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RESEARCH ON CORELATION OF OCHRATOXIN A WITH STARCH AND PROTEIN LEVELS IN MAIZE

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Abstract

This study comprises the incidence of Ochratoxin A in Maize grains from the 2010 harvest. The samples were gathered before storage of harvest and the co-relation between mycotoxin contamination and the relative quantity of various important bio chemicals present in the maize composition was identified. Among 30 samples of early and semi-early hybrids, 24 were positive, while two were found containing OTA quantity above approved levels of 5 ng/g (19.92 and 11.72 ng/g). A decrease in the quantity of the starch is directly related with the presence of OTA in samples. The presence of OTA in the tested samples showed no effect on the quantity of proteins.

INTRODUCTION

Mycotoxins are secondary metabolites, important for the survival of producing fungus and may appear at different stages of food chain starting from the field harvest till their storage whenever optimum conditions are available for their growth. Humans are exposed to the effects of mycotoxins in various ways: through the ingestion of contaminated food products (predominant way), contact and inhalation. Mycotoxicosis has two forms: acute and chronic; usually with symptoms being relatively serious. Mycotoxicosis may interfere with immune system such as to increase the susceptibly to infectious diseases. Ochratoxin A is well known for its effects/nephrotoxicity.

A variety of foods are susceptible to mycotoxin contamination. However, cereals and millets which form the staple food for many population groups are most prominent. Mycotoxins that have been investigated so far include aflatoxins, ochratoxins, various fusarium toxins and ergot alkaloids. Considerably high levels of these mycotoxins have been detected in cereals that have been harvested and left in the field during heavy rains or floods or improperly stored without adequate drying. The levels encountered under normal circumstances are otherwise relatively low. Among the mycotoxins, much attention has been focused on aflatoxins produced by *Aspergillus flavus* and *A. parasitic* in stored grains. Aflatoxin B 1, the most toxic and abundantly found among the series of aflatoxins,

continues to be a major problem in risk commodities like groundnut, maize and chillies.

Ochratoxins are a group of mycotoxins produced as secondary metabolites by several fungi of the *Aspergillus* or *Penicillium* families and are weak organic acids consisting of a derivative of an isocoumarin [2]. The family of ochratoxins consists of three members, A, B, and C which differ slightly. Crops are infected in the field during growth, at harvest, in storage and in shipment under favourable environmental conditions especially when they are not properly dried. Ochratoxin A may be present in a foodstuff even when the visible mould is not seen.

Ochratoxin A (OTA) is found mainly in cereal and cereal products. This group of commodities has been reported to be the main contributors of ochratoxin A exposure in assessments carried out by the European Commission (5,10) accounting for 50% of total dietary exposure of ochratoxin A in European countries [3].

MATERIAL AND METHODS

In this investigation, 30 early and semi-early and 13 late hybrids of maize were analyzed. All samples were collected from the harvest of 2010 in October. Early and semi-early hybrids were collected from the county Braila, whereas late hybrids were collected from the county of Alba. It must be mentioned that in Braila conditions of cultivation were not identical. The year 2010 was extraordinarily hot and dry. Samples were later submitted in the stores of the firm KWS Seeds SA. The determinations and scientific calculations were carried out at the laboratories of Biochemistry, Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine, Bucharest, Romania.

For the washing of samples, columns of immunoaffinity Ochraprep (RBiopharm) were used which gave good separation of the material to be analysed. After grinding the maize samples, 25 grams of each sample were weighed, extracting solvent (acetonenitril+distilled water 60:40) was added and was homogenized at a rapid speed in a blender for two minutes and later filtered though filter paper Whatman 4.For Ochratoxin A analysis, 4 ml of the filtrate was taken. Samples were diluted with 44 ml tampon phosphate 20mM pH 7.0 and were put in Ochraprep columns. After the attachment of analyte, segregation of the balast substances was carried out using 20 ml tampon phosphate 20mM, pH 7.0. The elute of Ochratoxin A was obtained with 1,5 ml solution of methanol + acetic acid (98:2) at one drop per second followed by dilution with 1.5 ml ultra pure water. 3 ml of the resulting elute was injected in 100 µl chromatograph. Quantification was carried out through liquid chromatography with fluorescence detection. For separation, columns of chromatography composed of octadecilsilani, C_{18} – Symmetry 4.6 x 250 mm, with particle 5 µm, were used. Analyte separation took place at 40˚C and elution took place with a ternary mixture of acetonitril + water

