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ISOLATION AND IDENTIFICATION OF MYCOFLORA FROM MAIZE SEEDS AND THEIR IMPACTS ON PROTEIN QUALITY

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ABSTRACT

Maize is one of the major food crops around the world and it provides energy for human and animals. It grows throughout the year due to its photothermo insensitive character and highest genetic yield. The composition of maize contents are Moisture 14.9%, Fat 3.6%, Fibre 2.7 %, Minerals 1.5 % and Protein 11.5% respectively. The economic value of the protein contents are utilized in the production of food products. The various genera of fungi like. Aspergillus, Penicillium, Alternaria, Fusarium and Rhizopus were recorded from maize sample of Var. A and Var. B The dominant fungi A. flavus (760 which produce toxic substance like Aflatoxin B1. Aflatoxin B1 known to adverse the seed germination and decline the quality (Protein) contents. The inhibition of seed germination were maximum 74.67% and 75.67% in both the Var. A and Var B. at 2000 ppb concentration of aflatoxin B1 respectivily, whereas the maximum inhibition of protein contents (73.34% and 49.36 %) in Var. A and Var. B were also recored at 2000ppb concentration of aflatoxin B1, respectively

Therefore, the healthy maize seeds should be released to the farmers in order to the secure their outputs as well as to provide the quality of energetic food for the human beings.

Keywords: Maise (Zea Mays L.), Mycoflora, Aflatoxin B1, Amino acids Kits

1. INTRODUCTION

Maize is one of the major food crops around world, providing energy for human and animals as well as being an important industrial row material (Erenstein et.al.,2022).

Maize is called the 'Queen of cereal' as it is grown throughout the year due to its photo-thermo-insensitive character and highest genetic yield potential among the cereals.It play a significant role in the global livestock and poultry industries (Tanumihardjo et.al.,2019).

The composition of maize contents i.e. moisture 14.9%, Protein 11.1%, Fat 3.6%, Fibre 2.7 % and minerals 1.5% (Kaila and Jat 2015). The economic value of the protein contents is utilized in the production of food products (Paliwal,2000). Some seed components such as protein content, lipids, and carbohydrates are involved in the

germination and formation of seedlings providing carbon and nitrogen and, consequently, directly related to vigor (Andrade et al., 2020).

Protein also regulate gene expression help in nutrient transport and storage and protect seeds from pathogen and environmental stress, additionally they are involved in hormone regulation, affecting the synthesis, transport and signaling of plant hormones that are essential for germination and early seedling development (Bewley et.al.,2013, Huang et.al.,2015). Aflatoxin B1 is a toxic substance produced by A. flavus that can 'profoundly contaminated maize seeds in Darbhanga , District (Prasad et.al.,(1997). There are no cohesive reports, regarding above research in these areas of Darbhanga District. Therefore, an attempt has been made to record the cohesive of Mycoflora and its impact on germination and Protein Quality of maize seeds (Zea mays L.) in Darbhanga District, Bihar, India.

2. MATERIALS AND METHODS

Study area

Maize seeds (Zea mays L.) were collected from Sakri and Laheriasarai village of Darbhanga District, Bihar.

Aflatoxin The standards of aflatoxin was prepared in the Departmental Lab isolatiobn of Aspergillus flavus from cereals.

Isolation and Identification:

100 seeds were used for isolation and identification of seed borne fungi. Seeds were surface sterilized with 2% NaOCl for 1 minutes and rinsed with distilled water and plated on blotting papers by the methods of ISTA (1990). Enough space was kept between each seeds for proper growth of fungi and avoids cross contamination. Plates were inclubated at room temperature for one week followed by macro and microscopic identification (Clenny 2005, Singh SB et.al., 2018). fungal colonies were maintained on PDA media for use and identification.

3. SEED GERMINATION

Naturally contaminated maize seed samples (highly contamination one from each location). were used in complete experiment. All seed samples were now soaked in double distilled water overnight and plated on moist blotter paper as per ISTA 1966. All the plates were incubated at room temperature (28± 2 ▫ C) for 7 days the result of seed germination were taken by using following formula :

Germination Index:

G.I. = Total no. of germination seed /Total no. of seed observed * 100

Estimation of Quantitative protein:

Quantitative estimation of the protein in the control and treated seeds were done by methods of Lowery et.al., (1951).

