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Effect of aflatoxin B_1 on seed germination, seedling growth, chlorophyll and carotenoid contents in mustard (*Brassica juncea* L.) seeds

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Five different concentrations (100, 250, 500, 1000 and 2000 µg l) of aflatoxin B_1 were found to be inhibitory to seed germination and seedling growth (root and shoot lengths) of mustard seeds (variety Varuna). They also lowered the levels of chlorophyll and carotenoids in the emerging leaves during seedling growth. The inhibitory effect was directly correlated with the concentration of treated toxin.

Key words : Aflatoxin, B1 carotenoid, chlorophyll, mustard seeds, seed germination and seedling growth

INTRODUCTON

Mustard (*Brassica juncea* L.) or rai is the most important oil-seed crop of Northern India. This crop is extensively grown in Bihar but this state also provides ideal conditions for the natural contamination of mycotoxins in different agricultural crops (Sinha, 1993; Ahmad, 1999). Aflatoxin B_1 has earlier been found to restrict plant growth by inhibiting seed germination, seedling growth and other physiological processes of the crops (Sinha *et al*., 1996). Since aflatoxins were one of the major contaminants of mustard seeds in Bihar, an attempt has been made in this investigation to record various physiological changes induced by aflatoxin B_1 during germination of mustard seeds.

MATERIALS AND METHODS

Effect on seed germination and seedling growth

Mustard seeds (variety Varuna) were obtained from Oilseed section, Rajendra Agriculture University, Sabour, Bhagalpur. A stock solution of aflatoxin B_1 (Sigma, St. Louis, Missouri, USA) was initially prepared in 1 ml ethanol from which the dilutions (100, 250, 500, 1000, and 2000 µg/l) were made with distilled water. The seeds were soaked initially in sterile distilled water for one hour and subsequently in different concentrations of aflatoxin B_1 solutions for 20 hours. For each treatment 100 seeds were taken in triplate. The soaked seeds were placed on moist blotting paper and kept for germination in seed germinator at 28 ± 2 ° C.

For germination study, the seeds were observed on 4th day and germination Index (GI) was calculated by the following formula :

$$GI = \frac{\text{Total no. of seeds germinated}}{\text{Total no. of seeds observed}} \times 100$$

Seedling growth was recorded on 10th day by measuring the lengths of radicle and plumule.

Effect on chlorophyll and carotenoid contents

Chlorophyll and carotenoid contents of the newly emerged leaves were estimated by methods of Arnon (1949) and Davis (1969), respectively 250 mg leaf tissue was extracted in 5 ml 80 % acetone. Resulting green liquid/extract was transferred to Buchner funnel containing Whatman No. 1 filter paper. Extraction of tissue was repeated 2 – 3 times with 5 ml of 80 % acetone which was subsequently filtered into the flask containing initial extract. With another 5 ml of acetone (80 %), the morter, pestle and sides of the funnel were rinsed. Finally the volume of filtrate was made to 25 ml by adding extra amount of 80 % acetone. The optical density of the extract was recorded in spectrophotometer set at 480, 645 and 663nm against blank (80 % acetone). The amount of chlorophyll present in the extract was calculated by the following formulae in terms of mg/g dry

weight :

$$\text{mg chl-a/g tissue} = \frac{12.7\,(D663) - 2.69\,(D645) \times V}{1000 \times W}$$

$$\text{mg chl-b/g tissue} = \frac{22.9\,(D645) - 4.68\,(D663) \times V}{1000 \times W}$$

Total chlorophyll = chl-a + chl-b

where,

D = optical density at specific wave length

V = final volume of the 80 % acetone-chlorophyll extract

W = fresh weight in g of the tissue extracted

Determination of the total carotenoid contents of the tissue in presence of chlorophyll had been made by the method of Davis (1969). Contributions by chl-a and chl-b to the extinction at 480 nm were determined using the extinction coefficient of the chlorophyll at the wave length. The increase in absorbancy at 480 nm which is due to the carotenoid formation (ΔE Car 480) is given by

$$\Delta E\ Car480 = \Delta E\ 480 + 0.114\ \Delta E\ 663 - 0.638\ \Delta E\ 645$$

where, ΔE Car 480 = Total carotenoids

ΔE = Extinction coefficient

RESULTS AND DISCUSSION

Inhibitions in seed germination were apparent due to aflatoxin B_1 and these inhibitions were directly correlated with the concentrations (100, 250, 500, 1000, 2000 µg/l) of the toxin (Table 1). Maximum inhibition (81%) was recorded due to 2000 µg/l concentration of aflatoxin B_1. Inhibitions in seed germinations were highly significant due to aflatoxin B_1 treatment. Normal seedling growth (both shoot and root lengths) was also found to be reduced drastically due to the inhibitory effects of aflatoxin B_1 (Table 1).

The shoot length in control set was 5.72 cm which was reduced upto 1.09 cm (80.94 %) due to treatment with highest concentration (2000µg/l) of aflatoxin B_1. Likewise the root length was also inhibited by 78.75 % due to that concentration of aflatoxin B_1, respectively. These inhibitions were also highly significant (Table 1).