+acetic acid in the ratio of 51:47:2 v/v/v, at the rate of 1 ml/minute. Detection of Ochratoxin A took place with excitation at 333 nm and emission at 43 nm. The data was obtained and worked upon using the soft ware EMPOWERS. Wave standardization used for the processing of results was set by using ochratoxin A (RBiopharm) with the concentration of 1000 ng/ml. After dilution of the standard at 16.67 ng/ml, 5 values of concentrates were obtained by injecting another volume different than the standard. Practicing two injections for each of five levels of concentrates, a standard curve was obtained with linear co-efficient (r^2) 0.999983 and co-efficient of co-relation(r) 0.999992. Using the standard curve the results for the determined were calculated.

Determination of starch quantity by polarimetric method comprised of two stages namely: extraction of starch from plant leaves and polarimetric determination of starch concentration Solubility and extraction of the starch was done in acidic medium (HCL) at the boiling point in the presence of a substance to defecate. Defecating substances have a role for separation by precipitation from the extract for dosing substances which may influence negative dosing. Amino acids and proteins (which are optically active) were precipitated by using phospho-wolframate of sodium. Dosing was done through measuring the angle α, which is the angle of deviation from the plane of polarized light while passing through the solution containing starch with the help of Lippich polarimetre. Results were calculated using the following expression:

Starch %=$\alpha \cdot Vt \cdot 100/[\alpha]^{20}Dxlxp$ where α =measured angle, Vt=sample volume, [α] = standard angle (183.7 for maize),L = tube length (20 cm),P = sample mass (g)

Protein quantity was determined by calculating the transformed amount of plant nitrogen (Nt) into the structure of proteins (Kjeldahl method).

RESULTS AND DISCUSSION

All the 43 samples were tested for the quantity of Ochratoxin A present by methods described earlier. The values obtained for early and semi-early hybrids are given in table 1.

From the data obtained, it is observed that the samples were positive in a significantly big proportion i.e. 24 from 30 of samples (around 80%). It must also be mentioned that only two of the positive samples had the values for Ochratoxin A higher than the admissible value of law EC 1881/2006 of 5 ng/g, levels determined at 19.92 and 11.72 ng/g. Rest of the positive samples were below than 2 ng/g, maximum being under 0.5 ng/g. Negative samples were considered either as zero or the lower limit of quantity (LOQ) of 0.01 ng/g. Determination of the Ochratoxin A for late hybrids gave the results presented in table 2.

The present values indicate a very low incidence of Ochratoxin A in case of late hybrids. Out of 13 only 3 showed positive values and even these were extremely

small quantities approaching almost the standard limit. Co-relation of registered values for the amount of Ochratoxin A and starch for late and semi-late hybrids is shown in figure 1.

Table 1

Analysis for Ochratoxin A Quantity in early and semi-early hybrids

No.	Hybrid name	OTA (ng/g)	No.	Hybrid name	OTA (ng/g)	No.	Hybrid name	OTA (ng/g)
1	Sutesti HIB EXP 2	19.92	11	SUTESTI KWS 3381	0	21	SUTESTI KIXA 4388 STANZA	0.24
2	Sutesti HIB EXP 3	1.75	12	SUTESTI KWS 6474	0.008	22	SUTESTI KORAL 4375	0.0165
3	Sutesti HIB EXP 4	0.46	13	SUTESTI KWS 5383	0.56	23	SUTESTI KABOS	0.052
4	Sutesti HIB EXP 5	0.25	14	SUTESTI KXA 6485	0.016	24	SUTESTI AMANDA	0
5	Sutesti HIB EXP 6	0.082	15	SUTESTI KXA 5392	11.72	25	SUTESTI KITTY	0
6	Sutesti HIB EXP 7	0.028	16	SUTESTI KXA 5387	0.235	26	SUTESTI CUPIDON	0.011
7	Sutesti HIB EXP 8	0.11	17	SUTESTI KXA 6493	0.023	27	SUTESTI MIKADO	0
8	Sutesti HIB EXP 13	0.02	18	SUTESTI KXA 6542	0.021	28	SUTESTI GARBURE	1.28
9	SUTESTI KWS 1393	0.26	19	SUTESTI KXA 3376(sinatra)	1.89	29	SUTESTI LAUREAT	0.155
10	SUTESTI KWS 1394	0.005	20	SUTESTI Kalifo 3545	0.095	30	SUTESTI KAPSUS	0

