Chapter 18
Efficacy of Phytohormones on Mycotoxin Treated Maize Seeds (*Zea mays* L.)

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18.1 Introduction

Phytohormones are also known as plant growth regulators that influence several biological activities. These are important for growth and development of plants (Sembedner & Parthier, 1993). Phytohormones function as important chemical messengers as they modulate many cellular processes in plants and also coordinate different signaling pathways during exposure to mycotoxins (Vob et al., 2014).

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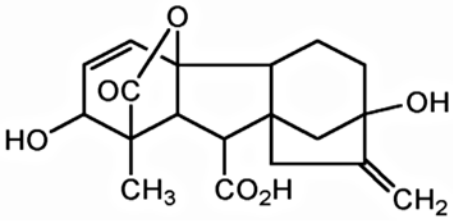
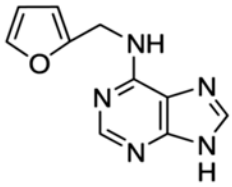
Maize is one of the most important staple food cereals grown throughout the world. It is commonly and directly consumed as processed food, indirectly as additive in most of food products and used as animal fodder (Lutz, 1994). Maize may be contaminated with storage fungi, producing mycotoxin that can be detrimental to health of human beings and animals. It is susceptible to a number of ear and kernel rots that can cause damage in humid areas.

Mycotoxins are naturally occurring toxins by certain fungi. These are found in food and can cause a variety of health effects, posing a serious health threat to both humans and live stocks. These fungi grow rapidly on a variety of natural substrates, and consumption of fungi-contaminated food can be detrimental (Kpodo et al., 1996). The crops that are frequently affected by *Aspergillus* spp. include cereals (corns, wheat, rice, and millet), oilseeds (pea nut, soya bean, sunflower), and spices (coriander, chilly, pepper, black pepper, turmeric, and ginger). The production of mycotoxin depends on high moisture content (20–25%) and high relative humidity (70–90%) (Shah et al., 2010).

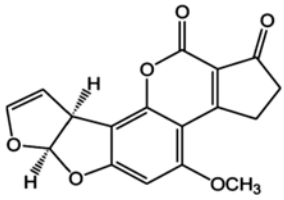
The fungi-producing mycotoxins fall broadly into two groups that invade before harvest commonly called field fungi, and those that occur only after harvest called storage fungi. The four major aflatoxins are called B_1, B_2, G_1, and G_2 based on their fluorescence under UV light. Aflatoxin B_1 is the most potent, natural, highly toxic, and carcinogenic secondary metabolite produced by *Aspergillus flavus* on agricultural crops (Lentopoloys et al., 2003). It is evident that aflatoxin is known for phytotoxicity with respect to seed germination and inhibition of root and hypocotyl elongation (Daskek & Llwellyn, 1983). AFB_1 was found at the highest concentration in contaminated food and feed. The different concentrations of AFB_1 inhibited chlorophyll, carotenoids, protein, and lipid contents and reduced the growth and germination of *Zea mays* L. and *Vicia faba* seeds (El-Naghy et al., 1999). It is properly documented that mycotoxin produced by specific filamentous fungi also causes significant reduction in crop yield and economic losses (Bhatnagar & Garcia, 2001). Aflatoxin content may vary with the season and storage time. It was observed that toxin contamination was higher during the rainy season and increased with increasing storage time (Ahmad, 1993). Aflatoxins are found in food chain in different pathways and make important detrimental damages in the metabolism of organisms (Hela et al., 2000). The seeds have been shown to be naturally contaminated with various levels of aflatoxin in the field (Ayalew, 2010) and during storage (Bilgrami & Sinha, 1992). Aflatoxins cause economic loss at all levels of food and feed production, processing, and distribution.



Chemical structure of Gibberellin (GA_3)



Chemical structure of Kinetin (Kn)



Chemical structure of Aflatoxin (AFB_1)

This article discusses hormonal action on maize seed, the action of mycotoxin, and finally the reversal effect with the application of phytohormones leading to the production of crops in the future.

18.2 Material and Methods

18.2.1 Collection of Healthy Seeds

The healthy seeds of maize (Madhuri-01) were obtained from Dayal Traders Manures and seed storage house, Darbhanga, India. The seeds were evaluated physically and were surface sterilized with 0.1% $HgCl_2$ for 2 min and then washed with sterilized distilled water.

