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Aflatoxin induced variations in protein and nucleic acid contents of mustard (*Brassica juncea* L. var. Pusa bold) seeds

K. K. SINHA AND M. S. AHMAD
University Department of Botany, T. M. Bhagalpur University, Bhagalpur 812007

Aflatoxins are secondary toxic metabolites produced by the toxigenic strains of *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare on various agricultural commodities. Besides causing quadruple threats to animals and human beings these toxins have also been shown to influence the physiological and biochemical processes of crop plants. Aflatoxins have been analysed as natural contaminants of various crops including mustard from Bihar State at different stages of crop development, harvesting and storage. Earlier aflatoxin B_1 was found to inhibit seed germination, seedling growth and chloroplast pigments of mustard (*Brassica juncea* L. var. Pusa bold) seeds. In this investigation five different concentrations (viz., 100, 250, 500, 1000 and 2000 µg/l) of aflatoxin b_1 were found to inhibit the synthesis of protein and nucleic acids at varying levels. The percentage inhibition in these components varied with the concentration of treated toxin. Maximum inhibition in protein, DNA and RNA levels was recorded as 28.33, 39.72 and 52.29% at 2000 µg/l concentration of aflatoxin B_1. Gel electrophoretic studies also revealed variations in the quality of protein due to toxin treatment.

Key words : Aflatoxin B_1, DNA and RNA, protein, mustard seeds

INTRODUCTION

Oil seeds have great significance in the life of human beings. Because of their specific flavour and palatability, different oil-seeds are used by the people in different parts of the world. In India, particularly in Northern parts, mustard (*Brassica juncea* L.) or rai is the most important oil-seed crop. The oil content of the seeds ranges from 30 to 40% and its oil is main cooking medium in this part of the country. Besides adding a special flavour and palatibility to food it also acts as a lubricating agent to body tissue.

Mustard seeds like other oil-seeds harbour several mycotoxin producing fungi. These have also been found to be contaminated with aflatoxins and other mycotoxins during storage (Sinha, 1996 ; Ahmad, 1999). Toxic effects of different mycotoxins on physiological and biochemical processes of crop seeds have been studied by several workers. Protein and nucleic acids are the important components of any seed. Their synthesis has also been influenced by the presence of mycotoxins in the seeds. Besides inhibiting the synthesis of protein during plant metabolism, aflatoxin and aflatoxin producing fungi have been shown to degrade the protein level of maize seeds (Bilgrami *et al.*, 1981), dry fruits (Bilgrami *et al.*, 1983) and fleshy fruits (Sinha and Singh, 1982, 1984; Singh and Sinha, 1982).

Role of nucleic acids in plant metabolism is also well known. However toxic effects of mycotoxins on nucleic acid metabolism of plant systems have not been studied in detail. Darlier aflatoxins have been shown to interfere with the nucleic acid metabolism by many workers.

An attempt has, therefore, been made in this part of investigation to record the influence of aflatoxin B_1 on the synthesis of protein and nucleic acids during seed germination of mustard (var. Pusa bold). Besides recording the levels of protein and nucleic acids, qualitative analysis of protein was also done by gel electrophoresis.

MATERIALS AND METHODS

Seeds of mustard (var. Pusa bold) were obtained from the Oil-seed Division, Rajendra Agriculture University, Sabour. A stock solution of aflatoxin B_1 (Sigma, St. Louis, Missouri, USA) was 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 steeped initially in destilled water for 1 hr and subsequently in different concentrations of aflatoxin B_1 for 20 hr. For each treatment, 100 seeds were taken in triplicate. The steeped seeds were subsequently left for germination in moist blotting paper at 28 ± 2°C. On seventh day quantitative and qualitative estimations of the protein ingerminated seeds were done by the spectrophotometric method (Lowry *et al.,* 1951) and the disc electrophoretic method (Ornstein and Davis, 1964), respectively. The nucleic acid contents of the control and treated seeds were estimated by the method of Gottlieb and Tripathi (1968). The results were subjected to one way analysis of variance.

RESULTS AND DISCUSSION

A significant decline in protein content of the seed was observed due to influence by different concentrations of aflatoxin B_1 (Table 1). Per cent inhibitions in protein levels was 3.50, 5.91, 13.93, 23.39 and 28.33% at 100, 250, 500, 1000 and 2000 µg/l concentrations of aflatoxin B_1 respectively.

