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Dose-dependent suppression of photosynthetic pigments in maize (*Zea mays* L.) seedlings exposed to aflatoxin B1

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Abstract

Maize (*Zea mays* L.) is a key cereal crop in Northern India, yet its early growth can be compromised in aflatoxin-prone agroclimates where *Aspergillus*-associated contamination is frequent. The present study evaluated the phytotoxic influence of aflatoxin B1 on major photosynthetic pigments in maize seedlings under controlled conditions. Uniform seeds were exposed to five aflatoxin B1 concentrations (100, 250, 500, 1000, and 2000 µg/L) and germinated for ten days; pigment contents were quantified from newly emerged leaves using standard spectrophotometric methods. Aflatoxin B1 caused a clear, concentration-dependent decline in chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids relative to the untreated control. At 2000 µg/L, chlorophyll a decreased by 70.15%, chlorophyll b by 64.29%, total chlorophyll by 68.60%, and carotenoids by 76.19%. Intermediate doses produced graded inhibition, confirming a strong dose-response relationship. The magnitude of carotenoid depletion suggests pronounced impairment of photoprotective capacity, which may aggravate photo-oxidative stress and reduce overall photosynthetic competence during the seedling stage. These results demonstrate that aflatoxin B1 toxicity extends beyond food and feed safety concerns to direct physiological disruption in maize, with likely implications for seedling vigor and stand establishment in contaminated regions such as Bihar. The findings support the urgency of adopting integrated mitigation strategies including improved storage, fungal management, and resistant cultivars to minimize mycotoxin exposure in maize-based production systems.

Keywords: Aflatoxin B1, maize, chlorophyll a, chlorophyll b, carotenoids, phytotoxicity, photosynthetic pigments, *Zea mays*, Bihar, seedling physiology

Introduction

Maize (*Zea mays* L.), commonly known as corn, constitutes a major pillar of agricultural production in Northern India, particularly in Bihar, where it contributes substantially to household food security and rural livelihoods (Kumar *et al.* 2014) [8]. Despite its agronomic importance, maize cultivation in Bihar is persistently challenged by the state's warm and humid conditions, which favour the proliferation of mycotoxin-producing fungi, especially *Aspergillus flavus* and *A. parasiticus*. These fungi frequently infest maize grains and lead to contamination with aflatoxins (Sinha and Sinha 1990; Reddy *et al.* 2010) [13, 11]. Among the aflatoxins, aflatoxin B1 is regarded as the most potent and is widely reported as a recurrent contaminant of maize, raising concerns not only for consumer health but also for crop performance and physiological integrity (Pitt and Hocking 2009; Bhat and Vasanthi 2003) [10, 3].

Importantly, aflatoxin B1 is not merely a post-harvest food safety issue; it can also exert direct phytotoxic effects during early plant development. Evidence suggests that exposure to aflatoxin B1 interferes with fundamental metabolic pathways, including the biosynthesis and stability of photosynthetic pigments such as chlorophylls and carotenoids molecules that are indispensable for photosynthetic function and seedling establishment (Mehan 1971; Kang 1970) [9, 7]. Chlorophyll a and chlorophyll b are central to light harvesting, while carotenoids contribute both to accessory light absorption and to photoprotection through quenching of reactive oxygen species, thereby safeguarding the photosynthetic apparatus under stress (Taiz and Zeiger 2010) [14].

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Previous investigations have documented pigment suppression under aflatoxin stress in crops such as *Abelmoschus esculentum* and mustard, indicating that toxin-induced disruption of photosynthetic machinery may represent a broader phytotoxic mechanism (Kang 1970 ; Sinha *et al.* 1992) [7, 12]. However, systematic quantification of these effects in maize seedlings remains relatively limited. Given Bihar's dependence on maize and its heightened vulnerability to aflatoxin contamination, clarifying the extent of pigment inhibition is essential for assessing potential risks to seedling vigour and, ultimately, yield stability. Therefore, the present study quantifies the influence of graded aflatoxin B1 exposure on chlorophyll and carotenoid contents in maize seedlings to better elucidate its physiological consequences and to support the development of mitigation strategies for aflatoxin-affected agroecosystems.

Materials and Methods

Maize seeds (*Zea mays* L.) of uniform quality were obtained from the Crop Section of Rajendra Agricultural University, Sabour, Bhagalpur, India, a hub of maize cultivation in Bihar. Aflatoxin B1 (Sigma-Aldrich, St. Louis, MO, USA) was prepared as a stock solution in 1 mL ethanol and diluted with sterile distilled water to concentrations of 100, 250, 500, 1000, and 2000 µg/L. A control treatment used distilled water alone to establish baseline pigment levels.

