



EFFECT OF AFLATOXIN AND ZEARALENONE ON NUCLEIC ACID AND PROTEIN CONTENTS IN MUSTARD SEEDS (*BRASSICA JUNCEA* L. VAR. KRANTI).

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Mycotoxins, particularly aflatoxin B₁ and zearalenone, have been seen as natural contaminants of various crops including mustard at different stages of the crop development, harvesting and storage. In this investigation five different concentrations (viz., 100, 250, 500, 1000 and 2000 µg/l) of above two mycotoxins viz., aflatoxin B₁ and zearalenone were found to reduce the levels of nucleic acid and protein in germinating mustard seeds. The inhibitory effects of those mycotoxins were observed at all concentrations of the treated toxins.

Key words:-Mustard seeds/Aflatoxin B₁, zearalenone/Protein, DNA and RNA.

According to Food and Agricultural Organisation (FAO, 1977) 25 percent of the world food crops are affected by mycotoxin contamination every year. Oil seeds are of great economic importance and play a significant role in the life of human beings. Mustard is extensively grown in Bihar state in India but this state also provides ideal environmental conditions for the natural contamination of aflatoxins in different agricultural crops (Kumar and Sinha, 1992, Ahmad and Sinha, 2002a). Aflatoxin B₁ has earlier been found to inhibit seed germination, seedling growth and other physiological processes of the crops (Sinha *et al.*, 1992, Prasad *et al.*, 1996). Role of nucleic acid 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. Earlier mycotoxins have been shown to interfere with the nucleic acid metabolism by many workers (Sinha and Kumari, 1990; Sinha and Prasad, 1996). Since aflatoxins and zearalenone have been analysed as natural contaminants of mustard seeds in Bihar (Ahmad and Sinha, 2002b) so an attempt has been made in this investigation to record various

physiological changes induced by these two mycotoxins on the synthesis of protein and nucleic acids during seed germination of mustard. Besides recording the levels of protein and nucleic acids.

MATERIALS AND METHODS

Seeds of mustard (var.Kranti) were obtained from the Oil-seed Division, Rajendra Agriculture University, Sabour Campus, India. A stock solution of aflatoxin B₁ (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 glass distilled water. Stock solutions of zearalenone were also prepared like aflatoxin B₁. The seeds were steeped initially in distilled water for 1 hr. and subsequently in different concentrations of aflatoxin B₁ and zearalenone for 20 hrs. For each treatment, 100 seeds were taken in triplicate. The steeped seeds were subsequently left for germination on moist blotting paper at 28± 2°C. On 7th day, quantitative estimations of the protein in seeds were done by spectrophotometric methods (Lowry *et al.*, 1951). The results were subjected to one way analysis of variance.

RESULTS AND DISCUSSION

A visual estimation revealed that seed germination and seedling growth of aflatoxin B₁ and zearalenone treated mustard seeds showed a gradual inhibition in comparison to the control seeds. Percent inhibitions in protein levels were 3.70, 6.70, 8.98, 22.40 and 37.34% as well as 1.09, 3.08, 5.02, 12.19 and 32.65% at 100, 250, 500, 1000 and 2000 µg/l concentrations of aflatoxin B₁ and zearalenone,

Table-1: Effect of different concentrations of aflatoxin B₁ on protein and nucleic acid (DNA and RNA) contents of mustard seeds.

Conc. of Afl. B ₁ (mg/l)	Protein content (mg/100mg)	DNA content (mg/100mg)	RNA content (mg/100mg)	% inhibitions in		
				Protein-	DNA-	RNA
0	23.70± 0.45	13.69± 0.177	41.85± 0.279	3.70	3.79	2.10
100	22.81± 0.10	13.17± 0.291	40.97± 0.198	6.70	5.25	5.68
250	22.11± 0.19	12.97± 0.167	39.47± 0.247	8.98	12.85	11.23
500	21.57± 0.41	11.93± 0.276	37.15± 0.233	22.40	25.56	25.42
1000	18.39± 0.28	10.19± 0.185	31.21± 0.397	37.34	44.85	52.28
2000	14.85± 0.57	7.55± 0.319	19.97± 0.677			
t=	17.47488	25.17289	60.11592			
r=	-0.9935142	-0.9968587	-0.9994471			
d.f.	4	4	4			

Table-2: Effect of different concentrations of zearalenone on protein and nucleic acid (DNA and RNA) contents of mustard seeds.

