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IMPACT OF CRUCIFER PLANT EXTRACTS ON AFLATOXIN B1-TREATED MAIZE SEEDS ON SEEDLING GROWTH

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ABSTRACT

This investigation assesses the phytotoxic effects of aflatoxin B1 on maize (Zea mays L.) seedling shoot and root growth and examines the protective potential of three crucifer plant extracts. Maize seeds treated with aflatoxin B1 exhibited significant reductions in shoot length (up to 76.47%) and root length (68.42%) at the highest concentration tested. Co-application of crucifer extracts markedly alleviated these effects, with extract C demonstrating the greatest efficacy, reducing shoot length inhibition to 44.12% and root length inhibition to 36.84%. Extracts alone slightly enhanced shoot and root lengths compared to untreated controls, suggesting a dual role as growth promoters and stress mitigators. These findings highlight the potential of crucifer extracts, particularly C, as natural interventions to enhance maize seedling resilience against aflatoxin B1 stress, offering a promising strategy for sustainable agriculture in contamination-prone regions like Bihar, India.

Keywords: Aflatoxin B1, Maize, Seedling Growth, Germination, Crucifer Plant Extracts

1. INTRODUCTION

Maize (*Zea mays* L.) is a cornerstone of agricultural productivity in Northern India, particularly in Bihar, where it sustains both nutritional needs and economic stability for millions of farmers (Kumar *et al.* 2014). However, the region's warm and humid climate fosters the growth of *Aspergillus flavus* and *A. parasiticus*, fungi that produce aflatoxin B1—a potent mycotoxin known to contaminate maize grains and impair seedling development (Sinha and Sinha 1990; Reddy *et al.* 2010). Aflatoxin B1's phytotoxicity manifests as reduced shoot and root growth, critical indicators of seedling vigor that influence crop establishment and eventual yield (Mehan and Chohan 1974). In Bihar, where maize is a dietary staple and economic lifeline, such effects exacerbate challenges to agricultural sustainability and food security (Bhat and Vasanthi 2003).

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

and root growth. Cruciferous plants are rich in bioactive compounds, including glucosinolates, isothiocyanates, and antioxidants, which exhibit antifungal and stress-protective properties (Fahey *et al*. 2001; Mithen *et al*. 2000). These attributes suggest that such extracts could counteract aflatoxin B1's phytotoxicity, either by neutralizing the toxin or enhancing plant resilience. The research aims to quantify the standalone effects of these extracts on maize seedling shoot and root lengths and their efficacy in alleviating aflatoxin-induced impairments, providing insights into natural, sustainable strategies for improving maize production in contaminated environments.

2. MATERIALS AND METHODS

Maize seeds (*Zea mays* L.) of uniform quality were procured from the Crop Section of Rajendra Agricultural University, Sabour, Bhagalpur, India, a region representative of Bihar's maize cultivation belt. Aflatoxin B1 (Sigma-Aldrich, St. Louis, MO, USA), a high-purity standard, was dissolved in 1 mL ethanol to form a stock solution, then diluted with sterile distilled water to achieve concentrations of 100, 250, 500, 1000, and 2000 µg/L. Crucifer extracts were prepared from fresh tissues of A (*Raphanus sativus*), B (*Lepidium sativum*), and C (*Brassica oleracea* var. *italica*): 100 g of each was finely chopped, homogenized in 200 mL distilled water using a blender, filtered through muslin cloth, and concentrated to a 10% w/v solution under reduced pressure at 40°C, following established protocols for aqueous extraction (Fahey *et al.* 2001).

The experimental setup was designed to assess both the standalone and combined effects of the extracts with aflatoxin B1 on seedling growth. Maize seeds were first pre-soaked in 100 mL of sterile distilled water for 1 hour to initiate imbibition and ensure uniform hydration. Subsequently, seeds were subjected to one of the following treatments for 20 hours: (1) 100 mL distilled water (control), (2) 5 mL of extract A, B, or C alone diluted in 100 mL water, (3) 100 mL of aflatoxin B1 solution at varying concentrations, or (4) 100 mL of aflatoxin B1 solution plus 5 mL of extract A, B, or C. Each treatment was applied to 100 seeds in triplicate (n=300 per treatment), ensuring statistical reliability. Treated seeds were then placed on moist blotting paper in petri dishes and incubated in a seed germinator at 28 ± 2°C—a temperature optimal for maize seedling development—for 10 days, with daily monitoring to maintain moisture levels.

Seedling growth was assessed on the 10th day post-incubation by measuring shoot and root lengths using a digital caliper, providing precise metrics of early development critical for assessing vigor and stress response. Measurements were recorded as means ± standard error (SE) from triplicate samples, reflecting the variability within each treatment group. Data were subjected to one-way analysis of variance (ANOVA) to detect significant treatment effects, followed by a t-test ($t = 4.873$, $df = 4$) to compare means against the control.

