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Effects of Phytohormones on Reversing the Inhibitory Action of Aflatoxin B1 on the Growth of Maize Seeds (*Zea mays* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. Author GP designed the study, performed the statistical analysis, wrote the protocol, data interpretation and wrote the first draft of the manuscript. Author VKM managed the analyses of the study, edited and finalized the draft into manuscript format, literature searches and correspondence of the manuscript. Author NK managed the technical searches. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

The present study is aimed to investigate the effect of phytohormones namely gibberellic acid (GA_3) and kinetin on aflatoxins B1 treated maize seeds var madhuri-01. Maize seeds were treated with aflatoxins B1 separately and in combination of phytohormones at a concentration of 2.0 ppm each. Reversal of inhibitory effect of aflatoxin B1 on the seed germination, root, shoot length, chlorophyll a, b and carotenoid of maize seed in presence of phytohormone was observed. The inhibitory effect of aflatoxins B1, on seed germination, root length, shoot length, chlorophyll a, chlorophyll b and

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carotenoid content were 78.57%, 65.03%, 63%, 74%, 72% and 85%, respectively. However, when the maize seeds were treated in combination of aflatoxins B1 with kinetin and aflatoxin B1 with GA_3, reversal of the inhibitory effect of aflatoxin B1 was observed to great extent. Taken together, the reversal of phytoinhibitory effect of aflatoxin B1 by GA_3 and kinetin could be of agricultural significance.

Keywords: Chlorophyll; carotenoids; aflatoxin-B_1; phytohormones and Zea mays L.

1. INTRODUCTION

Maize is one of the largest and most important cereal crops produced in the world. In 2012, the total production of maize was 872 million metric tons. Maize (also known as corn) is a domesticated cereal grain that has been grown as food and animal feed for tens of thousands of years. Maize (*Zea mays* L.) is an important crop for human consumption, particularly in developing countries, where this cereal can represent up to 65% of the total calories and 53% of the protein intake [1]. Maize is an important cereal crop and consumed by almost entire population in India in various ways. It is cultivated in all the three seasons' viz. Kharif (Monsoon), winter and summer in India.

Inhibitory effect of aflatoxin B1 on various cereal crops has been well documented [2,3,4,5]. The inhibitory effect of mycotoxins results in severe losses of maize productivity. The previous studies showed that the role of phytohormones like GA_3 and kinetin as anti- toxicants as well as the phytostimulatory agents. Adverse effect of aflatoxins B1 on physiological process of *Zea mays*, *Vigna radiate,* such as reduction in seed germination, seedling growth has been well documented [6,7]. Aflatoxins B1 is known to interfere with the synthesis of GA_3 which stimulates the activities of lipase and α-amylase enzyme, primarily required during seed germination and seedling growth [8]. Literature survey shows that GA_3 plays a key role in seed dormancy breaking, seedling growth and induce seed germination in soyabean seeds [9]. Brown et. al. (1986) [10] proposed that GA_3 acted the most active group of hormones in the breaking of dormancy in *Protea Campacta R-BR*, cytokinin (kinetin) also act activity in stimulating of radicles growth in green gram [2,3].Since only the few reports are available about the impact of phytohormones on mycotoxins treated seeds, the present work was designed to find out if the additional dose of GA_3, and kinetin can reverse the inhibitory effect of aflatoxins -B_1 on germination and growth of maize seedlings.

2. MATERIALS AND METHODS

Seeds of maize var. Madhuri-01 were obtained from Dayal Traders Manures and seed storage house, Kadirabad, Darbhanga, Bihar, India. aflatoxins-B_1 and phytohormones such as GA_3 and kinetin were obtained from the local Scientific Stores, Darbhagna of sigma, USA. The concentration of aflatoxins-B1, GA_3 and kinetin used was 2.0 ppm only. Since the effects of 5 different concentrations of aflatoxins B1 (0.1, 0.25, 0.50, 1.0 and 2.0 ppm) on seed germination, seedling growth, chlorophylls and carotenoids synthesis of maize seeds were studied during Ph.D. work [11]. The maximum inhibition in above parameters were recorded at 2.0 ppm of aflatoxins B1 and hence, already established concentration of aflatoxin B1 (2.0 ppm) was used in the present study [11]. Stock solution was initially prepared in 1.0 mL ethanol from which the dilution (2.0 gm^{-3} change to mL) was made in sterile double distilled water solutions of aflatoxins B1 toxin and phytohormones such as GA_3 and kinetin were mixed separately and in combination also, at a ratio of 1:1 (v/v), at 2.0 ppm to investigate their combined effects. Maize seeds were soaked in double distilled water for 1 h and subsequently in different combinations of these toxins and phytohormones for 20 h. One hundred healthy maize seeds were selected for each treatment in triplicate set. The soaked seeds were subsequently placed on moist blotting paper and incubated in seed germinator at 18±2 ºC. Seed germination index (GI) was calculated after 5-days of incubation according to the formula as given below.