Conc. of Zearl. (mg/l)	Protein content (mg/100mg)	DNA content (mg/100mg)	RNA content (mg/100mg)	% inhibitions in		
				Protein-	DNA-	RNA
0	23.70± 0.45	13.69± 0.177	41.85± 0.279	1.09	6.28	3.87
100	23.44± 0.75	12.83± 0.27	40.23± 0.65	3.08	14.08	4.92
250	22.97± 0.19	11.77± 0.14	39.79± 0.21	5.02	23.95	11.78
500	22.51± 0.17	10.41± 0.26	36.92± 0.13	12.19	34.47	23.58
1000	20.81± 0.13	8.97± 0.10	31.98± 0.10	32.65	58.50	52.47
2000	15.96± 0.37	5.68± 0.14	19.89± 0.19			
t=	14.00486	11.74741	28.24379			
r=	-0.9899564	-0.9858151	-0.9975022			
d.f.	4	4	4			

respectively (Tables-1&2). Variations in the seed protein due to mycotoxins have earlier been worked out in *Brassica* sp. (Vaughan *et al.*, 1966), maize (Sinha and Kumari, 1989, Prasad *et al.*, 1996) and mung (Kumari, 1988).

It is also evident from Table-1 that aflatoxin B₁ reduced the nucleic acid contents (DNA and RNA) of mustard seeds. In the control the total amount of DNA and RNA was measured as 13.69±0.177 and 41.85±0.279 and it was reduced to 7.55±0.319 and 19.97±0.677µg/100mg at higher concentrations (2000 µg/l) of aflatoxin B₁ treatment. Similarly zearalenone also caused significant inhibitions in nucleic acid contents of mustard seeds which were upto 58.50 and 52.47% in DNA and RNA contents, 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 B₁ in various crops like wheat (Sinha and Sinha, 1995), maize (Prasad *et al.*, 1996) and gram (Sinha, 1996).

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REFERENCES

Ahmad M S & Sinha K K 2002a Mycotoxin contamination of mustard (*Brassica juncea* L.) seeds at the time of harvest. *J Mycopath Research* **40**(2)59-162.

- Ahmad M S & Sinha K K 2002b Mycotoxin contamination of mustard (*Brassica juncea* L.) seeds during storage. *Indian Phytopath* **53(3)** 299-302.
- F A O 1977 *Mycotoxins*. Report of the joint FAO/WHO/UNEP Conference on Mycotoxins. Nairobi Sept 19-27, 1977 (FAO Food and Nutrition Paper 2 p105).
- Kumar N & Sinha K K 1992 Natural occurrence of mycotoxins in some stored food grains. *J Indian Bot Soc* **71** 191-194.
- Lowry O H Rosenbrough N J Farr A L & Randall R J 1951 Protein measurement with the folin phenol agent. *J Biol Chem* **193** 265-275.
- Prasad G Sinha K K & Ali M M 1996 Effect of aflatoxins B₁ on chlorophyll, nucleic acid and protein contents in maize. *Biologia Plantarum* **38(1)** 47-50.
- Sinha K K 1996 *Mycotoxin induced abnormalities in crop plants*. M D Publishers Pvt Ltd New Delhi p 124.
- Sinha K K & Kumari P 1989 Effect of aflatoxins B₁ on biochemical changes in mung seeds (variety Pusa-119). *Microbiol letters* **40** 145-149.
- Sinha K K & Kumari P 1990 Some physiological abnormalities induced by aflatoxins B₁ in mung seeds (*Vigna radiata* variety Pusa Baishakhi). *Mycopathologia* **110(2)** 72-79.
- Sinha K K & Sinha A K 1995 Effects of aflatoxins B₁ on some biochemical changes in some seeds of wheat varieties. *Indian Phytopath* **48(2)** 123-131.
- Sinha K K & Prasad G 1996 Effect of citrinin on pigment, nucleic acid and protein contents in maize. *Biologia Plantarum* **38(1)** 47-50.
- Sinha K K Kumar N & Prasad G 1992 Use of mustard (*Brassica juncea* L.) and gram (*Cicer arietinum* L.) seedling germination inhibition assay for aflatoxins B₁. *Mycopathologia* **121** 175-178.
- Vaughan J G Waite A Boulter D & Waisters S 1966 Comparative studies of the seed proteins of *Brassica campestris*, *B. oleracea* and *B. niger*. *J Exp Bot* **17** 332-334.