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Short Communication

Mycotoxins Induced Changes in Protein and Nucleic Acid Contents of Germinating Mustard var. Pusa Mahak Seeds

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Oil seeds are of great economic importance and play a significant role in the life of human beings. Twenty five percent of the world food crops are affected by mycotoxin contamination every year [1]. The ideal climatic conditions for growing mustard (*Brassica juncea* L.) in Bihar, India also provides perfect environment for the natural contamination of aflatoxins in different agricultural crops [2, 3]. Aflatoxin B_1 and citrinin have been reported to inhibit seed germination, seedling growth and other physiological processes of various crops [2, 4-6]. Role of nucleic acid in plant metabolism is also well known [7]; however, toxic effects of mycotoxins on nucleic acid metabolism of plant systems have not been studied in detail. Aflatoxins have also shown to interfere with the nucleic acid metabolism by many workers [7, 8]. Aflatoxins and citrinin have been found to be natural contaminants of mustard seeds in Bihar [3]. Therefore, in this investigation an attempt was made to record various physiological changes induced by these two mycotoxins on the synthesis of protein and nucleic acids during seed germination of mustard. Additionally, the levels of protein and nucleic acids were recorded and qualitative analysis of protein was done by Gel electrophoresis.

Seeds of mustard var. Pusa Mahak were obtained from the Oilseed Division of Bihar Agriculture University, Sabour Campus, India. A stock solution of aflatoxin B_1 (Sigma, St. Louis, Missouri, USA) was prepared in 1ml ethanol from which the dilutions (100, 250, 500, 1000 and 2000 µg /l) were made with double distilled water. Stock solutions of citrinin were also prepared like aflatoxin B_1. The seeds were steeped initially in distilled water for 1 hr. and subsequently in different concentrations of aflatoxin B_1 and citrinin for 20 hrs. For each treatment, 100 seeds were taken in triplicate. The steeped seeds were subsequently were subjected to germination test in moist blotting paper at 28±2°C. On 7^{th} day, quantitative and qualitative estimations of the protein in seeds were done by spectrophotometric methods [9] and disc-electrophoretic methods [10], respectively. The results were subjected to one-way analysis of variance.

Seed germination and seedling growth of mustard were inhibited significantly by the varied concentrations of aflatoxin B_1 and citrinin. A visual examination showed gradual death of the seedlings in toxin treated seeds. The normal growth rate of roots and shoots of seedlings in toxin treated seeds were also reduced in comparison to control. The maximum inhibition in germination (84.52 and 80.48%) was found at 2000µg/l of aflatoxin B_1 and citrinin, respectively. The percentage germination inhibition recorded in lower levels of toxins were found to 73.80, 46.42, 35.71, 14.28% with aflatoxin B_1 and 73.17, 31.70, 18.29, 9.75% with citrinin at 1000, 500, 250 and 100µg/l concentrations, respectively. Seedling growth (both shoot and root lengths) was also found to be drastically reduced due to the inhibitory effects of aflatoxin B_1 and citrinin (Fig. 1 & 2).

The shoot lengths in control were found to be 5.76 and 4.46 cm, which were reduced by 80.55

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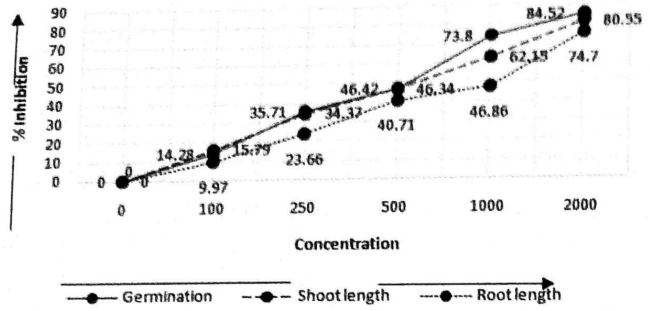

Fig. 1. Effect of different concentrations of aflatoxin B_1 on germination and seedling growth of mustard seeds

