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MYCOLOGICAL AND MYCOTOXICOLOGICAL INVESTIGATIONS ON MAIZE SAMPLES COMING FROM CLUJ AND ALBA COUNTIES

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Abstract: A number of 36 samples from Alba - and 34 from Cluj counties obtained in the 2001 and 2002 harvests were randomly collected in October 2002 and analysed for mycotic load and for zearalenone and ergosterol as fungal mass indicator. Following the mycological examination; there has been found a moderate mycotic charge with a number of 14 samples and intense mycotic load with 11 samples. The highest mycetes occurence have belonged *Fusarium; Rhisopus; Alternaria; Aspergillus* genera. Ergosterol was detected with all samples with a maximium value of 72 mg/kg; zearalenone was found in 54% of the total of analysed maize samples reaching values of up to 2250µg/kg; indicating a high degree of fungal contamination as well as a severe zearalenone contamination.

INTRODUCTION

Fusarium genus contains a large number of species of fungi; producing various mycotoxins such as zearalenone; toxic trichothecenes; fumonisins. These mycotoxins produce important diseases in farm animals; and trichothecenes have been also major causes of human diseases. (1). Ergosterol is the dominant sterol in fungi. It is not formed; or only traces of it are formed; by higher plants. An important reason for its suitability as a chemical indicator of fungal growth in feedstuffs is that is generally formed by the fungi which occur in feedstuffs.(8). Zearalenone is a nonsteroidal estrogenic mycotoxin produced by several *Fusarium* species. It has been implicated in numerous mycotoxicoses in farm animals; especially in pigs.(5). Zearalenone is found in a number of cereal such as maize; barley;oats and wheat. Maize is one of the most important grains in Europe and plays an important role in human and animal nutrition. Cluj and Alba counties are two important maize-growing regions of Transylvania; with a moderate continental climate with prevailing westerly winds. Crop rotation in this area is normally between corn and wheat.(3).

MATERIAL AND METHOD

Corn samples for feed obtained from 2001 and 2002 harvest were collected randomly in October 2002 in Cluj and Alba counties; and analysed for zearalenone (ZON) as well as for ergosterol (Erg) as fungal mass indicator. A number of 36 samples were obtained from Alba - and 34 from Cluj counties. The 2001 samples were collected from pig - and poultry breeding farms; with these maize is part of combined feeds meant for the feeding of these species; as well as in the husbandries.In so far as the 2002 samples are concerned; part of these were harvested directly from maize fields; and another came from corn cribs on farms and husbandries.

Partial samples were collected using spears and scoops. Three incremental samples were drawn from all layers of the crop container. Multiple subsamples were pooled; mixed throughly and quartered. Opposite quarters were rejected and the remainder mixed anew to obtain one final reduced sample of 500g. For mycological examination- preparation of samples and insemination on solid Sabouraud medium; incubation at 24^0C. Reeding of results at 3- and 5 days from incubation. Expression of the result**:** CFU/g product. Identification of prevailing fungi carried out for the stereomicroscopic examination of the cultural characters and microscopic of the preparations on slides; stained with Bleu Cotton.Ergosterol was appraised by HPLC/UV-detection after saponification according to Schwadorf and Müller (7). For zearalenone analysis the sample was extracted with a mixture of acetonitrile and water; sample cleaning was carried out on immunoaffinity column. Identification and quantification of ZEA was carried out by HPLC with fluorescence detection(6). Its detection limit was 2 µg/kg.

RESULTS AND DISCUSSIONS

Following the mycological examination; there has been found a moderate mycotic load with a number of 14 samples; in these the CFU/g product was comprised between 60 thousand and 300 thousand and an intense mycotic charge was noticed with 11 maize samples; in these the CFU/g varied between 300 thousand and 6;600 thousand. The highest mycetes occurrence have belonged *Fusarium; Rhisopus; Alternaria; Aspergillus* genera.

Ergosterol was detected with all samples analysed. The highest occurrence of infestation with ZON was recorded in 2001 both in Alba county (20 samples) and in Cluj (9 samples) county;as well. The increased occurrence correlates with the high concentrations of ZON.