Table 2

Analysis for Ochratoxin A Quantity in late hybrids

No.	Hybrid name	OTA (ng/g)	No.	Hybrid name	OTA (ng/g)
1	FRATIA 92 EXP 1	0	8	AMADEO	0
2	FRATIA 92 EXP 2	0	9	FRATIA RONALDINHO	0
3	FRATIA LOSC	0	10	FRATIA LAUREAT	0
4	FRATIA 92 EXP 9	0	11	FRATIA GAVOTT	0.025
5	FRATIA 92 EXP 11	0	12	FRATIA SEVERO	0
6	FRATIA 92 KXA 5373	0.015	13	FRATIA CARERRA	0.034
7	FRATIA KXA 5375	0			

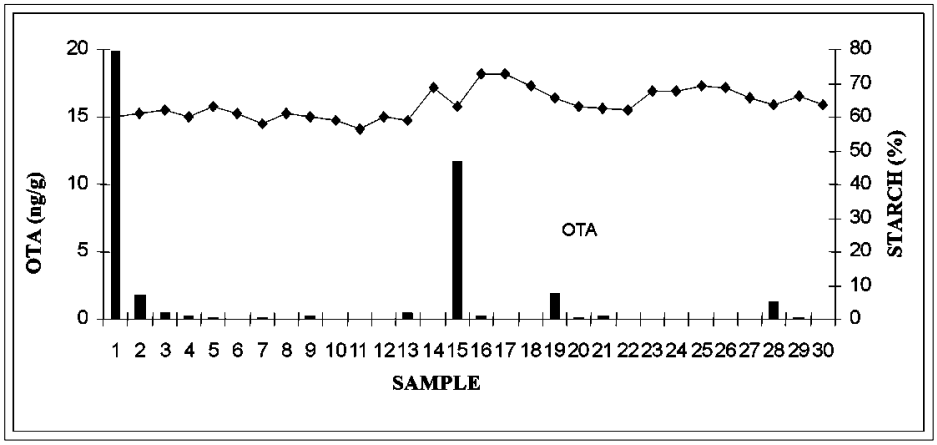

Fig. 1. Influence of contamination with Ochratoxin A upon Starch Quantity in early and semi early hybrids

The above graph presents a corelation between the quantities of Ochratoxin A and starch. It is observed that the samples contaminated with high quantities of Ochratoxin A contain reduced amounts of starch. This thing can be explained by the fact that the fungi need the resources of carbon, for their growth, glucose being the easiest source to be used. In case of studies on late hybrids, the co relation between two parameters observed is presented in figure 2.