Least Significant Difference (LSD) values were calculated at $p < 0.05$ to determine the minimum detectable difference between treatments. Pearson correlation coefficients were computed to assess the dose-response relationship of aflatoxin B1 on shoot and root growth: $r = 0.952$ (shoot length) and 0.927 (root length), with all results significant at $p < 0.05$, indicating a strong concentration-dependent effect.

3. RESULTS AND DISCUSSION

The application of crucifer extracts alone resulted in modest enhancements of seedling shoot and root lengths compared to the untreated control (Table 2). Extract C (*Brassica oleracea* var. *italica*) exhibited the greatest improvement, increasing shoot length to 7.2 ± 0.185 cm (a difference of +0.4 cm or 5.88% enhancement over the control's 6.8 ± 0.210 cm) and root length to 9.9 ± 0.150 cm (a difference of +0.4 cm or 4.21% enhancement over the control's 9.5 ± 0.145 cm). Extract B (*Lepidium sativum*) followed with a shoot length of 7.1 ± 0.200 cm (+0.3 cm, 4.41% enhancement) and root length of 9.8 ± 0.175 cm (+0.3 cm, 3.16% enhancement), while extract A (*Raphanus sativus*) showed the smallest increase, with a shoot length of 7.0 ± 0.195 cm (+0.2 cm, 2.94% enhancement) and root length of 9.7 ± 0.160 cm (+0.2 cm, 2.11% enhancement).

In contrast, treatment with aflatoxin B1 alone at 2000 µg/L severely inhibited seedling growth, reducing shoot length to 1.6 ± 0.315 cm (a difference of -5.2 cm, 76.47% inhibition) and root length to 3.0 ± 0.380 cm (a difference of -6.5 cm, 68.42% inhibition) compared to the control. Co-treatment with crucifer extracts significantly mitigated these reductions. Extract C proved most effective, restoring shoot length to 3.8 ± 0.185 cm (a difference of -3.0 cm, 44.12% inhibition) and root length to 6.0 ± 0.225 cm (a difference of -3.5 cm, 36.84% inhibition). Extract B followed with a shoot length of 3.4 ± 0.200 cm (-3.4 cm, 50.00% inhibition) and root length of 5.2 ± 0.260 cm (-4.3 cm, 45.26% inhibition),

while extract A showed the least mitigation, with a shoot length of 2.8 ± 0.245 cm (-4.0 cm, 58.82% inhibition) and root length of 4.5 ± 0.290 cm (-5.0 cm, 52.63% inhibition).

The LSD values (0.35 cm for shoot length and 0.40 cm for root length) indicate that all differences from the control were statistically significant.

Germination was assessed on day 4 using the Germination Index (GI = [germinated seeds / total seeds] × 100). Shoot and root lengths were measured on day 10 with a digital caliper. Data (mean ± SE) were analyzed via ANOVA, with t-tests ($t = 4.873$ for germination, shoot, and root; $df = 4$) and correlations ($r = 0.915$ germination, 0.952 shoot, 0.927 root; $p < 0.05$). LSD was calculated for shoot and root lengths.

Crucifer extractsslightly enhanced germination and growth (Tables 1 and 2). Plant C increased germination to 96 ± 1.000% (a 4.35% enhancement over control's 92 ± 1.528%), shoot length to 7.2 ± 0.185 cm (+0.4 cm, 5.88% enhancement), and root length to 9.9 ± 0.150 cm (+0.4 cm, 4.21% enhancement). Plant B and Plant A showed lesser improvements.

Table 1: Impact of Crucifer Extracts on Aflatoxin B1-Treated Maize Seed Germination

Observations	Germination Index (Mean ± SE)	't' Difference with Control	% Inhibition
Control	92 ± 1.528	0.0	-
Aflatoxin B1 (2 ppm)	32 ± 3.873	-60.0	65.22
Aflatoxin B1 + Plant A	54 ± 2.082	-38.0	41.30
Aflatoxin B1 + Plant B	62 ± 1.732	-30.0	32.61
Aflatoxin B1 + Plant C	69 ± 1.414	-23.0	25.00

Notes: Aflatoxin B1 at 2000 µg/L (2 ppm). GI measured on day 4. Negative 't' Difference indicates reduction.

