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IMPACT OF CRUCIFER PLANT EXTRACTS ON CHLOROPHYLL AND CAROTENOID CONTENTS OF AFLATOXIN B1 TREATED MAIZE SEEDS

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ABSTRACT

This research evaluates the phytotoxic effects of aflatoxin B1 on the chlorophyll and carotenoid contents of maize (*Zea mays* L.) seedlings and examines the protective potential of three crucifer plant extracts: A (*Raphanus sativus*), B (*Lepidium sativum*), and C (*Brassica oleracea* var. *italica*). Aflatoxin B1 treatment at 2000 µg/L reduced chlorophyll a by 0.470% (70.15%), chlorophyll b by 0.180% (64.29%), total chlorophyll by 0.652% (68.60%), and carotenoids by 0.016% (76.19%) compared to controls. Co-treatment with crucifer extracts significantly mitigated these losses, with extract C achieving the greatest restoration, reducing total chlorophyll inhibition to 0.330% (34.74%) and carotenoid inhibition to 0.009% (42.86%). Extracts alone slightly increased pigment levels, with C enhancing total chlorophyll by 0.060% (6.32%) and carotenoids by 0.003% (14.29%). These findings suggest that crucifer extracts, particularly C, serve as both enhancers and mitigators, offering a sustainable approach to counter aflatoxin B1-induced photosynthetic stress in maize cultivation, especially in aflatoxin-prone regions like Bihar, India.

Keywords: Aflatoxin B1, Maize, Chlorophyll A, Chlorophyll B, Carotenoids, Crucifer Plant Extracts

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1. INTRODUCTION

Maize (*Zea mays* L.) is a critical cereal crop in Bihar, India, underpinning both food security and economic stability, yet its production is increasingly threatened by aflatoxin B1 contamination from *Aspergillus* spp., exacerbated by the region's humid climate (Sinha and Sinha 1990; Kumar *et al.* 2014). Beyond its well-known risks to human and animal health via contaminated grains, aflatoxin B1 exerts significant phytotoxic effects on maize seedlings, notably by depleting chlorophyll and carotenoid contents—essential pigments for photosynthesis and photoprotection (Reddy *et al.* 2010). These reductions impair light absorption, energy conversion, and antioxidant defenses, compromising seedling vigor and potentially reducing yield (Taiz and Zeiger 2010). In Bihar, where maize is a dietary staple, such physiological disruptions pose a dual threat to agricultural productivity and food safety (Bhat and Vasanthi 2003).

This study investigates the potential of three crucifer plant extracts—A (*Raphanus sativus*), B (*Lepidium sativum*, garden cress), and C (*Brassica oleracea* var. *italica*, broccoli)—to mitigate aflatoxin B1's impact on maize seedling

chlorophyll (a and b) and carotenoid contents. Cruciferous plants are renowned for their rich profiles of glucosinolates, isothiocyanates, and antioxidants, compounds known to neutralize oxidative stress and inhibit fungal toxins (Fahey *et al.* 2001; Mithen *et al.* 2000). By assessing the extracts' standalone effects and their efficacy in combination with aflatoxin B1, this research seeks to elucidate their dual roles as pigment enhancers and stress protectants. The findings aim to offer a natural, sustainable strategy to bolster maize photosynthetic capacity and resilience in aflatoxin-contaminated agroecosystems like Bihar, addressing a critical need for environmentally friendly agricultural interventions.

2. MATERIALS AND METHODS

Maize seeds (*Zea mays* L.) were obtained from the Crop Section of Rajendra Agricultural University, Sabour, Bhagalpur, India, ensuring relevance to Bihar's maize-growing conditions. Aflatoxin B1 (Sigma-Aldrich, St. Louis, MO, USA) was prepared as a stock solution in 1 mL ethanol, then diluted with sterile distilled water to concentrations of 100, 250, 500, 1000, and 2000 µg/L. Crucifer extracts were produced from fresh tissues of A (*Raphanus sativus*), B (*Lepidium sativum*), and C (*Brassica oleracea* var. *italica*): 100 g of each was blended in 200 mL distilled water, filtered through muslin cloth, and concentrated to a 10% w/v solution at 40°C under reduced pressure, preserving bioactive integrity (Fahey *et al.* 2001).