Seeds were pre-soaked in 100 mL sterile distilled water for 1 hour to initiate imbibition, then immersed in 100 mL of aflatoxin B1 solutions (or water for controls) for 20 hours, ensuring toxin uptake. Each treatment comprised 100 seeds in triplicate (n=300 per concentration). Treated seeds were placed on moist blotting paper in petri dishes and incubated at 28 ± 2 °C in a seed germinator for 10 days, allowing seedling development under controlled conditions.

Chlorophyll and carotenoid contents were measured in newly emerged leaves on day 10, following established protocols. For chlorophyll, 250 mg of fresh leaf tissue was homogenised in 5 mL of 80% acetone using a mortar and pestle. The extract was filtered through Whatman No. 1 paper in a Buchner funnel, with extraction repeated 2-3 times using additional 5 mL aliquots of 80% acetone. The mortar, pestle, and funnel were rinsed with 5 mL of 80% acetone, and the final volume was adjusted to 25 mL. Absorbance was recorded at 645 nm and 663 nm using a spectrophotometer (UV-Vis, Shimadzu) against an 80% acetone blank. Chlorophyll a and b contents (mg/100 mg fresh weight) were calculated using Arnon's equations (Arnon 20):

$$\text{mg chlorophyll a / 100 g tissue} = \frac{12.7 (A_{663}) - 2.69 (A_{645}) \times V}{1000 \times W} \times 100$$

$$\text{mg chlorophyll b / 100 g tissue} = \frac{22.9 (A_{645}) - 4.68 (A_{663}) \times V}{1000 \times W} \times 100$$

Total chlorophyll = chlorophyll a + chlorophyll b

Where A = absorbance, V = final volume (25 mL), and W = tissue weight (0.25 g).

Carotenoid content was determined at 480 nm using Davis's method (Davis 35) [5], accounting for chlorophyll interference:

$$\Delta E_{\text{Car 480}} = A_{480} + 0.114A_{663} - 0.638A_{645}$$

Results were expressed as mg/100 mg fresh weight (%). Measurements were replicated thrice, and data are presented as means ± standard error (SE).

Observed data were analyzed using one-way analysis of variance (ANOVA), with a t-test (t-values: chlorophyll a = 13.974, chlorophyll b = 11.704, total chlorophyll = 13.737, carotenoids = 6.945; df = 4) and Pearson correlations (r-values: chlorophyll a = 0.989, chlorophyll b = 0.986, total chlorophyll = 0.989, carotenoids = 0.961). Significance was assessed at $p < 0.05$.

Results and Discussion

Aflatoxin B1 significantly reduced chlorophyll a, chlorophyll b, and total chlorophyll contents in maize seedling leaves, with inhibition increasing with concentration (Table 1). Control seedlings exhibited 0.670 ± 0.0015% chlorophyll a, 0.280 ± 0.0070% chlorophyll b, and 0.950 ± 0.0085% total chlorophyll. At 2000 µg/L, these values dropped to 0.200 ± 0.0012% (70.15% inhibition), 0.100 ± 0.0045% (64.29% inhibition), and 0.298 ± 0.0057% (68.60% inhibition), respectively. Intermediate concentrations showed graded declines (e.g., 1000 µg/L: 44.78%, 30.36%, 40.63% inhibition for chlorophyll a, b, and total, respectively), with strong correlations ($r = 0.989$ for chlorophyll a and total chlorophyll, $r = 0.986$ for chlorophyll b). Carotenoid levels followed a similar trend, decreasing from 0.021 ± 0.0010% in controls to 0.005 ± 0.0009% at 2000 µg/L (76.19% inhibition). Intermediate reductions ranged from 9.52% (100 µg/L) to 61.90% (1000 µg/L), with a correlation of $r = 0.961$. All reductions were statistically significant ($p < 0.05$).

Table 1: Effects of Aflatoxin B1 on Chlorophyll and Carotenoid Contents in Maize Seedlings

Conc. of Aflatoxin B1 (µg/L)	% Chlorophyll a (Mean ± SE)	% Chlorophyll b (Mean ± SE)	% Total Chlorophyll (Mean ± SE)	% Carotenoids (Mean ± SE)	% Inhibition in Chl a	% Inhibition in Chl b	% Inhibition in Total Chl	% Inhibition in Carotenoids
0 (Control)	0.670 ± 0.0015	0.280 ± 0.0070	0.950 ± 0.0085	0.021 ± 0.0010	-	-	-	-
100	0.620 ± 0.0020	0.265 ± 0.0045	0.885 ± 0.0065	0.019 ± 0.0012	7.46	5.36	6.84	9.52
250	0.580 ± 0.0035	0.245 ± 0.0050	0.825 ± 0.0080	0.017 ± 0.0008	13.43	12.50	13.16	19.05
500	0.490 ± 0.0025	0.220 ± 0.0060	0.710 ± 0.0085	0.013 ± 0.0007	26.87	21.43	25.26	38.10
1000	0.370 ± 0.0018	0.195 ± 0.0055	0.564 ± 0.0070	0.008 ± 0.0006	44.78	30.36	40.63	61.90
2000	0.200 ± 0.0012	0.100 ± 0.0045	0.298 ± 0.0057	0.005 ± 0.0009	70.15	64.29	68.60	76.19