100 mg of seed flour were crused in 100 ml of acetate buffer (pH 4.8) and centrifused 2 ml of the test solution was taken from the supernatant. To this 10 ml of alkaline reagent (prepared by mixing 50 ml of 2 % Na2Co3 solution in 0.1 N NaOH solution and 1 ml of 0.5% CuSO4 in 1 % Na- K-tartarate solution) was mixed thoroughly for 10 minutes, 1 ml of diluted Folin – Ciocalteaue reagent (1: 3 in distilled water) was added, after 10 minutes the extinction was read at 600 nm against the blank prepared by albumin.

Estimation of Qualitative Protein:

The qualitative analysis was measured with the help of amino quant followed by thin layer chromatography (TLC) plate methods.

The qualitative analysis was done by disc electrophorelic methods (Ornstein and Davis, 1964. Gels were scanned by LKB Ultracan – XL- Enhances Laser Densito-meter) (LKB, Bromma, Sweden). The least significant difference at 1 and 5 % levels

(LSD01 and LSD05) were determined following the procedure of Dospekhov (1984).

4. RESULT AND DISCUSSION

Isolation and Identifification of Mycoflora associated with Maize seeds:

Each maize seed plate was carefully examined for fungal growth and identified mycoflora were noted which include Aspergillus flavus, Aspergillus nigar, Fusarium spp., Penicillium spp., and Rhizopus (Table: 1). Aspergillus flavus was dominantly found in all the seed samples collected from the two villages taken under experiment.

Table 1 Mycoflora associated with collected maize samples (var. A and Var. B) from different locations of Darbhanga District.

Fungal Mycoflora	Maize Var, A (Pioneer)			Maize Var. B (White maize X-08)		
	Jale	Laheriasarai	Mean (S.E)	Jale	Laheriasarai	Mean(S.E)
Aspergillus flavus	76	**57**	**66.5±6.36**	**67**	**52**	**59.5±7.5**
A.nigar						
Alterneria spp.	**20**	**17**	**18.5±1.5**	**18**	**04**	**11.0±7.0**
Fusarium spp.	**5**	**4**	**4.5±0.5**	**9**	**6**	**7.5±1.5**
Penicillum spp.						
Rhizopus spp.	**4**	**6**	**5.0±1.0**	**6**	**0**	**3.0±3.0**
	7	**8**	**7.5±0.5**	**0**	**8**	**4.0±4.0**
	13	**3**	**8.0±5.0**	**9**	**0**	**4.5±4.5**

Seed Germination:

The effect of five different concentration of AFTB1 on seed germination of maize (Var. A and Var. B) were observed in TABLE- 2. A Significant fall in germination was noticed at 2ppb concentration of AFTB1. A Significant fall in seed germination was noticed at 2ppb concentration of AFB1. The maximum inhibition in seed germination were 9.57%, 18.34%, 33.42%, 51.10% and 74.67% in Var. A maize seeds at 100, 250, 500, 1000, 2000 ppb concentration of AFTB 1, respectively in Jale village of Darbhanga District and Var. B. 8.26%, 20.11%, 40.16%, 15.12% 75.76 % and 78.97% at 2ppb concentration.

More or less similar results were observed in Laherai sarai of Darbhanga District.

Table 2 Effect of AFTB1 on Maize Seeds (Var-A and Var. –B) Germination.