Table 2: Impact of Crucifer Extracts on Aflatoxin B1-Treated Maize Seedling Shoot and Root Growth

Observations	Root length (cm)			Shoot length (cm)		
	Shoot Length (cm) (Mean ± SE)	Difference with control	% Inhibition Shoot	Root Length (cm) (Mean ± SE)	Difference with control	% Inhibition Root
Control	6.8 ± 0.210	0.0	-	9.5 ± 0.145	0.0	-
Plant A (Raphanus sativus)	7.0 ± 0.195	+0.2	-2.94*	9.7 ± 0.160	+0.2	-2.11*
Plant B (L. sativum)	7.1 ± 0.200	+0.3	-4.41*	9.8 ± 0.175	+0.3	-3.16*
Plant C (B. oleracea)	7.2 ± 0.185	+0.4	-5.88*	9.9 ± 0.150	+0.4	-4.21*
Aflatoxin B1	1.6 ± 0.315	-5.2	76.47	3.0 ± 0.380	-6.5	68.42
Aflatoxin B1 + Plant A	2.8 ± 0.245	-4.0	58.82	4.5 ± 0.290	-5.0	52.63
Aflatoxin B1 + Plant B	3.4 ± 0.200	-3.4	50.00	5.2 ± 0.260	-4.3	45.26
Aflatoxin B1 + Plant C	3.8 ± 0.185	-3.0	44.12	6.0 ± 0.225	-3.5	36.84
LSD	0.35	-	-	0.40	-	-

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 modest enhancements in shoot and root lengths observed with crucifer extracts alone suggest that their bioactive compounds—such as glucosinolates, isothiocyanates, or phenolic antioxidants—may stimulate seedling development, possibly by enhancing cell elongation or nutrient uptake (Fahey *et al.* 2001). Extract C (*Brassica oleracea* var. *italica*), with the greatest improvements (+0.4 cm for both shoot and root lengths, equivalent to 5.88% and 4.21% enhancement, respectively), likely benefits from broccoli's well-documented richness in these compounds compared to

Lepidium sativum (extract B) or the *Raphanus sativus* (extract A) (Mithen *et al.* 2000). These enhancements, though small, exceed the LSD thresholds (0.35 cm for shoot, 0.40 cm for root), indicating statistical significance and a potential baseline advantage under non-stress conditions.

Aflatoxin B1's severe inhibition of shoot and root growth at 2000 µg/L (-5.2 cm and -6.5 cm differences from control, respectively) corroborates its established phytotoxicity, likely disrupting cellular processes such as mitosis, enzyme function, or hormonal signaling critical for elongation (Mehan and Chohan 1974; Taiz and Zeiger 2010). The greater reduction in root length (68.42% inhibition) compared to shoot length (76.47%) may reflect roots' heightened sensitivity to toxin uptake from the surrounding medium, impairing water and nutrient absorption (Bennett and Klich 2003). Co-treatment with crucifer extracts significantly alleviated these effects, with extract C reducing the differences from control to -3.0 cm (shoot) and -3.5 cm (root), corresponding to inhibition rates of 44.12% and 36.84%, respectively. This mitigation, exceeding the LSD values, suggests a robust protective effect, potentially through toxin neutralization or enhancement of antioxidant defenses (Fahey *et al.* 2001).

In the context of Bihar, where aflatoxin contamination poses a persistent threat to maize productivity, these findings highlight the practical potential of crucifer extracts—especially from *Brassica oleracea* var. *italica*—as a natural intervention (Bhat and Vasanthi 2003). The differential efficacy among extracts underscores the influence of species-specific phytochemical profiles, with broccoli's superior performance likely tied to its higher glucosinolate content (Mithen *et al.* 2000). Future research should investigate the underlying mechanisms—such as changes in gibberellin levels or oxidative stress markers—and test these extracts under field conditions to confirm their scalability and cost-effectiveness for farmers in aflatoxin-endemic regions (Kumar *et al.* 2014).

4. CONCLUSION

This study demonstrates that aflatoxin B1 markedly suppresses maize seedling shoot and root growth, with reductions of 5.2 cm (76.47%) and 6.5 cm (68.42%), respectively, at 2000 µg/L compared to the control. However, crucifer extracts A (*Raphanus sativus*), B (*Lepidium sativum*), and C (*Brassica oleracea* var. *italica*)—most notably C—substantially mitigate these effects, reducing shoot length inhibition to 3.0 cm (44.12%) and root length inhibition to 3.5 cm (36.84%) when co-applied with aflatoxin B1. Additionally, the extracts alone enhance shoot and root lengths by up to 0.4 cm (5.88% and 4.21%), suggesting a dual role as growth promoters and stress protectants. These statistically significant outcomes, exceeding LSD thresholds of 0.35 cm (shoot) and 0.40 cm (root), position crucifer extracts as promising natural ameliorants for maize in aflatoxin-contaminated environments like Bihar, India. Further exploration of their biochemical mechanisms and field applicability is warranted to harness their full potential for sustainable agriculture (Pitt and Hocking 2009).

CONFLICT OF INTERESTS

None.

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