18.2.2 Collection of Aflatoxin B1 and Phytohormones

The stock solution of AFB_1 was obtained from Sigma, USA. Pure phytohormones such as GA_3 and Kn were obtained from the local scientific stores, Darbhanga, India, and stored before analysis.

18.2.3 Preparation of Stock Solution

The stock solution of AFB_1 and phytohormones were prepared separately in ethanol from, which the dilution (i.e., 0.1, 0.25, 0.5, 1.0, and 2.0 ppm) was made in sterilized distilled water. Solutions of aflatoxin B_1 and phytohormones (2 ppm) were also mixed in different ratios (1:1, 1:2, 2:1, 1:3, 3:1 v/v) in order to record the combined effects.

The seeds were soaked initially in distilled water for 1 h and subsequently in different combinations of AFB_1 and phytohormones for 20 h. For each treatment, 100 seeds were taken in triplicates. The soaked seeds were then placed on moist blotter paper in sterilized petriplates and were kept for germination under automatic regulated seed germinator at 28 ± 2 °C (McLean et al., 1995; Sinha, 1990).

18.2.4 Seed Germination Index (GI)

It was calculated after 5 days of incubation according to the formula as given below:

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

The seedling growth (root and shoot lengths) was determined on the 7th day by measuring the lengths of radicle and plumule.

The data were analyzed statistically, i.e., t-test for seed germination and F-test for seedling growth. Statistical calculations were carried out using the ANOVA test (Dospekov, 1984).

18.2.5 Quantitative Estimation of Starch

About 200 mg freshly grounded sample was thoroughly shaken in 80% warm ethanol. After 5 min, the supernatant was decanted and centrifuged. Starch was estimated from the residue by adding 5 ml distilled water and mixing it thoroughly with 6.5 ml of 52% perchloric acid (prepared by adding 270 ml of 70% perchloric acid

into 100 ml of distilled water). This was followed by constant stirring for 10 min. After 15 min, again 20 ml of distilled water was added and centrifuged and then the supernatant was decanted. The extraction was repeated thrice and the supernatant was mixed together. The volume was raised to 100 ml by adding extra distilled water (stock solution). One milliliter of this stock solution was mixed with 10 ml at 0.1% anthrone reagent (760 ml conc. H_2SO_4 was diluted up to 1 l and 1 g anthrone dissolved) and heated at 100 °C for 12 min. The bluish-green solution was cooled at room temperature. Optical density was recorded at 630 nm under spectrophotometer. Readings were compared with the standard curve of starch prepared through similar procedure.

18.2.5.1 Assay of α-Amylase

It was assayed according to the method of Bernfeld (Bernfeld, 1955). One milliliter of starch solution was taken in experimental and control tubes. Approximately 0.5 ml of enzyme source (homogenate, i.e., 100 mg powder/ml acetate buffer, pH 4.8) was added in experimental and control tubes, but in the latter, the homogenate was previously dipped in boiling water bath for 5 min before being added. Both experimental and control tubes were incubated for 30 min at 37 °C. After incubation, both were dipped in boiling water bath for 5 min and subsequently cooled in running tap water. Three milliliter of KI solution (prepared by adding 25.4 mg I_2 and 400 mg KI in 100 ml of distilled water) was added in both tubes and mixed with the help of cyclomixer. The supernatant was used as crude extract for the extraction of enzyme. Colorimetric reading was taken at 620 nm, and difference of control and experimental reading gave the activity of α-amylase which was expressed in the value of optical density. Data recorded at each stage were the average of the three replicates.

18.2.6 Quantitative Estimation of Protein

It was done by the method of Lowry in both control and treated seeds (Lowry et al., 1951). Approximately 100 mg of seed flour were crushed in 100 ml of acetate buffer (pH 4.8) and centrifuged. The test solution (2 ml) was taken from the supernatant. To this, 10 ml of alkaline reagent (prepared by mixing 50 ml of 2% Na_2CO_3 solution in 0.1 N NaOH solution and 1 ml 0.5% $CuSO_4$ in 1% Na-K tartrate solution) was mixed thoroughly and was allowed to stand at room temperature for 10 min. One milliliter of diluted Folin–Ciocalteu reagent (1:3 in distilled water) was added. After 10 min, the extraction was added at 600 nm against the blank prepared by albumin.