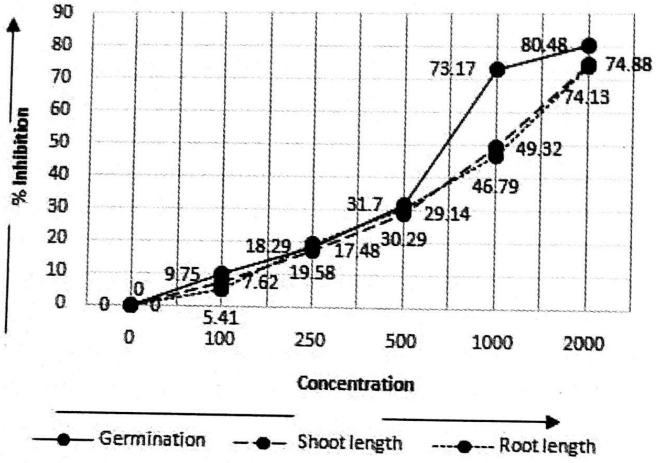

Fig. 2. Effect of different concentrations of citrinin on germination and seedling growth of mustard seeds

and 74.88% due to treatment with highest concentration (2000µg/l) of aflatoxin B_1 and citrinin, respectively. Likewise 74.70 and 74.13% reduction was observed in root length due to the concentration of aflatoxin B_1 and citrinin, respectively. These inhibitions were also found to be highly significant.

Percent inhibitions in protein levels were 3.94, 7.76, 9.95, 21.26 and 44.02% as well as 1.50, 3.00, 6.70, 13.61 and 31.38% at 100, 250, 500, 1000 and 2000 µg/l concentrations of aflatoxin B_1 and citrinin, respectively. Protein quality as estimated through gel electropherogram revealed the presence of 8 bands (4 major, 3 minor, 1 diffused) having different Rf values in germinated seeds. It was clearly evident that the quality of protein in the treated seeds was affected by aflatoxin B_1 and citrinin (Fig. 3 & 4).

It was revealed that the maximum numbers of

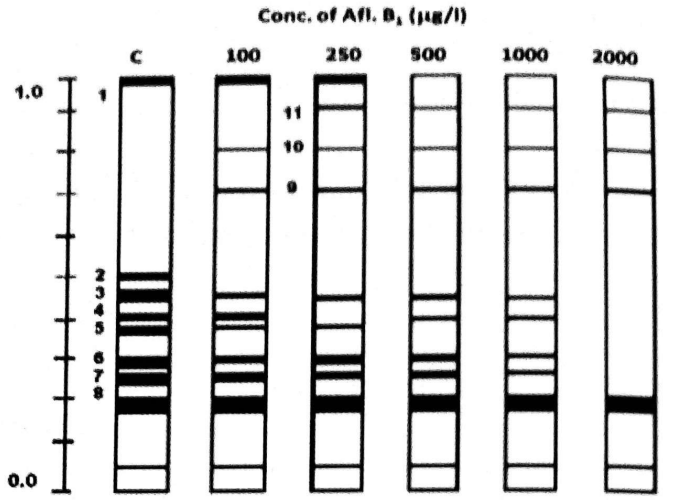

Fig. 3. Gel electropherogram showing the protein profiles of mustard seeds (var. Pusa Mahak) due to aflatoxin B_1 treatment

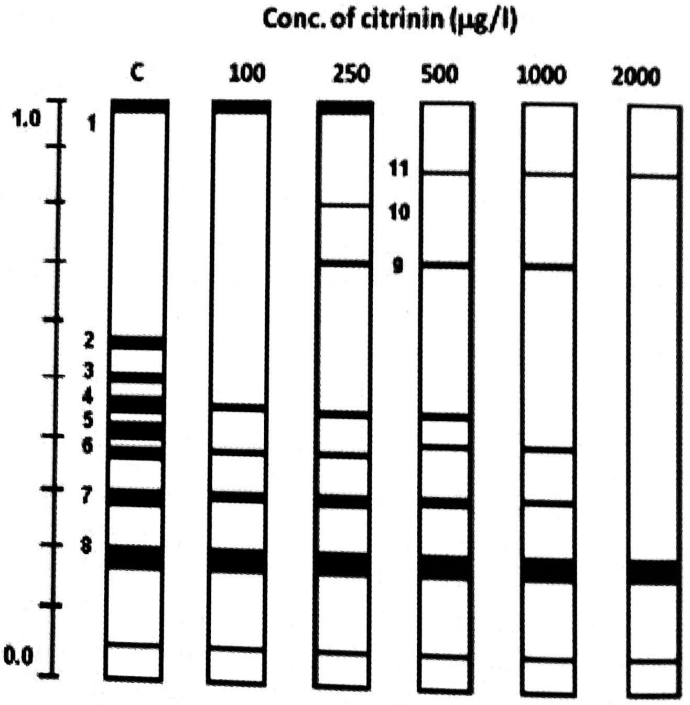

Fig. 4. Gel electropherogram showing the protein profiles of mustard seeds (var. Pusa Mahak) due to citrinin treatment