* t = 13.974 (chl a), 11.704 (chl b), 13.737 (total chl), 6.945 (carotenoids); df = 4; r = 0.989 (chl a), 0.986 (chl b), 0.989 (total chl), 0.961 (carotenoids). All values significant ($p < 0.05$).

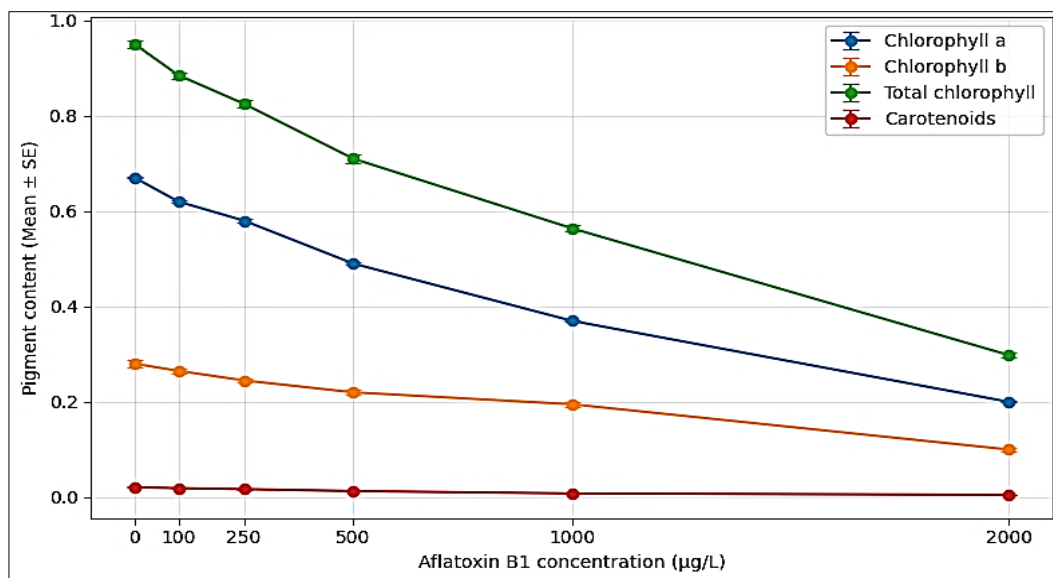


Fig 1: Effect of Aflatoxin B1 on Photosynthetic Pigments in Maize Seedlings

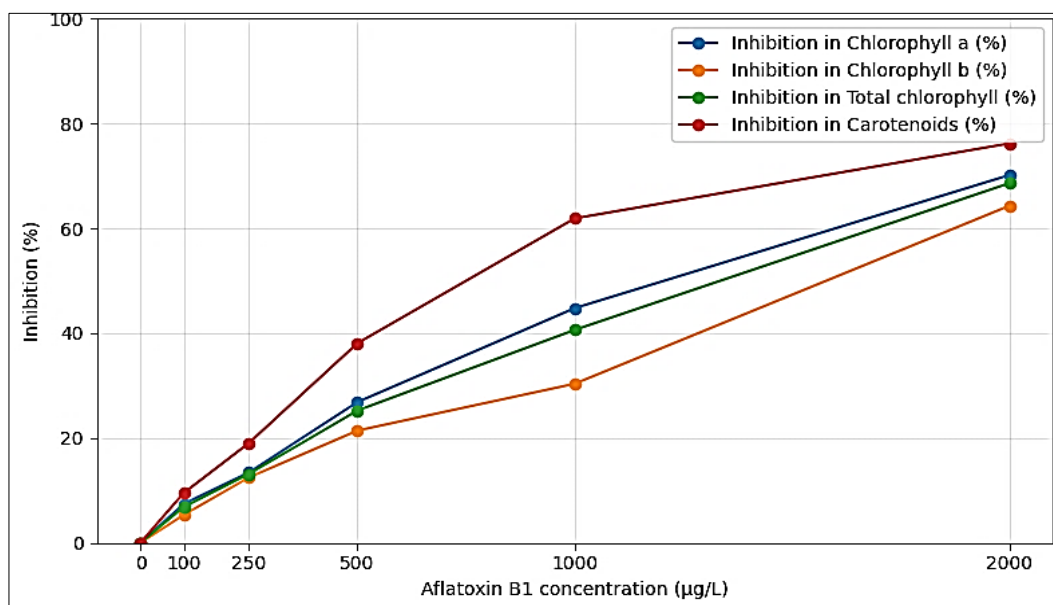


Fig 2: Dose-Response: Percent Inhibition of Pigments vs Aflatoxin B1 Concentration