18.3 Results and Discussion

The effects of phytohormones (GA_3 and Kn) and AFB_1on individual and combined ratios were observed on the seed germination of maize (Table 18.1; Figs. 18.1 and 18.2). A significant fall in seed germination at all concentrations of AFB_1 was noticed, but the maximum inhibition (79%) in all parameters was observed at the highest concentration (2 ppm) of AFB_1. The seed germination decreased by prolonged soaking intervals than the control treatment. When aflatoxin was mixed with GA_3, then inhibition was reversed up to 20.40%. The same case was noticed when aflatoxin was mixed with Kn. The toxin functioned as anti-gibberellin by inhibiting DNA synthesis (Cavusoglu & Solusoglu, 2015). This is due to the fact that GA_3 is capable of breaking the dormancy and inducing seed germination and seedling growth.

The effects of phytohormones (GA_3 and Kn) and AFB_1 on the length of roots were observed (Table 18.2). The root length showed positive response to GA_3 and Kn and grew up to 12.11 cm and 11.76 cm, respectively, but when AFB_1 was applied separately, then the root length grew up to 3.42 cm showing 65% inhibition. But when AFB_1 and GA_3 were treated, the root length was reversed up to 8.92 cm, and similar response (8.65 cm) showed if AFB_1 and Kn were applied.

The effects of phytohormones (GA_3 and Kn) and AFB_1 on the length of shoots were observed (Table 18.3). The shoot length showed positive response to GA_3 and Kn and grew up to 8.89 cm and 8.76 cm, respectively, but when AFB_1 was applied separately, then the shoot length grew up only 2.25 cm showing 63% inhibition. AFB_1 restricted the growth of plant by inhibiting seed germination (Reddy et al., 2010). But when AFB_1 and GA_3 were treated, then the shoot length was reversed up to 4.94 cm, and similar response (4.83 cm) showed if AFB_1 and Kn were applied.

The effects of phytohormones (GA_3 and Kn) and AFB_1 on the content of starch were observed (Table 18.4). Starch is accumulated during seed development, and germination is characterized by its degradation. The dissolution of insoluble starch to soluble maltose-dextrins occurs in the presence of α-amylase which catalyzes the hydrolysis of glucosidic linkage (Prasad et al., 2018a). A fluctuation in the starch content was observed in maize seeds due to the treatment of phytohormones and

Table 18.1 Impact of hormones with respect to seed germination on AFB_1-treated maize seeds

Observations	Seed germination (Mean value ± SE)	Difference with control	Percentage Inhibition
Control	98 ± 0.47	–	–
GA_3 (2 ppm)	100 ± 1	2	2
Kn (2 ppm)	100 ± 1	2	2
AFB_1 (2 ppm)	21 ± 0.57	13.50	79
AFB_1 + GA_3 (2 ppm v/v)	78 ± 0.47	22.27	20.40
AFB_1 + Kn (2 ppm v/v	79 ± 0.74	27.02	19.38