protein bands were at 100 µg/l concentration of aflatoxin B_1 (Fig. 3). Band No. 9 & 10 appeared on the gel while there was disappearance of bands no. 2&3. Even at 250 µg/l concentration one new band (No.11) appeared with the disappearance of another band at no.4. At 500 µg/l concentration bands no. 9, 10 & 11 also appeared and at the maximum concentration i.e., 2000 µg/l only 2 minor and 1 diffused band remained in the seeds. In

citrinin treated seeds, five bands appeared at 100 µg/l concentration while the band nos. 2, 3 & 4 present in the control seeds disappeared (Fig. 4). At 250 µg/l new bands; nos. 9 & 10 appeared. A new band (no.11) appeared at the higher concentrations of citrinin treatment. Only 3 bands were present at 2000 µg/l concentration of this toxin.

Aflatoxin B_1 and citrinin were found to reduce 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 and citrinin. Electrophoretic variations in the seed protein due to mycotoxin have earlier been worked out in *Brassica* sp. [3, 11, 14, 15], maize [5, 12] and mung [13].

It is also evident from table 1 that aflatoxin B_1 reduced the nucleic acid contents (DNA and RNA) up to 45.36% and 52.11% in mustard seeds. In the control set, the total amount of DNA and RNA was measured as 14.55±0.127 and 43.75±0.197µg/100 mg and it was reduced to 7.95±0.298 and 20.95±0.580 µg/100 mg at higher concentrations (2000 µg/l) of the toxin treatment.

Similarly citrinin also caused significant inhibitions in nucleic acid contents of mustard seeds which were up to 53.40% and 43.56% in

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

Conc. of Afl. B_1 (µg/l)	Protein content (µg/100mg)	DNA content (µg/100mg)	RNA content (µg/100mg)	Percent inhibitions Protein	DNA	RNA
0	24.60±0.35	14.55±0.127	43.75±0.197			
100	23.630.09	14.27±0.213	41.81±0.169	3.94	1.92	4.43
250	22.69±0.17	13.87±0.161	40.33±0.234	7.76	4.90	7.81
500	22.15±0.39	12.17±0.259	38.19±0.221	9.95	16.35	12.70
1000	19.37±0.33	11.85±0.166	32.11±0.295	21.26	18.55	26.60
2000	13.77±0.65	7.95±0.298	20.95±0.590	44.02	45.36	52.11
t=	26.02023	11.47916	49.85087			
r=	-.997059	-.9851592	-.9991962			
d.f.	4	4	4			

Table 2. Effect of different concentrations of citrinin on protein and nucleic acid (DNA and RNA) contents of mustard seeds (var. Pusa Mahak)

Conc. of Zearl. (µg/l)	Protein content (µg/100mg)	DNA content (µg/100mg)	RNA content (µg/100mg)	Percent inhibitions Protein	DNA	RNA
0	24.60±0.35	14.55±0.127	43.75±0.197			
100	24.97±0.66	13.60±0.29	42.25±0.57	1.50	6.52	3.42
250	23.86±0.21	12.79±0.19	41.65±0.19	3.00	12.09	4.80
500	22.95±0.17	11.39±0.21	39.53±0.17	6.70	21.71	9.64
1000	21.25±0.19	9.85±0.19	35.77±0.19	13.61	32.30	18.24
2000	16.88±0.39	6.78±0.17	24.69±0.17	31.38	53.40	43.56
t=	21.46133	12.00202	20.42067			
r=	-.9956858	-.9863984	-.9952381			
d.f.	4	4	4			

DNA and RNA contents, respectively at 2000 µg/l concentration of the toxin (Table 2). Earlier workers have also recorded inhibitions in DNA and RNA levels at this concentration of aflatoxin B_1 in various crops like wheat 61.90 and 57.04%, [16], maize 58.11 and 53.55%, [5] and gram (56.79 and 51.26%, [17]. Lillehoj and Ciegler [18] demonstrated remarkable effects of various levels of aflatoxin B_1 on the syntheses of DNA and RNA in *Flavobacterium aurantiacum*. They observed that a concentration of 50 µg/l of aflatoxin B_1 completely blocked DNA synthesis in 4hr incubation reducing the RNA by less than 15% at that time.

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