Aflatoxin B1 exerted a profound inhibitory effect on photosynthetic pigments in maize seedlings, with chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids declining in a dose-dependent manner. The maximum reductions at 2000 µg/L (70.15% for chlorophyll a, 64.29% for chlorophyll b, 68.60% for total chlorophyll, and 76.19% for carotenoids) indicate severe disruption of pigment synthesis, likely linked to restricted seedling growth observed in prior studies (Mehan 1971; Kang 1970) [9, 7]. These findings align with reports of chlorophyll inhibition in *Abelmoschus esculentum* (Kang 1970) [7] and mustard (Sinha *et al.* 1992) [12], suggesting a conserved phytotoxic mechanism across species. The slightly greater inhibition of chlorophyll a versus chlorophyll b may reflect differential sensitivities in biosynthetic pathways, with chlorophyll a being more susceptible to toxin-induced oxidative damage (Taiz and Zeiger 2010; Bennett and Klich 2003) [14, 2].

Carotenoid depletion, peaking at 76.19%, exceeds chlorophyll losses, possibly due to their role as antioxidants, rendering them more vulnerable to aflatoxin-induced

reactive oxygen species (Das *et al.* 2015; Pitt and Hocking 2009) [4, 10]. This imbalance could impair photoprotection, exacerbating photosynthetic stress. The strong correlations ($r > 0.96$) underscore a robust dose-response relationship, consistent with aflatoxin's known metabolic interference (Reddy *et al.* 2010) [11]. Since maize is a dietary staple and aflatoxin contamination is prevalent in Bihar (Bhat and Vasanthi 2003; Kumar *et al.* 2014) [3, 8], these reductions threaten seedling vigor and yield potential by limiting their efficiency (Taiz and Zeiger 2010) [14]. Future research should investigate molecular targets, such as chloroplast enzyme activity or lipid peroxidation (Das *et al.* 2015) [4], and explore mitigation strategies like resistant cultivars or biocontrol agents (Pitt and Hocking 2009) [10]. These efforts are critical to sustaining maize production in contaminated regions.

Conclusion

The present investigation establishes that aflatoxin B1 significantly compromises the photosynthetic pigment content of maize (*Zea mays* L.) seedlings, with reductions in

chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids intensifying across a concentration gradient of 100 to 2000 µg/L. At the highest concentration, chlorophyll a declined by 70.15%, chlorophyll b by 64.29%, total chlorophyll by 68.60%, and carotenoids by 76.19%, reflecting a profound disruption of pigment synthesis and accumulation. These results, corroborated by robust statistical correlations ($r = 0.989$ for chlorophyll a and total chlorophyll, $r = 0.986$ for chlorophyll b, $r = 0.961$ for carotenoids), indicate that aflatoxin B1's phytotoxicity extends beyond growth inhibition to impair fundamental photosynthetic processes, likely through chloroplast damage or oxidative stress (Das *et al.* 2015; Taiz and Zeiger 2010) ^[4, 14].

For maize cultivation in Bihar, India, a region marked by high aflatoxin prevalence, these pigment losses pose a dual threat: diminished seedling vigor and reduced photosynthetic capacity, both of which could curtail crop productivity (Kumar *et al.* 2014; Reddy *et al.* 2010) ^[8, 11]. The greater inhibition of carotenoids compared to chlorophylls suggests heightened vulnerability of photoprotective mechanisms, potentially amplifying photooxidative damage under field conditions (Pitt and Hocking 2009) ^[10]. These observations align with prior studies documenting pigment suppression in crops like *Abelmoschus esculentum* and mustard (Kang 1970; Sinha *et al.* 1992) ^[7, 12], reinforcing aflatoxin B1's broad phytotoxic profile, though maize appears less severely affected than some dicots. The study's findings emphasize the necessity of addressing aflatoxin contamination to preserve maize's physiological integrity and yield potential. Future investigations should explore the toxin's specific biochemical targets such as enzymes in the chlorophyll biosynthesis pathway (e.g., magnesium chelatase) or carotenoid degradation pathways and assess the efficacy of mitigation approaches, including resistant germplasm, fungal biocontrol, or antioxidant treatments to bolster seedling resilience (Pitt and Hocking 2009; Das *et al.* 2015) ^[4, 10]. Such efforts are critical to mitigating the agricultural and economic risks posed by aflatoxin B1 in maize-dependent regions, ensuring the sustainability of this vital crop.

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