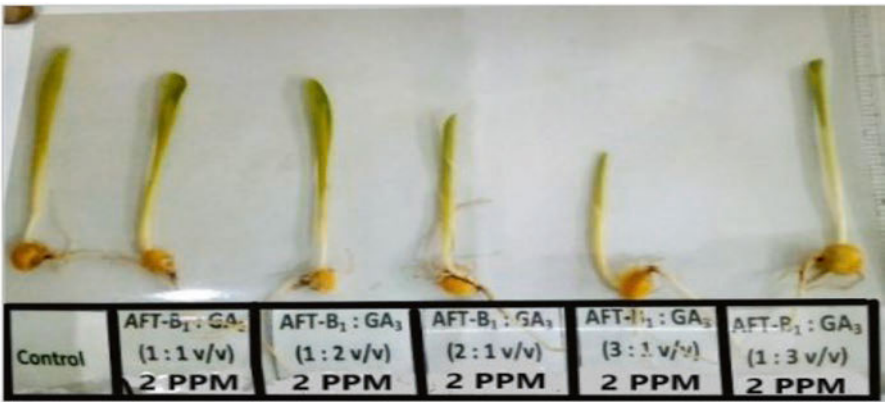


Fig. 18.1 Combined effect of AFB_1 and GA_3 (2 ppm v/v) on maize seedling growth

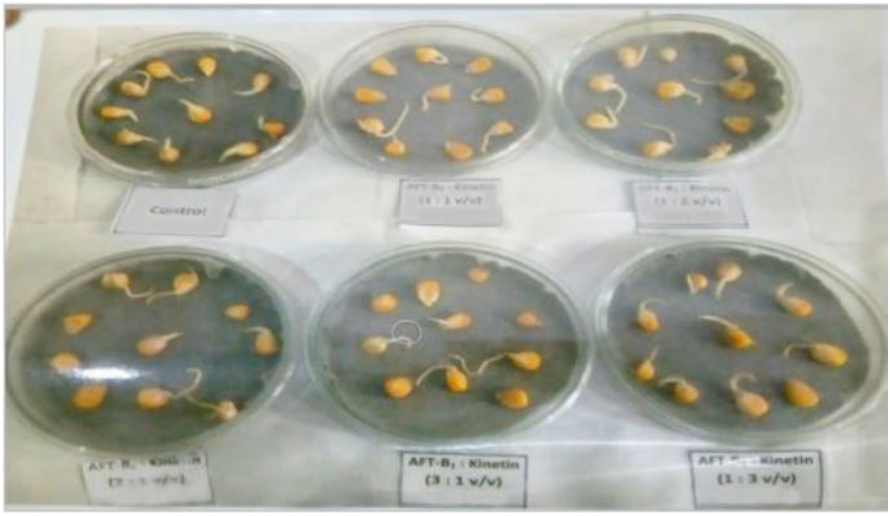


Fig. 18.2 Combined effect of AFB_1 and Kn (2 ppm v/v) on maize seed germination

mycotoxin. The maximum amount of starch (62.41 mg/100 mg) was recorded when maize seeds were treated with AFB_1 at 2 ppm concentration. The amount was decreased considerably up to 12.25 mg/100 mg and 13.12 mg/100 mg when AFB_1 was mixed with GA_3 and Kn, respectively.

The effects of phytohormones (GA_3 and Kn) and AFB_1 on the content of α-amylase were observed in different time duration (Table 18.5). The enzyme activity was completely lost in control set as well as GA_3 and Kn treatment in maize seeds after 90-min interval. However, enzyme activity was also lost in maize seeds due to treatment of GA_3 and Kn individually after 60-min interval.(Sakdeo 2016). The maximum inhibition as a result of AFB_1 treatment was observed. With increase

Table 18.2 Impact of hormones with respect to root length on AFB_1-treated maize seeds

Observations	Root length (cm)	Difference with control	Percentage Inhibition
Control	9.78 ± 0.12	–	–
GA_3 (2 ppm)	12.11 ± 1.00	2.33	23.82
Kn (2 ppm)	11.76 ± 0.15	0.35	20.24
AFB_1 (2 ppm)	3.42 ± 0.09	8.69	65.03
AFB_1 + GA_3 (2 ppm v/v)	8.92 ± 0.07	3.19	8.79
AFB_1 + Kn (2 ppm v/v	8.65 ± 0.04	3.46	11.55

Table 18.3 Impact of hormones with respect to shoot length on AFB_1-treated maize seeds

Observations	Shoot length (cm)	Difference with control	Percentage inhibition
Control	6.15 ± 0.24	–	–
GA_3 (2 ppm)	8.89 ± 0.08	2.74	44.50
Kn (2 ppm)	8.76 ± 0.09	2.61	42.00
AFB_1 (2 ppm)	2.25 ± 0.08	3.40	63.00
AFB_1 + GA_3 (2 ppm v/v)	4.94 ± 0.12	0.12	19.00
AFB_1 + Kn (2 ppm v/v	4.83 ± 0.12	1.32	21.00

Table 18.4 Impact of hormones on starch content on AFB_1-treated maize seeds

Observations	Starch content (mg/100 mg)
Control	4.75 ± 0.13
GA_3 (2 ppm)	0.93 ± 0.17
Kn (2 ppm)	1.14 ± 0.24
AFB_1 (2 ppm)	62.41 ± 0.23
AFB_1 + GA_3 (2 ppm v/v)	12.25 ± 0.18
AFB_1 + Kn (2 ppm v/v)	13.12 ± 0.10

