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THE IMPACT OF CRUDE AFLATOXIN B_1 ON PHYSIOLOGICAL AND BIOCHEMICAL PROCESSES IN MAIZE (*ZEA MAYS* L.) VARIETIES

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ABSTRACT

Aflatoxin B_1 was extracted from a locally isolated strain of Aspergillus flavus and applied to three commonly available varieties of maize (Zea mays L.) viz : Ganga 5, Nutan 517 & Nutan 101. An inhibition in seed germination between 24.0 to 38.7%, maximum being in case of Nutan 101, was recorded. Seedling growth, comprising root and shoot lengths also had a marked reduction in all the three varieties. The Suppression in root length was between 25% to 33.0% and shoot length 21.4 to 27.6%. The synthesis of chlorophyll in the emerging leaf and the protein contents in the treated seeds also recorded a significant reduction. The depletion was 45.3, 27.8 and 45.4% for total chlorophyll and 52.8, 31.6 and 46.7% for protein formation in Ganga 5, Nutan 517 and Nutan 101. respectively.

KEYWORDS: *Aflatoxin B_1, Chlorophyll, Maize, Protein, Seed Germination, Seedling Growth*

Article History

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INTRODUCTION

Aflatoxins are toxic metabolic substances produced by the toxigenic strains of *Aspergillus flavus* on food and feed commodities (Bilgrami, 1984; Bakhiet and Musa, 2011; Masood and Kumar, 2012). Aflatoxin B_1 is the most potent hepatocarcinogen (IARC, 1993; Turner et al., 2000), has been widely studied among all the known aflatoxins. The toxin is known to contaminate a number of food crops, including maize and other cereals (Bilgrami and sinha, 1987; Bilgrami et al.,1992). While contaminating the crops, the quality of seeds is also affected by the toxin, as a result, the important nutrients get depleted.

In addition, the aflatoxin containing seeds may be a risk factor to the consumer particularly the downtrodden section of the society and the livestock, where poor growth, lesser feed conversion efficiency, increased mortality rate and greater susceptibility to disease have been recorded (Jones et al., 1981; Huff et al., 1986; Osweiler, 2000; Pimpukdee et al., 2004).

Maize, being a susceptible substrate of *A. flavus* infection and aflatoxin contamination has been evaluated by investigators at several levels (Sinha, 1990; Sinha and Kumari, 1991; Li etal., 2001). In the present investigation, three locally cultivated varieties of maize viz., Ganga 5, Nutan 517 and Nutan 101, were subjected to aflatoxin B_1 treatment. Important physiological and biochemical changes comprising effect on seed germination, seedling growth as well as chlorophyll and protein syntheses were analyzed under controlled conditions.

MATERIALS AND METHODS

The varieties of maize (*Zea mays* L.) viz; Ganga 5, Nutan 517 and Nutan 101 were obtained from the local government farmhouse of Bhojpur district, headquartered at Ara. The crude aflatoxin B_1 was extracted from a toxigenic Isolate of *Aspergillus flavus* using SMKY liquid medium (Diener and Davis, 1966). The isolate was collectedfrom the maize seed sample and had aflatoxin producing capacityof about 0.9 ppm.

The seed samples were surface sterilized with 0.1% mercuric chloride solution for 2 minutes and subsequently washed with sterile distilled water. After proper washingthe test samples were put in sterile distilled water for 1 hr and subsequently soaked in freshly prepared aqueous aflatoxin B_1 extract for 20 hrs. For control soaking was done in distilled water.

A total of ten seeds each of treated and control lots were placed separately in each Petri plates containing moistened blotting paper with a thin layer of sterile water soaked cotton. Germination counts were recorded after an incubation of 72 hrs. A total of 3 replicates of 100 seeds of each experimental variety of maize were taken.

The seedling growth was measured on the 7^{th} day by recording the root and shoot lengths. The experiment was repeated three times with two replicates of 20 seeds each.

