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EFFECT OF MYCOTOXINS ON PIGMENT SYNTHESIS IN MUSTARD (*Brassica juncea* L.) SEEDS (VAR.PUSA BOLD)

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ABSTRACT

Mycotoxins are toxic secondary metabolites produced by fungi that usually belong to the genera like *Aspergillus*, *Penicillium* and *Fusarium*. These can be produced on a wide range of agricultural commodities under varied ecological coditions worldwide. Mycotoxin contaminated products cause significant agro-economic and trade problems at every stages of crop cultivation up to marketing.The significance of mycotoxin in International trade is being recognized both by the developed and developing countries by which the export of agricultural commodities is also being affected. Some mycotoxins particularly aflatoxin, citrinin and zearalenone have been analysed as natural contaminats of various crops including mustard from Bihar state at different stages of the crop development, harvesting and storage. In this investigation effects of five different concentrations (viz.,100,250,500,1000 and 2000µg/l) of aflatoxinB_1,citrinin and zearalenone were evaluated against synthesis of different pigments (viz., chlorophyll and carotenoids) in the emerging leaves of mustard seeds (var. Pusa bold) during seedling growth.The inhibitory effects of those myctoxins were observed at all concentrations which were directly correlated with the concentration of the treated toxins. Maximum inhibitions in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were 78.5, 56.7 67.6 and 84.9% in aflatoxinB1 treated seeds whereas 57.8, 38.3, 61.9 and 68.9% and 64.4, 42.7, 59.2 and 76.1% inhibitions were recorded by citrinin and zearalenone, respectively.

KEY WORDS: Mycotoxins, Chlorophyll, Carotenoid and Mustard seeds.

INTRODUCTION

The oil-seeds are used for various edible and industrial purposes. Among all oil-seeds, mustard (*Brassica juncea* L.) is one of the most important crop in Bihar but this state also provides ideal environmental conditions for the natural contamination of mycotoxins in different agricultural crops (Sinha, 1993; Ahmad, 1999 and 2007). 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*., 1992, Sinha, 1993 and Prasad *et al*., 1996). Since mycotoxins were one of the major contaminants of mustard seeds in Bihar (Ahmad, 1999), an attempt has been made in this investigation to record various physiological changes induced by aflatoxinB_1,citrinin and zearalenone on chlorophyll and carotenoid syntheses in the cotyledonary leaves of mustard (var. Pusa bold).

MATERIALS AND METHODS

Seeds of mustard (var. Pusa bold) were obtained from Bihar Agricultural University, Sabour, Bhagalpur. A stock solution of aflatoxin B_1 (obtained from Sigma, St.Louis, Missouri, USA) was initially prepared in 1ml ethanol from which the dilutions (100, 250, 500, 1000 & 2000 µg/l) were made with distilled water.Stock solutions of citrinin and zearalenone were also prepared like aflatoxin B_1.

Chlorophyll and carotenoid contents of the newly emerged leaves were estimated by the method of Arnon (1949) and Davis (1969), respectively. 250 mg leaf tissue was extracted in 5 ml 80 % of 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 80 % of acetone, which was subsequently filtered into the flask containing initial extract. With another 5 ml of acetone (80 %) the mortar-pestle and sides of 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 663 nm against blank (80 % acetone). The amount of chlorophyll present in the extract was calculated by following formulae in terms of mg/g dry weight:

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

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

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 (1996). Contributions by chl-a andchl-b to the extinction at 480 nm were determined using the extinction coefficient of the chlorophyll at that wavelength. The increase in absorbancy at 480 nm which is due to the carotenoid formation (E Car 480) is given by

E Car 480 = E Car 480 + 0.114 E 663 – 0.63 E 645

Where,
E car 480 = Total carotenoids
E = Extinction coefficient.

All the results were subjected to one way analysis of variance.