Obsarvations	Var. A (Pioneer (Concentration (ppb)	Var. B (White maize X08) Concentration (ppb)
	Control 100 250 500 1000 2000	Control 100 250 500 1000 2000
Germination index (S.E)	92% 83% 74% 62% 43% 23%	89% 82% 70% 52% 39% 20%
	(0.05) (1.27) (2.06) (0.47) (0.53) (0.84)	(0.04) (2.06) (2.43) (0.43) (0.57) (0.69
t-Difference with control		
% inhibition	---- 9 18 30 49 69	------ 7 19 37 50 69
	----- 9.57 18.34 33.42 51.10 74.67 %	----- 8.26 20.11 40.16 15.12 75.67

A highly significant fall in the levels of protein of maize seedlings was recorded with the treatment of different concentration of aflatoxin B1 in Table -3 and 4. The variation in the control and that of corresponding LSD 05 (Least Significant Difference at 5% levels). Values in Tables revealed significant inhibitory effects of those toxins on protein levels. The percent inhibition in protein were recorded 24.43 %, 33.05 %, 43.66%, 61.79%, and 73.34% at 100, 250, 500, 1000, 2000 PPB Aflatoxin B1 (Var. A) in Laheriasari respectively.

Similarly inhibition in protein were also recored 5.78% , 12.19%, 26.34%, 38.21 %, 49.36% in Jale at different concentration of aflatoxin B1respectivily.

Table-3 Effect of Aflatoxin B1 on Protein content of maize seedling obtained from Laheraisarai and Jale of Darbhanga District (Var. A)

	Protein (mg/100 mg) Var. A (Pioneer)					
Concentration	Laheriasarai			Jale		
$AFTB_1$ ppb	Amount	Diff.with control	% inhibition	Amount	Diff. with control	% inhibition
Control	8.42 (0.10)	------	-----	8.43 (0.17)	-------	-----
100	7.15 (0.08)	1.27	24.43%	7.46 (0.13)	0.97	5.78%
250	6.04 (0.01)	2.38	33.05%	7.40 (0.11)	1.03	12.19%
500	4.75 (0.06)	3.67	43.66%	6.12 (0.06)	2.31	26.34%
1000	3.19 (0.05)	5.23	61.79%	5.21 (0.05)	3.22	38.21%
2000	2.22 (0.08)	6.22	73.34%	3.59 (0.07)	4.84	49.36%
LSD_{01}			6.21			5.28
LSD_{05}			4.42			3.78

Table 4 Effect of Aflatoxin B1 seed treatment on protein content of maize seedling obtained samples collected from Laheraiasarai and Jale Darbhanga District (Var. B)

	Protein (mg/100 mg) Variety – B (White Maize X-08)					
Conc. Of	Laheraisarai			Jale		
$AFTB_1$	AMOUNT	Diff. with control	% inhibition	AMOUNT	Diff. with Control	% Inhibition
CONTROL	8.16 (0.03)	-----	-----	8.45 (0.04)	------	---------
100	7.12 (0.02)	1.04	3.06%	7.14 (0.05)	1.31	5.78%
250	5.59 (0.04)	2.57	15.40%	6.00 (0.07)	2.45	33.05%
500	3.52 (0.03)	4.64	28.66%	4.75 (0.06)	3.70	43.66%
1000	2.18 (0.05)	5.98	44.15%	3.19 (0.05)	5.26	61.79%
2000	2.12 (0.07)	6.04	72.41%	2.19 (0.09	6.26	73.31%
LSD_{01}			6.19			5.21
LSD_{05}			3.76			3.59

Figure in parentheses are S.E.

All values are significant at both 1 and 5 % levels

The presence of Aflatoxins can result in stunted growth and reduced seedling vigor due to the diminished availability and functional of necessary proteins for growth and metaboloism (Chowdhary et, al.,2008). Aflatoxin contamination of seeds were widely used by the grower that can degrade seed quality and reduce Plant health.

The above investigation means impact of Aflatoxin B1 on seed germination as well as protein synthesis support in previous finding by (Keller et.al., 1975; Sinha & kumari,1990 and G. Prasad &N. Kumari et.al.,2018).

5. CONCLUSION

The AFTB1 can disrupt protein synthesis and degrade exitisting proteins, impairing the function of ribosomes hindering the translocation process. It also induces oxidative stress and damage cellular structure.

Seed could play an important role in the epidemic in field for high quality and quantity so that healthy seeds of maize should be released to farmers in order to secure their outputs to return high economy.

CONFLICT OF INTERESTS

None.

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