The experiment was structured to evaluate both the standalone and combined effects of the extracts with aflatoxin B1 on seedling pigment contents. Seeds were pre-soaked in 100 mL sterile distilled water for 1 hour to initiate imbibition, then subjected to one of four treatments for 20 hours: (1) 100 mL distilled water (control), (2) 5 mL of extract A, B, or C diluted in 100 mL water, (3) 100 mL aflatoxin B1 solution at varying concentrations, or (4) 100 mL aflatoxin B1 solution plus 5 mL of extract A, B, or C. Each treatment was applied to 100 seeds in triplicate (n=300), followed by incubation on moist blotting paper in petri dishes at 28 ± 2°C for 10 days, a duration sufficient for pigment development in maize seedlings under controlled conditions.

On day 10, chlorophyll and carotenoid contents were quantified in newly emerged leaves to assess photosynthetic capacity. For chlorophyll, 250 mg of fresh leaf tissue was homogenized in 5 mL 80% acetone using a mortar and pestle, filtered through Whatman No. 1 paper in a Buchner funnel, and extracted repeatedly until colorless, with the final volume adjusted to 25 mL using additional 80% acetone. Absorbance was measured at 645 nm and 663 nm using a UV-Vis spectrophotometer (Shimadzu) against an 80% acetone blank. Chlorophyll a and b contents were calculated according to Arnon's equations (Arnon 20):

mg chlorophyll a / 100 g tissue = $\frac{12.7\ (A663) - 2.69\ (A645)\ x\ V}{1000\ x\ W}$ x 100

mg chlorophyll b / 100 g tissue = $\frac{22.9\ (A645) - 4.68\ (A663)\ x\ V}{1000\ x\ W}$ x 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), correcting for chlorophyll interference:

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

Results were expressed as % w/w (mg/100 mg fresh weight). Data were recorded as means ± standard error (SE) from triplicate samples and analyzed using one-way analysis of variance (ANOVA) to detect treatment effects. A t-test was applied to compare means (t = 13.974 for chl a, 11.704 for chl b, 13.737 for total chl, 6.945 for carotenoids; df = 4), with Pearson correlations ($r > 0.96$) confirming dose-response relationships, all significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

Crucifer plants extracts alone enhanced chlorophyll and carotenoid contents compared to the control (Table 1). Extract C (*Brassica oleracea* var. *italica*) showed the greatest increase, raising chlorophyll a to 0.710 ± 0.0016% (a difference of +0.040%, 5.97% enhancement), chlorophyll b to 0.300 ± 0.0055% (+0.020%, 7.14% enhancement), total chlorophyll to 1.010 ± 0.0070% (+0.060%, 6.32% enhancement), and carotenoids to 0.024 ± 0.0008% (+0.003%, 14.29% enhancement). Extract B (*Lepidium sativum*) followed with chlorophyll a at 0.700 ± 0.0020% (+0.030%, 4.48% enhancement), chlorophyll b at 0.295 ± 0.0060% (+0.015%, 5.36% enhancement), total chlorophyll at 0.995 ± 0.0075%

(+0.045%, 4.74% enhancement), and carotenoids at 0.023 ± 0.0011% (+0.002%, 9.52% enhancement). Extract A (*Raphanus sativus*) exhibited the smallest enhancement, with chlorophyll a at 0.690 ± 0.0018% (+0.020%, 2.99% enhancement), chlorophyll b at 0.290 ± 0.0065% (+0.010%, 3.57% enhancement), total chlorophyll at 0.980 ± 0.0080% (+0.030%, 3.16% enhancement), and carotenoids at 0.022 ± 0.0009% (+0.001%, 4.76% enhancement).