Chlorophyll content of newly emerged leaf was estimated by the method of Arnon (1949). Total chlorophyll was calculatedby combining the values of chl.a and b. The quantitative estimation of total protein was done by the method of Lowary etal., (1951).

RESULTS AND DISCUSSIONS

Seed germination was inhibited up to 38.7% in Nutan 101, whereas it was 33.0% in Ganga 5 and 24.0% in Nutan 517 (Table – 1). Seedling growth comprising root and shoot lengths also showed a marked reduction in all the varieties. An inhibition of 25.1, 21.6 and 33% in root length and 21.4, 19.2 and 27.6% in shoot length were recorded for Ganga 5,Nutan 517 and Nutan 101, respectively (Table – 2).

Table 1: Effect of Crude Aflatoxin on Seed Germination in Maize

Variety of Maize	Aflatoxin Treatment	Percent Germination (S,E)	Percent Inhibition In Germination	'T' Difference Value
Ganga-5	Control treated	97 (1.7) 61 (2.8)	- 33.0	9.0
Nutan-517	Control treated	96 (1.1) 73 (2.6)	- 24.0	8.3
Nutan-101	Control treated	93 (1.5) 57 (2.9)	- 38.7	10.9

Values are significant at 1% level

Table 2

Table 2: Effect of crude aflatoxin on seedling growth in maize

Variety of maize	Aflatoxin Treatment	Root length (cm)		Shoot length (cm)	
		Mean ±S.D.	% inhibition	Mean ±S.D.	% inhibition
Ganga -5	Control	11.73 ±1.00	-	10.13 ±0.73	-
	Treated	08.79 ±0.65	25.1	7.96 ±0.56	21.4
Nutan -517	Control	12.77 ±0.65	-	10.08 ±0.61	-
	Treated	10.01 ±0.71	21.6	8.15 ±0.61	19.2
Nutan-101	Control	11.78 ±0.83	-	10.29 ±0.49	-
	Treated	7.89 ±0.55	33.0	7.45 ±0.58	27.6

Seed germination and seedling growth are the manifestations of several important biochemical mechanismswhich start with the imbibition of water by the seed and resultant enzymatic activity. Sinha (1994) elaboratelystudied the effect of aflatoxin B_1 and zearalenone on physiological responses of common crop plants and concluded that the reduction in the germination of the seed was directly proportional to the concentration of the mycotoxins. Furthermore, the overall process of the emergence of radicaland plumule have been found to be influenced by a number of chemical compounds, some of which may occur internally. These compoundsincluded terpenes, lactones, phenolics, aldehydes, etc (Steward and Krikorian, 1971). Aflatoxin B_1 which occurs mostly as internal seed contaminant probably interferes the overall process of germination as well as seedling growth.

The toxic effect of aflatoxin on chlorophyll synthesis was evident in the emerging leaves. A marked reduction in the contents of chlorophyll a and b were recorded in all the three varieties (Figure 1). A depletion of 45.3,27.8 and 45.4% in total chlorophyll contents were recorded in Ganga 5, Nutan 517 and Nutan 101, respectively. The effect of aflatoxin B_1 on protein content was also prominent. The inhibition was maximum in Ganga 5 (52.8%) followed by Nutan 101 (46.7%) and Nutan 517 (31.6%) (Figure 2).

The synthesis of chlorophyll, which is the necessity of autotrophic mode of nutrition in plants, was severely affected by the toxin treatment. Sinha and Kumari (1991) also observed a substantial inhibition in chlorophyll formation in maize due to aflatoxin B_1. Chohan (1983) is of the opinion that mycotoxins check the synthesis of chlorophyll by restricting the growth hormone-induced synthesis of RNA, DNA, and protein in the leaf. Recently, Deepavali and Nilima (2012) recorded a depletion of chlorophyll synthesis in some maize varieties treated with aflatoxin. A variation in chl. a and b syntheses in the treated varieties reflects that the compound interferes at the biosynthetic level which helps the formation of chlorophyll b either from chlorophyllide a or from chl.a.