Table 18.5 Impact of hormones on α-amylase content on AFB_1-treated maize seeds

	α-amylase activity			
Observations	0 min	30 min	60 min	90 min
Control	0.73 ± 0.09	0.34 ± 0.09	0.14 ± 0.08	–
GA_3 (2 ppm)	0.56 ± 0.12	0.24 ± 0.08	–	–
Kn (2 ppm)	0.62 ± 0.07	0.26 ± 0.12	–	–
AFB_1 (2 ppm)	0.97 ± 0.07	0.61 ± 0.07	0.30 ± 0.07	0.12 ± 0.04
AFB_1 + GA_3 (2 ppm v/v)	0.65 ± 0.09	0.31 ± 0.10	0.13 ± 0.04	0.09 ± 0.57
AFB_1 + Kn (2 ppm v/v)	0.73 ± 0.17	0.34 ± 0.08	0.21 ± 0.07	0.08 ± 0.04

in time incubation, the α-amylase activity gradually decreased in both germinated and nongerminated seeds.

The effects of phytohormones (GA_3 and Kn) and AFB_1 on the content of protein were observed in different time duration (Table 18.6). A highly significant fall in the level of protein in maize seedlings was observed during the treatment of different

Table 18.6 Impact of AFB_1 on protein content of maize seeds

Concentration of AFB1 (ppm)	Protein (mg/100 mg)	Difference with control	Percentage Inhibition
0.00	8.43 ± 0.10	–	–
0.10	7.14 ± 0.02	1.29	15.30
0.25	5.88 ± 0.02	2.55	30.24
0.50	4.65 ± 0.03	3.78	44.88
1.00	3.19 ± 0.05	5.24	62.15
2.00	2.23 ± 0.08	6.20	73.44

Table 18.7 Combined effects of AFB1 and GA3 on protein content of maize seeds

Concentration of AFB_1: GA_3 (2 ppm v/v)	Protein content (mg/100 mg)		
	Amount (Mean value ± SE)	Difference with control	Percentage inhibition
0:0	8.35 ± 0.04	–	–
1:1	4.86 ± 0.06	3.49	41.79
1:2	5.59 ± 0.05	2.76	33.05
2:1	3.87 ± 0.02	4.48	53.65
1:3	7.12 ± 0.05	1.23	14.73
3:1	3.19 ± 0.01	5.16	61.79

Table 18.8 Combined effects of AFB1 and Kn on protein content of maize seeds

Concentration of AFB_1: Kn (2 ppm v/v)	Protein content (mg/100 mg)		
	Amount (Mean value ± SE)	Difference with control	Percentage inhibition
0:0	8.45 ± 0.04	–	–
1:1	4.76 ± 0.06	3.69	43.66
1:2	5.56 ± 0.05	2.89	34.20
2:1	3.85 ± 0.02	4.60	54.43
1:3	7.10 ± 0.05	1.35	15.97
3:1	3.17 ± 0.01	5.28	62.48

concentration of AFB_1. The percentage inhibition was found to be the maximum at (2 ppm) concentration of AFB_1. Protein is inhibited due to nonavailability of mRNA.

The biochemical effect of AFB_1 in combination with GA_3 on protein content was noticed when treated in various combination ratios (Table 18.7). The maximum and minimum inhibitions were observed when concentrations of AFB_1 and GA_3 are in the ratio of 3:1 and 1:3, respectively (Prasad et al. 2018b).

The biochemical effect of AFB_1 in combination with Kn on protein content was noticed when treated in various combination ratios (Table 18.8). The maximum and minimum inhibitions were observed when concentrations of AFB_1 and GA_3 are in the ratio of 3:1 and 1:3, respectively (Dilip et al., 2017; Pratiwi et al., 2015).

18.4 Conclusion

Maize is an important agricultural crop and has suffered from various fungal diseases resulting in the loss of its productivity. Phytohormones have played pivotal roles in increasing the length of root, shoot, and seed germination. Definitely aflatoxin has caused much damage to the yield of maize crop if applied separately. The reversal effect was noticed if phytohormones and aflatoxin were applied in combination and has gained much prominence with respect to the yield in such infected maize crops. If this method is applied by farmers, then it will be financially fruitful due to high yields as well as beneficial for the consumers with respect to the protein quality intake.

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