Aflatoxin B1 alone at 2000 µg/L significantly reduced pigment levels: chlorophyll a to 0.200 ± 0.0012% (a difference of -0.470%, 70.15% inhibition), chlorophyll b to 0.100 ± 0.0045% (-0.180%, 64.29% inhibition), total chlorophyll to 0.298 ± 0.0057% (-0.652%, 68.60% inhibition), and carotenoids to 0.005 ± 0.0009% (-0.016%, 76.19% inhibition). Co-treatment with crucifer extracts mitigated these reductions. Extract C was most effective, restoring chlorophyll a to 0.450 ± 0.0015% (-0.220%, 32.84% inhibition), chlorophyll b to 0.200 ± 0.0040% (-0.080%, 28.57% inhibition), total chlorophyll to 0.620 ± 0.0060% (-0.330%, 34.74% inhibition), and carotenoids to 0.012 ± 0.0006% (-0.009%, 42.86% inhibition). Extract B followed with chlorophyll a at 0.410 ± 0.0018% (-0.260%, 38.81% inhibition), chlorophyll b at 0.180 ± 0.0045% (-0.100%, 35.71% inhibition), total chlorophyll at 0.590 ± 0.0070% (-0.360%, 37.89% inhibition), and carotenoids at 0.011 ± 0.0007% (-0.010%, 47.62% inhibition). Extract A showed the least mitigation, with chlorophyll a at 0.350 ± 0.0020% (-0.320%, 47.76% inhibition), chlorophyll b at 0.160 ± 0.0050% (-0.120%, 42.86% inhibition), total chlorophyll at 0.510 ± 0.0065% (-0.440%, 46.32% inhibition), and carotenoids at 0.009 ± 0.0008% (-0.012%, 57.14% inhibition).

Table 1: Impact Of Crucifer Plant Extracts On Aflatoxin B1-Treated Chlorophyll And Carotenoid Contents Of Maize Seeds (Zea Maize)

Treatment	% Chl a (Mean ± SE)	Difference with control	% Inhibition	% Chl b (Mean ± SE)	Difference with control	% Inhibition	Total Chl (a+b)	% Inhibition	% Carotenoids	Difference with control	% Inhibition
Control	0.670 ± 0.0015	0.0	-	0.280 ± 0.0070	0.0	-	0.950 ± 0.0085	-	0.021 ± 0.0010	0.0	-
Plant A (Raphanus sativus)	0.690 ± 0.0018	+0.020	-2.99*	0.290 ± 0.0065	+0.010	-3.57*	0.980 ± 0.0080	-3.16*	0.022 ± 0.0009	+0.001	-4.76*
Plant B (L. sativum)	0.700 ± 0.0020	+0.030	-4.48*	0.295 ± 0.0060	+0.015	-5.36*	0.995 ± 0.0075	-4.74*	0.023 ± 0.0011	+0.002	-9.52*
Plant C (B. oleracea)	0.710 ± 0.0016	+0.040	-5.97*	0.300 ± 0.0055	+0.020	-7.14*	1.010 ± 0.0070	-6.32*	0.024 ± 0.0008	+0.003	-14.29*
Aflatoxin B1	0.200 ± 0.0012	-0.470	70.15	0.100 ± 0.0045	-0.180	64.29	0.298 ± 0.0057	68.60	0.005 ± 0.0009	-0.016	76.19
Aflatoxin B1 + Plant A	0.350 ± 0.0020	-0.320	47.76	0.160 ± 0.0050	-0.120	42.86	0.510 ± 0.0065	46.32	0.009 ± 0.0008	-0.012	57.14
Aflatoxin B1 + Plant B	0.410 ± 0.0018	-0.260	38.81	0.180 ± 0.0045	-0.100	35.71	0.590 ± 0.0070	37.89	0.011 ± 0.0007	-0.010	47.62
Aflatoxin B1 + Plant C	0.450 ± 0.0015	-0.220	32.84	0.200 ± 0.0040	-0.080	28.57	0.620 ± 0.0060	34.74	0.012 ± 0.0006	-0.009	42.86

Notes: Data for aflatoxin B1 treatments are at 2000 µg/L. "Difference with Control" is calculated as treatment mean minus control mean (positive values indicate enhancement, negative values indicate reduction). Negative % inhibition (*) indicates enhancement relative to control.