RESULTS AND DISCUSSION

Visual chlorosis of the cotyledonery leaves of mustard was confirmed from Tables –1, 2, 3 & Fig.I showing the gradual depletion in chlorophyll as well as carotenoid levels due to toxic effect of varied concentrations of aflatoxin B_1 and zearalenone. Percentage chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid contents were 0.6638, 0.2098, 0.8736 and 0.0133, respectively (Table-1). However, there was gradual depletion in chlorophyll as well as carotenoid levels as were recorded as 78.5 and 56.7 %, respectively, at 2000 µg/l concentration of aflatoxin B_1 followed by 39.8 and 26.7 %, 25.0 and 16.3 %, 12.2 and 8.3 %, 6.0 and 3.6 % inhibitions at 1000, 500, 250 as well as 100 µg/l concentrations. 2000 µg/l of aflatoxin B_1 depleted total chlorophyll and carotenoid contents by 67.6 and 84.9 %, respectively.

As is evident from Table-2, maximum inhibitions in chlorophyll-a and chlorophyll-b levels were recorded as 57.8 and 38.3 %, respectively, at 2000 µg/l concentration of citrinin followed by 36.3 and 25.3 %, 22.1 and 11.4 %, 7.9and 5.7 %, 5.0 and 3.0 % inhibitions at 1000, 500, 250 as well as 100 µg/l concentrations of the same toxin, respectively. The highest concentration (2000 µg/l) of citrinin depleted total chlorophyll and carotenoidcontents by 61.9 and 68.9 %, respectively.

As is evident from Table-3, maximum inhibitions in chlorophyll-a and chlorophyll-b levels were recorded as 64.4 and 42.7 %, respectively, at 2000 µg/l concentration of zearalenone followed by 39.4 and 27.7 %, 26.8 and 13.1 %, 8.7and 5.3 %, 5.5 and 4.3 % inhibitions at 1000, 500, 250 as well as 100 µg/l concentrations of the same toxin, respectively. The highest concentration (2000 µg/l) of citrinin depleted total chlorophyll and carotenoidcontents by 59.2 and 76.1 %, respectively. 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 all toxins (aflatoxin B_1,citrinin and zearalenone).

As is evident from Tables-1,2&3 chlorophyll and carotenoid syntheses in the emerging leaves were also inhibited drastically due to toxin treatments (aflatoxin B_1,citrinin and zearalenone) although the rate of inhibition was variable at different levels of toxin treatment. At the same time, it was also noted that the higher concentration (2000 µg/l) of both aflatoxin B_1 was always more lethal in reducing different types of pigments of the mustard when compared with that of citrinin and zearalenone.

Restricted seedling growth coupled with certain genetic as well as metabolic disturbance due to toxins may be the reason behind the reduction of pigment levels of the leaves of the mustard. Earlier workers have also recorded inhibitions in chlorophyll levels due to aflatoxins. Kang (1970) observed significant inhibition in chlorophyll syntheses in the cotyledonary leaves of *Abelmoschusesculentum* due to aflatoxin B_1 depending upon its concentration. Aflatoxin B_1 has been known to exert deleterions effect on chlorophyll syntheses in *Lepidiumsativum* (Schoental and White, 1965), *Raphanussativus* as well as *Sorghum vulgare*(Mehan and Chohan, 1974), *Vignaradiata* (Sinha and Kumari, 1989) and in wheat (Sinha, 1991).

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Table-1
Effect of aflatoxin B_1 on chlorophyll-a,b,Total chlorophyll and carotenoid contents of mustard seedlings