With Erg; the highest frequency was recorded in 2001 (22 samples) in Alba county i.e.; a mean of 3;52 mg/kg and a standard deviation of 2;90 very close to that in Cluj county in 2002 (21 positive samples) with a mean of its level of 2;21 mg/kg and a standard deviation of 1;66 (table1).

Table 1.

Mean values and dispersion indices of levels Erg and ZON

County	**Year/parameter**		**n**	**X ± s_X**			**s**
Alba	2001	Erg (mg/kg)	22	3;52	±	0;61	2;90
		ZON(µg/kg)	20	242;45	±	98;96	442;58
	2002	Erg (mg/kg)	14	2;14	±	0;65	2;43
		ZON(µg/kg)	3	26;66	±	20;67	35;80
Cluj	2001	Erg (mg/kg)	13	11;27	±	6;09	21;96
		ZON(µg/kg)	9	802;55	±	332;24	996;74
	2002	Erg (mg/kg)	21	2;21	±	0;36	1;66
		ZON(µg/kg)	6	40;50	±	30;39	74;44

The highest occurrence of infestation with ZON was recorded in 2001 both in Alba county (20 samples) and in Cluj county (9 samples); the increased occurrence was accompanied by very high concentrations of these; as well.

A fairly significant correlation (r = 0.95) was recorded in 2002 in Alba county (table). Between the mycotoxin level studied and that of Erg were identified distinctly significant correlations in 2001 with both counties studied i.e.; Alba (r = 0;81) and Cluj (r = 0;80) (table 2).

Table 2.

Correlations between ZON (µg/kg)—and Erg (mg/kg) levels by years and counties

Specification	r(X;Y)	r	t
ZON-ERG;2001; AB	0;81	0;67	6;04
ZON-ERG;2002; AB	**0;95**	**0;91**	**3;29**
ZON-ERG;2001; K	0;80	0;65	3;64
ZON-ERG;2002; K	-0;58	0;33	-1;42

In so far as the highest concentration in ZON (2250µg/kg) is concerned; was encountered in Cluj county in 2001; in Alba county this was 1666 µg/kg.

Curtui et al. (2) analysed maize samples from Western Romania and detected zearalenone in 13 % of the samples; with a median content of 250 µg/kg and a maximum value of 1200 µg/kg.

In comparison with the situation in other European countries; it is possible to assert that; in the Romanian samples a rather high degree of zearalenone contamination is obvious. For example Lew et al (4) detected zearalenone contents between 40 and 90 µg/kg in samples from Austria in the harvest years 1996 - 1998 with maximum contents of ZEA not exceeding 340 µg/kg.

CONCLUSIONS

The results of the mycological examination of maize samples taken under study have revealed an increased mycotic load (60-300 thousand CFU/g) and very high (300-6;600 thousand CFU/g) to over 30% of the samples; although the maize did not display organoleptic alterations macroscopically that could be identified; between mycotic charge and the humidity of maize a direct correlation is found. The prevailing species of mycetes belonged to *Fusarium; Rhisopus*; *Alternaria; Aspergillus* genera. Ergosterol was detected with all the 70 samples (100%); the highest values (72 mg/kg) having been recorded with the maize cultivated in Cluj in the year 2001. Zearalenone was found with 54% of the total of analysed maize samples reaching values of up to 2250 µg/kg. The highest ZON charge was find in maize crops from the year 2001 in Cluj; where the rainfalls were higher than in Alba county.

Comparing the data obtained in the two years; i.e.; 2001 and 2002; respectively (samples from the harvest of 2001 were stored on the farms till harvest time) one can notice that the level of ergosterol as well as that of zearalenone; were higher in samples of the year 2001 than those of 2002. This can be explained that rainfall in April through October 2001 was 520 mm higher; as to 405 mm in 2002 along the same periods as well as that the storage of maize was improper; thing allowing for the development of *Fusarium* toxins during storage.

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