There is slight variation in the chlorophyll and carotenoids contents of the cotyledonary leaves of mustard variety. Percentage chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid contents were 0.6531, 0.2061, 0.8592 and 0.0132 respectively (Table 2). However, there was gradual depletion in chlorophyll as well as carotenoid levels due to toxic effect of varied concentrations of aflatoxin B_1.

Maximum inhibitions in chlorophyll-a and chlorophyll-b levels were recorded as 73.84 and 46.14%, respectively, at 2000 µg/l concentration of aflatoxin B_1 followed by 41.67 and 26.34%, 26.33 and 16.93%, 13.18 and 10.62%, 5.87 and 1.69% inhibitions at 1000, 500, 250 as well as 100 µg/l concentrations of the same toxin, respectively. The highest concentration (2000 µg/l) of aflatoxin B_1 depleted total chlorophyll and carotenoid contents by 67.20 and 81% respectively. Statistical statistical analysis of the data revealed that inhibitions in chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid contents were significant at all the concentrations of aflatoxin B_1.

As is evident from Table 1, aflatoxin B_1 inhibited seed germination and the inhibition was directly correlated with the concentration of the toxin. Drastic reduction was also observed in the seedling growth due to the inhibitory effects of aflatoxin B_1. Aflatoxins have earlier been reported to have inhibitory effects on seed germination and seedling growth of various plants by different workers. Mehan and Chohan (1974) recorded 100% inhibition in seed germination of *Phaseolus aureus* due to the toxins present in culture filtrate of toxigenic strain of *A. flavus*. Seed germination of cowpea, lettuce, *Lepidium sativum*, sorghum, maize and other crops has also been shown to be inhibited by the treatment with different levels of aflatoxins (Schoental and White, 1965; Adekunle and Bassir, 1973; Crisan, 1973; Tripathi, 1973; Tripathi and Mishra, 1983).

Because of restricted seedling growth chlorophyll level has been greatly reduced due to aflatoxin B_1. chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid were found to be inhibited although the rate of inhibition was variable at different levels of toxin treatment (Table 2). At the same time, it was also noted that the highest concentration

Table 1. Effect of aflatoxin B_1 on seed germination, seedling growth (root and shoot lengths) of mustard

Conc. of Afl. B_1 (µg/l)	% germination mean ± SE	Shoot length mean ± SE (cm)	Root length mean ± SE (cm.)	% inhibition in		
				Germination	Shoot length	Root length
0	94 ± 1.624	5.72 ± 0.283	9.79 ± 0.126	--	--	--
100	86 ± 1.673	5.28 ± 0.178	8.75 ± 0.19	8.51	7.69	10.62
250	81 ± 1.099	4.62 ± 0.163	6.94 ± 0.198	13.82	19.23	29.11
500	69 ± 1.938	3.45 ± 0.097	5.12 ± 0.187	26.59	39.68	47.70
1000	38 ± 2.507	2.01 ± 0.181	4.10 ± 0.217	59.57	64.86	58.12
2000	19 ± 2.564	1.09 ± 0.043	2.08 ± 0.421	79.78	80.94	78.75
t=	8.442	5.930	4.840	--	df= 4	--
r=	0.973	0.947	0.924			

(All values are highly significant at all the concentrations of toxin)

(2000µg/l) of aflatoxin B_1 was always more lethal in reducing different types of pigments in the seedling. Earlier workers have also recorded inhibitions in chlorophyll levels due to aflatoxins. Kang (1970) observed significant inhibition in chlorophyll synthesis in the cotyledonary leaves of *Abelmoschus esculentum* due to aflatoxin B_1 depending upon its concentration. This result was also confirmed by Mehan (1971) in his studies. Use of mustard and gram seedling germination inhibition assay has also been proposed for aflatoxin B_1 (Sinha *et al.*, 1992).

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Table 2. Effect of aflatoxin B1 on chlorophyll and carotenoid contents of mustard seedlings

Conc. of Afl. B_1 (µg/l)	% chl.a mean ± SE	% chl. b mean ± SE	% total chl. mean ± SE	% of carotenoid mean ± SE	% inhibition in			
					chl.a	chl.b	T.chl.	Carotenoid
0	0.6531 ± 0.0025	0.2061 ± 0.0075	0.8592 ± 0.0083	0.0132 ± 0.0010	--	--	--	--
100	0.6147 ± 0.0017	0.2026 ± 0.0035	0.8173 0.003	0.0124 ± 0.0007	5.87	1.69	4.87	6.06
250	0.5670 ± 0.0022	0.1892 0.0009	0.7562 ± 0.0028	0.0110 ± 0.0006	13.18	10.62	11.98	16.66
500	0.4811 ± 0.007	0.1712 ± 0.0005	0.6523 ± 0.0009	0.0098 ± 0.0007	26.33	16.93	24.08	25.75
1000	0.3809 ± 0.0008	0.1518 ± 0.0007	0.5327 ± 0.0008	0.0049 ± 0.0009	41.67	26.34	38.00	62.87
2000	0.1708 ± 0.0010	0.1110 ± 0.0056	0.2818 ± 0.0049	0.0024 ± 0.0008	73.84	46.14	67.20	81.81
t =	18.275	14.736	17.721	7.725		df= 4		
r =	0.994	0.991	0.994	0.968				

(All values are highly significant at all the concentrations of toxin)

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