Conc.OfAfl. B_1 (µg/l)	% Amount				% Inhibition			
	Chl. a Mean ± SE	Chl. B Mean ± SE	Total chl. (a+b) Mean ± SE	Carotenoid Mean ± SE	Chl. a	Chl. b	Total chl.	Carotenoid
0	0.6638 ± 0.0030	0.2098 ± 0.0038	0.8736 ± 0.0068	0.0133 ± 0.0016	-	-	-	-
100	0.6235 ± 0.0012	0.2021 ± 0.0024	0.8256 ± 0.0034	0.0129 ± 0.0018	6.0	3.6	5.4	3.0
250	0.5827 ± 0.0016	0.1923 ± 0.0012	0.7750 ± 0.0028	0.0118 ± 0.0002	12.2	8.3	11.2	11.2
500	0.4976 ± 0.004	0.1756 ± 0.0024	0.6732 ± 0.0064	0.0101 ± 0.0008	25.0	16.3	22.9	24.0
1000	0.3992 ± 0.0006	0.1536 ± 0.0008	0.5528 ± 0.0014	0.0052 ± 0.0012	39.8	26.7	36.7	60.9
2000	0.1424 ± 0.0022	0.0908 ± 0.0023	0.2332 ± 0.0045	0.0020 ± 0.0017	78.5	56.7	67.6	84.9
r	-.9977528	-.9984235	-.9980073	-.9778082				
t	29.78286	35.57604	31.63335	9.334598				
d.f.	4	4	4	4				

Table- 2
Effect of citrinin on chlorophyll a, b,Total chlorophyll and carotenoid contents of mustard seedlings.

Conc. Of Citrinin (µg/l)	% Amount				% Inhibition			
	Chl. a Mean ± SE	Chl. b Mean ± SE	Total chl. (a+b) Mean ± SE	Carotenoid Mean ± SE	Chl. a	Chl. b	Total chl.	Carotenoid
0	0.6258 ± 0.0007	0.2013 ± 0.0005	0.8271 ± 0.0012	0.0129 ± 0.0004	-	-	-	-
100	0.5943 ± 0.0014	0.1952 ± 0.010	0.7882 ± 0.0024	0.0126 ± 0.0008	5.0	3.0	4.7	2.3
250	0.5762 ± 0.0007	0.1898 ± 0.0007	0.7660 ± 0.0014	0.0119 ± 0.0006	7.9	5.7	7.3	7.7
500	0.4874 ± 0.0003	0.1783 ± 0.0016	0.6657 ± 0.0019	0.0110 ± 0.0005	22.1	11.4	19.5	14.4
1000	0.3985 ± 0.0009	0.1492 ± 0.0009	0.5477 ± 0.0018	0.0082 ± 0.0004	36.3	25.3	33.7	36.4
2000	0.2635 ± 0.0010	0.1241 ± 0.0005	0.3151 ± 0.0015	0.0040 ± 0.0009	57.8	38.3	61.9	68.9
r	-.9867613	-.9860025	-.9770665	-.9986431				
t	12.16875	11.8275	26.05337	38.353				
d.f.	4	4	4	4				

Table- 3
Effect of zearalenone on chlorophyll a, b,Total chlorophyll and carotenoid contents of mustard seedlings.

Conc. of Zear. (µg / l)	% Amount				% Inhibition			
	Chl. a Mean ± SE	Chl. b Mean ± SE	Total chl. (a+b) Mean ± SE	Carotenoid Mean ± SE	Chl. a	Chl. b	Total chl.	Carotenoid
0	0.6486 ± 0.0011	0.2010 ± 0.0007	0.8496 ± 0.0018	0.0134 ± 0.0007	_	_	_	_
100	0.6128 ± 0.0010	0.1923 ± 0.0019	0.8051 ± 0.0029	0.0130 ± 0.0003	5.5	4.3	5.2	3.0
250	0.5916 ± 0.0006	0.1902 ± 0.0005	0.7818 ± 0.0011	0.0121 ± 0.0003	8.7	5.3	7.9	9.7
500	0.4742 ± 0.0005	0.1745 ± 0.0012	0.6487 ± 0.0017	0.0106 ± 0.0006	26.8	13.1	23.6	20.8
1000	0.3926 ± 0.0009	0.1452 ± 0.0007	0.5378 ± 0.0016	0.0097 ± 0.0008	39.4	27.7	36.6	27.6
2000	0.2309 ± 0.0011	0.1151 ± 0.0009	0.3460 ± 0.0020	0.0032 ± 0.0003	64.4	42.7	59.2	76.1
r	-.9832148	-.9885293	-.9853407	-.9879699				
t	10.7778	13.09058	11.55161	12.77714				
d.f.	4	4	4	4				