$$GI = \frac{No. of\ germinated\ seeds}{No. of\ seed\ observed} X\ 100$$

Radical and plumule length was measured in centimeter after 7-days of growth. Chlorophyll-a, b and carotenoid contents were estimated according to the method described by Arnon (1949) [12] and Davis (1976) [13], respectively. The data were analyzed statistically that is "t-test" for seed germination and F-test for seedling growth as well as chlorophyll contents. Statistical calculations were carried out using ANOVA test [14].

3. RESULTS AND DISCUSSION

The effect of GA_3 and kinetin on aflatoxins B1 treated maize seed grown seedling germination, root and shoot length is represented in Table 1 and Table 2. The maximum inhibition in seed germination was 78.57% in presence of aflatoxins B1 only. However, minimum inhibition was observed with kinetin (19.38%) and GA_3 (10.20% in combination with aflatoxins B1. It was observed that aflatoxins B1 drastically inhibited seed germination, root and shoot length when applied separately. On the other hand, these parameters increased when treated in combination with GA_3 and kinetin phytohormone in presence of aflatoxins B1. Students t-test Para analysis showed significant effect ($P<0.01$) on seed germination due to aflatoxins B1 and with phytohormones treatments. The lowest inhibition recorded in root length and shoot length was 8.79% and 19.00% in GA_3 treated seeds, respectively. However, due to kinetin treatment the lowest inhibition in root and shoot length was 11.55% and 21.00%, respectively. The highest inhibition in root and shoot length was 65.03% and 63% respectively, in presence of aflatoxins B1 only. However, the treatment of aflatoxins-B1 with kinetin exhibited little bit greater inhibition as compared to the aflatoxins-B1 with GA_3 treatments. The longest root length was observed in GA_3 (12.11 cm) followed by kinetin (11.76 cm) treated maize seeds. However, the shortest root length was observed in aflatoxins B1 treated seeds followed by B1 with kinetin (8.92 cm) and aflatoxin B1 with GA_3 (8.65 cm) seeds of maize. The longest shoot length was observed in GA_3 (8.89 cm) followed by kinetin (8.76 cm) treated maize seeds. However, the shortest shoot length was observed in aflatoxins B1 treated B1 with Kinetin (4.83 cm) and B1with GA3 (4.94 cm) seeds of maize. It was observed that GA_3 and kinetin reverses the toxic and inhibitory effect of aflatoxins-B1 as shown in Table 2. The effect of aflatoxins B1 and phytohormones on the chlorophyll a, b and carotenoid is represented in Table 3. It was observed that aflatoxins B1 drastically inhibited chlorophyll a, b and carotenoid. On the other hand these parameters reversed when treated in combination with GA_3 and kinetin phytohormone in presence of aflatoxins B1, separately. The lowest inhibition was recorded in Chl-a, Chl-b, Total Chl (a+b) and carotenoids were 16.00%, 10.00%, 11.00% and 28.00% due to GA_3 treatment. However, due to kinetin treatment the inhibition in Chl-a,Chl-b, total Chl (a+b) and carotenoids was 18.00%, 20.00%, 19.00% and 35.00% in maize seeds. Aflatoxin B1 with kinetin showed more inhibition in comparison to the toxin with GA_3. The drastic reduction as well as enhancement of total chlorophyll and carotenoids content in aflatoxins-B1 treated seed was noticed due to stimulatory effects of GA_3 and kinetin. GA_3 also reverses the inhibition caused by the maximum concentration of aflatoxins-B1 in Cotton seeds. Our results are like the other reports [8]. Singh et. al. (1991) [15] also recorded the similar inhibition reversed effect of GA_3 caused due to aflatoxins B1 in the seeds of chick pea and pigeon pea [16]. There are only few reports are available on the stimulating effect of GA_3 on the chlorophyll synthesis [17]. Above result reveals that GA_3 and kinetin play crucial role in reversal of toxic effect of aflatoxins B1 treated seeds by breaking dormancy of seeds, which warrants further investigations.