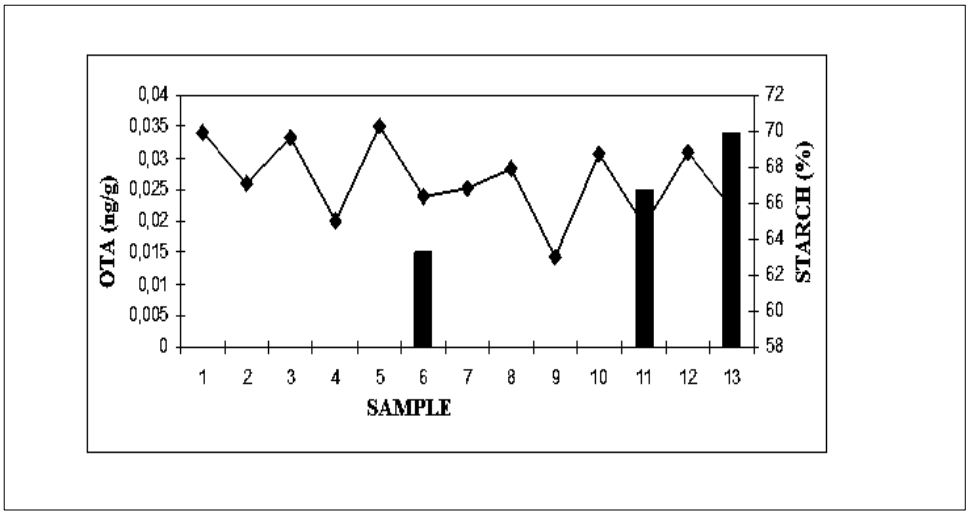

Fig. 2. Influence of contamination with Ochratoxin A upon Starch Quantity late hybrids

The corelation between the quantities of Ochratoxin A and starch for the samples of late and semi late hybrids is given in figure 3.

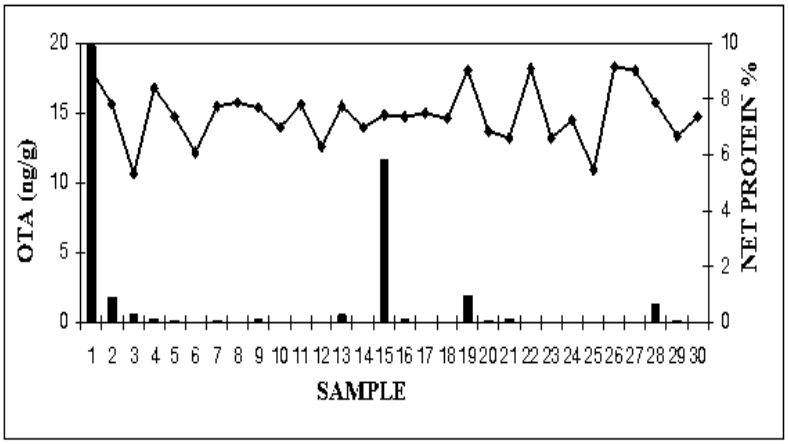

Fig. 3. Influence of contamination with ochratoxin A upon Net Protein quantity in early and semi early hybrids

Neither in late hybrids a co relation among the samples contaminated with ochratoxin was A and the quantity of gross protein was observed (Figure 4).

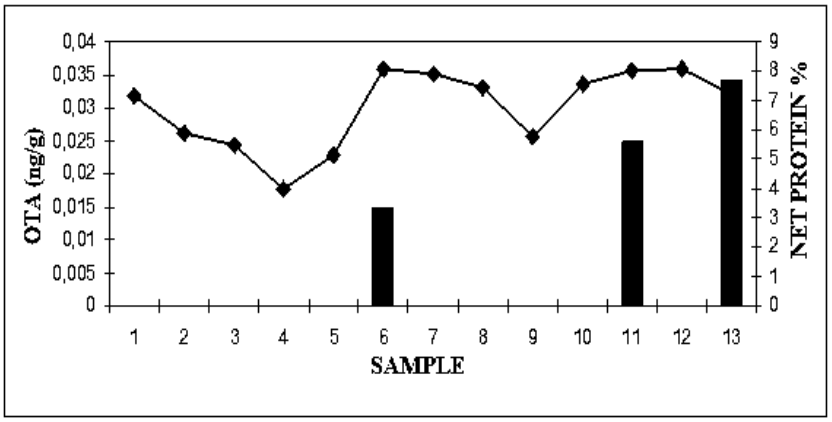

Fig. 4. Influence of contamination with ochratoxin A upon Gross Protein quantity in late hybrids

CONCLUSIONS

1. The presence of OTA has a direct effect on the quantity of starch in cells. A higher amount of OTA corresponds to decreased starch quantity. Similarly from the data, it is confirmed that amount of Ochratoxin A does not have any effect on quantity of net proteins.

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