The enhancement of chlorophyll and carotenoid contents by crucifer extracts alone suggests a stimulatory effect on photosynthetic pigment synthesis, potentially driven by their antioxidant properties or nutrient contributions (Fahey *et al.* 2001). Extract C (*Brassica oleracea* var. *italica*) exhibited the most pronounced increase, elevating chlorophyll a by 0.040% (5.97%), chlorophyll b by 0.020% (7.14%), total chlorophyll by 0.060% (6.32%), and carotenoids by 0.003% (14.29%), likely reflecting broccoli's higher glucosinolate and vitamin content compared to *Lepidium sativum* (extract B) or the *Raphanus sativus* (extract A) (Mithen *et al.* 2000). These enhancements, though modest, indicate a potential role for crucifer extracts in boosting photosynthetic capacity under non-stress conditions, with carotenoids showing a relatively larger proportional increase, possibly due to their role in photoprotection (Taiz and Zeiger 2010).

Aflatoxin B1's severe reduction of pigment levels at 2000 µg/L—chlorophyll a by 0.470% (70.15%), chlorophyll b by 0.180% (64.29%), total chlorophyll by 0.652% (68.60%), and carotenoids by 0.016% (76.19%)—aligns with its known disruption of chloroplast function and pigment biosynthesis pathways (Kang 1970; Das *et al.* 2015). The greater inhibition of carotenoids (76.19%) compared to total chlorophyll (68.60%) may reflect their vulnerability as sacrificial antioxidants under toxin-induced oxidative stress, a pattern consistent with prior mycotoxin studies (Pitt and Hocking 70). Co-treatment with crucifer extracts significantly mitigated these losses, with extract C reducing the differences from control to -0.220% for chlorophyll a (32.84% inhibition), -0.080% for chlorophyll b (28.57% inhibition), -0.330% for total chlorophyll (34.74% inhibition), and -0.009% for carotenoids (42.86% inhibition). This substantial recovery suggests that extract C's bioactive compounds—possibly isothiocyanates or phenolics—may neutralize aflatoxin B1's oxidative effects or stabilize chloroplast membranes (Mithen *et al.* 2000).

In Bihar, where aflatoxin contamination jeopardizes maize yield and quality, these findings position crucifer extracts—particularly from *Brassica oleracea* var. *italica*—as a dual-purpose tool for enhancing pigment levels and counteracting phytotoxicity (Bhat and Vasanthi 2003). The differential efficacy among extracts highlights the importance of species-specific phytochemical profiles, with broccoli's superior performance likely tied to its rich antioxidant capacity (Kumar *et al.* 2014). Future research should explore the molecular mechanisms—such as upregulation of chlorophyll biosynthesis enzymes (e.g., magnesium chelatase) or carotenoid stabilization pathways—and evaluate field-scale applications to confirm their practicality for farmers facing aflatoxin challenges (Taiz and Zeiger 2010).

4. CONCLUSION

This study confirms that aflatoxin B1 at 2000 µg/L severely depletes maize seedling pigments, reducing chlorophyll a by 0.470% (70.15%), chlorophyll b by 0.180% (64.29%), total chlorophyll by 0.652% (68.60%), and carotenoids by 0.016% (76.19%) relative to controls. However, crucifer extracts A (*Raphanus sativus*), B (*Lepidium sativum*), and C (*Brassica oleracea* var. *italica*)—most notably C—markedly ameliorate these losses, with C limiting reductions to -0.220% for chlorophyll a (32.84%), -0.080% for chlorophyll b (28.57%), -0.330% for total chlorophyll (34.74%), and -0.009% for carotenoids (42.86%). Alone, the extracts enhance pigment contents by up to 0.060% (6.32%) for total chlorophyll and 0.003% (14.29%) for carotenoids, indicating a dual capacity to promote photosynthesis and mitigate stress. These results underscore the potential of crucifer extracts, particularly from broccoli, as natural protectants for maize in aflatoxin-contaminated regions like Bihar, India, meriting further investigation into their biochemical mechanisms and practical deployment in sustainable agriculture (Pitt and Hocking 2009).

CONFLICT OF INTERESTS

None.

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