Protein is the building block of all living organisms. Therefore, inhibition in the formation of metabolically active protein, as is evident from the present investigation, suppresses several biochemical processes some of which may change the pattern of growth of seedlings and hence the overall growth of the plants.

Sinha (1994) also recorded a sharp decline in protein contents of aflatoxin B_1 and zearalenone treated seeds of maize, gram and mustard. A variation of the toxin effect at the level of variety was recorded for each physiological and biochemical parameters studied in the present investigation. It reflects that each variety responds differently when it comes at the biochemical level due to a minor shift in the genetic makeup.

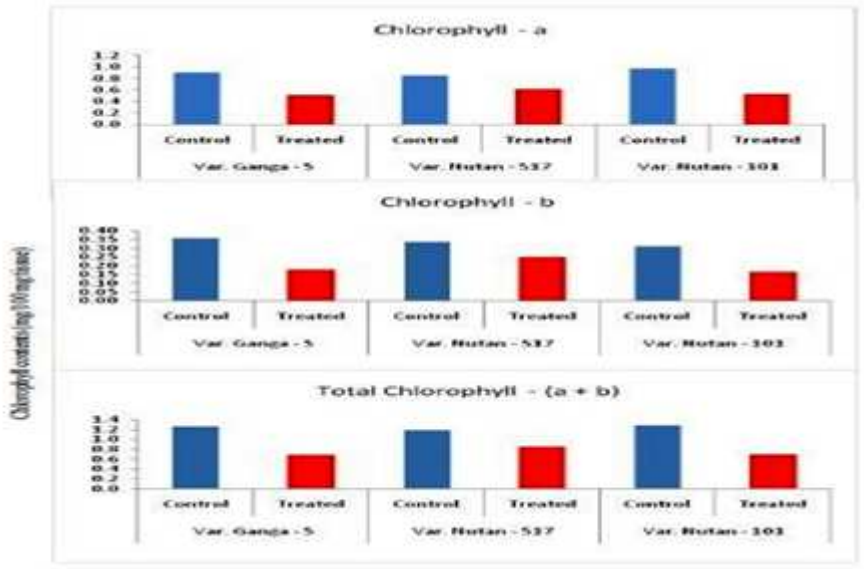

Figure 1: Effect of Crude Aflatoxin on Chlorophyll a,b and Total Chlorophyll Contents in Maize Seedlings

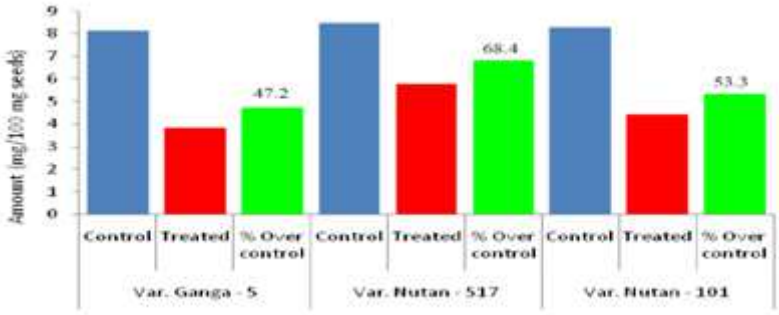

Figure 2: Effect of Crude Aflatoxin on Protein Contents in Germinating Maize Seeds

CONCLUSIONS

Several chemicals including aflatoxin B_1 (AFB_1) interfere with the emergence of radical and plumule, thus affecting the overall process of seed germination and seedling growth. The toxic effects of AFB_1 continue and suppress the syntheses of other compounds like chlorophyll and protein. In the Present dissertation a significant effect in the syntheses of chlorophyll and protein as well as seed germination and seedling growth were recorded with the treatment of crude AFB_1 at the varietal level of Maize.

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