Table 1. Impact of GA_3 and kinetin on aflatoxin B1 treated maize seeds #

Observations	Control	GA_3	Kinetin	aflatoxin B_1	aflatoxin B_1 + GA_3	Aflatoxin B_1 + Kinetin
Seed germination (Index X ± S.E).	98 ± 0.47	100 ± 1	100 ± 1	21 ± 0.57	88 ± 0.47	79 ± 0.74
% inhibition	-	-	-	78.57%	10.20%	19.38%
"t" Difference with Control	–	– 2.00	– 2.00	13.50	22.27	27.02
% Inhibition	–	– 2.00	– 2.00	78.57	10.20	19.38

 # p (0.01) (t – test), Significant at all the treatments and combinations of toxins and phytohormones

Table 2. Effect on root and shoot length of phytohormone GA3 and Kinetin on aflatoxin B1 treated maize seeds

Observations	Root length (cm)			Shoot length (cm)		
	X ± S.E	Difference with control	% inhibition	X ± S.E.	Difference with control	% Inhibition
Control	9.78 ± 0.12	–	–	6.15 ± 0.24	–	–
GA_3	12.11 ± 1.00	2.33	– 23.82	8.89 ± 0.08	– 2.74	– 44.55
Kinetin	11.76 ± 0.15	0.35	– 20.24	8.76 ± 0.09	– 2.61	– 42.00
Aflatoxin-B_1	3.42 ± 0.09	8.69	65.03	2.25 ± 0.08	3.90	63.00
Aflatoxin-B_1 + GA_3	8.92 ± 0.07	3.19	8.79	4.94 ± 0.12	1.21	19.00
Aflatoxin-B_1 + Kinetin	8.65 ± 0.04	3.46	11.55	4.83 ± 0.12	1.32	21.00
$LSDO_1$	–	0.33	3.80	–	0.42	14.62
$LSDO_5$	–	0.23	2.71	–	0.29	10.41

** Non significant at 1 and 5% level*
*** Non Significant at only 5% level, all other values are significant at both 1 and 5% level*

Table 3. Effects of GA_3 and kinetin on aflatoxin B_1 treated cholophylles carotenoid contents of Maize seeds #

Observations (Concen.)	%Chl-a X ± S.E.	Difference with control	% inhibition	%chl – b X ± S.E.	Difference with control	% inhibition	Total Chl (a+b)	% inhibition	% Carotenoids	Difference with control	% inhibition
Control	0.880 ± 0.073	–	–	0.328± 0.159	–	–	1.216± 0.014	–	0.014± 0.023	–	–
GA_3	0.979 ± 0.039	–0.099	– 11.25	1.159± 0.014	–1.262	–61.58	1.571± 0.029	–24.00	0.027± 0.023	–0.013-	–92.00
Kinitin	0.926 ± 0.025	0.046	–4.60	1.488± 0.019	–1.160	–51.21	1.422± 0.029	16.00	0.026± 0.014	–0.012	–85.00
aflatoxins-B_1	0.226 ± 0.023	0.654	74.00	0.274± 0.023	0.054	72.00	0.316± 0.014	74.00	0.002± 0.014	0.012	85.00
aflatoxins-B_1 + GA_3	0.737 ± 0.039	0.143	16.00	0.293± 0.053	0.035	10.00	1.081± 0.014	11.00	0.010± 0.023	0.004	28.00
aflatoxins-B_1 + Kinitin	0.715 ± 0.039	0.165	18.00	0.261± 0.014	0.067	20.00	0.980± 0.014	19.00	0.009± 0.014	0.005	35.00

#% = mg/100mg
Significant at 1 & 5% level

4. CONCLUSION

Application of phytohormones like GA_3 and Kinetin could significantly reverse the inhibitory effect of aflatoxin B1 of maize seedlings. That could be of agricultural importance and warrants further investigations.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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