin in different combinations on respiration quotient, R.Q., in maize seeds germination for 24, 48 and 72 h.

Discussion

The results obtained confirmed the findings on the toxic effects of aflatoxin and other mycotoxins on seed germination, seedling growth (Prasad *et al.* 1994, Sinha *et al.* 1993) as well as on Chl synthesis (Sinha and Kumari 1990, Prasad *et al.* 1994). The inhibition in pigment content in this investigation might be attributed to the inhibition of grana formation in the chloroplast (*cf.* Slowatizky *et al.* 1969).

Changes in starch and sugar contents (*cf.* also Prasad 1995) might be connected with the inhibition of α -amylase activity. Harris (1976) correlated the degradation of starch with the activation of starch hydrolysing enzymes like α -amylase during seed germination, which convert the starch molecules into simple sugars. Because of reduction in α -amylase activity, higher levels of starch were recorded in the toxin treated seeds. This might also be the reason for low contents of sugars in the treated seeds. In pea, transient changes in α -amylase activity were correlated with the changes in the rate of starch hydrolysis (Morohashi *et al.* 1989). Maximum content of starch in high toxin treated seeds also suggested least participation of this enzyme because of inhibitions in seeds germination due to aflatoxin B₁ (Prasad *et al.* 1994).

The suppression of protein and nucleic acid synthesis might be due to the inhibition in chromatin bound DNA dependent polymerase activity by toxins (Tripathi and Mishra 1983). Variations in the polyacrylamide gel electrophoresis pattern of soluble protein of *Aspergillus parasiticus* and *A. oryzae* in peanut seeds have been reported by Ory and Cherry (1972).

Reduction in RQ of germinating seeds due to toxin treatment might be attributed to the production of CO₂ because of the breakdown of reserve food materials in the stored seeds by the action of fungi (Vaidehi *et al.* 1983). Prasad (1995) observed that the least starch degradation in toxin treated seeds resulted ultimately in the least RQ

values. The fluctuation in RQ in the present investigation might be due to the presence of stored fats in embryo, which may itself be drawn into metabolism before the starch reserves of the endosperm are mobilised (Stiles and Leach 1973). The accumulation of products of anaerobic metabolism (due to utilization of fats as substrates in germination and their subsequent oxidation as a result of the splitting of seed coats) may also be responsible for the RQ fluctuations.

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