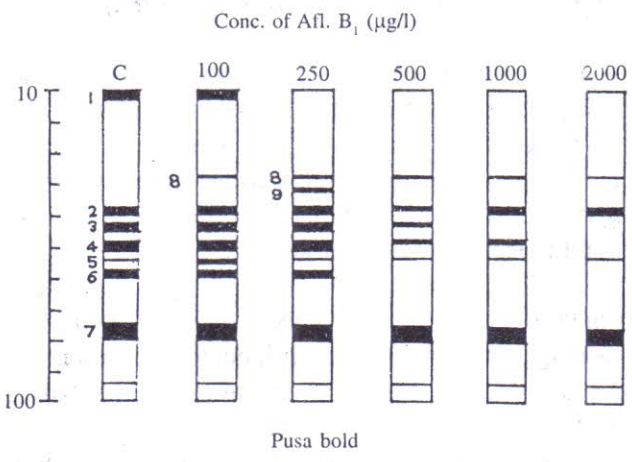

Fig. 1 : Gel Electrophorogram showing Protein profiles of Mustard seeds due to aflatoxin B_1 treatments.

Protein quality as estimated through electrophorogram (Fig. 1) revealed the presence of 7 bands (3 major, 3 minor, 1 diffused) having different Rf values in germinated seeds of mustard. The quality of protein in the treated seeds was affected by aflatoxin B_1. It iis apparent from Fig. 1 that at 100 µg/l concentration of aflatoxin B_1, maximum number of bands was noted. Band no. 8 appeared on the gel at this stage which remained present upto 1000 µg/l concentration. At 250 µg/l concentration band No. 1 disappeared with the appearance of a new band (No. 9). AT 500 µg/l concentration, only 2 major, 2 minor and i diffused bands were present. Maximum inhibition in number of bands was noted at 2000 µg/l.

Aflatoxin B_1 was found to reduce drastically the number of bands of protein profile at different concentrations. At lower concentration some new bands were synthesized, which were found to be diffused at higher concentrations. The newly synthesized bands might be defensive one, acting against aflatoxin B_1. Electrophoretic variations in the seed protein due to mycotoxin have earlier been worked out in *Brassica* sp. (Vaughan *et al.,* 1966), maize (Sinha and Kumari, 1989a, Prasad *et al.,* 1996) and mung (Kumari, 1988).

Table 1 : Effect of different concentrations of aflatoxin B_1 on protein and nucleic acid (DNA and RNA) contents of mustard seeds.

Conc. of Afl. B_1 (µg/l)	Protein content (µg/100ml)	DNA content (µg/100ml)	RNA content (µg/100ml)	% inhibitions in Pro.	DNA	RNA
0	23.68	13.09±0.169	41.21±0.530			
100	22.85±0.16	12.85±0.0245	40.49±0.192	3.50	1.83	1.76
250	22.27±00.12	12.67±0.178	39.21±0.242	5.91	3.20	4.85
500	20.38±0.46	11.34±0.341	36.92±0.343	13.93	13.36	10.41
1000	18.14±0.77	9.98±0.177	30.91±0.438	23.39	39.72	52.29
2000	16.97±0.49	7.89±0.332	19.66±0.589	28.33	39.72	52.29
t =	5.635	10.840	27.136			
r =	0.942	0.983	0.997			
df =	4	4	4			

It is also evident from Table-1 that aflatoxin B_1 depleted nucleic acid contents (DNA and RNA) of mustard seeds. The total amount of DNA and RNA was measured as 13.09 and 41.21 µg/l 100 ml which was reduced to 7.89 and 19.66 µg/l 100 ml at higher concentration (2000 µg/l) of the toxin treatment (Table 1). DNA and RNA synthesis was

earlier recorded due to aflatoxin B_1 in cotton-seed (Black and Altschul, 1965), maize (Sinha and Kumari, 1989b, 1990) and gram (Sinha, 1996). Lillehoj and Ciegler (1967) demonstrated remarkable effects of various levels of aflatoxin B_1 on the synthesis of DNA and RNA in *Flavobacterium aurantiacum*. They also observed that a concentration of 50 µg/l of aflatoxin B_1 completely blocked DNA synthesis in 4 hour incubation while reducing the RNA by less than 15% at that time.

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