THE DETECTION AND QUANTIFICATION OF MYCOTOXINS DIFURANICE IN MEDICINAL SPECIES

DECELAREA ŞI CUANTIFICAREA MICOTOXINELOR DIFURANICE ÎN SPECII MEDICINALE

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Abstract. *Species (medicinal species) is a pharmaceutical form made up of mixtures of different plant organs (plant products), dried, which is used in therapy in the form of infusions, decoctions, macerates, syrups, tinctures, glycerine extracts, oil extracts, wine etc. In temperate continental area in which our country stands, saprophyte fungi live on the medicinal herbs so the medicinal species are often seen as different mycotoxins, metabolites of fungi. The highest incidence in this type of phyto is represented by mycotoxinsdifuranice (aflatoxins and sterigmatocistins), ochratoxin and patulin. The objective of this work consists in the qualitative and quantitative determination of mycotoxins in samples of medicinal species from pharmacies, freelance producers deprived of knowledge of toxicokinetics and toxicodynamics active principles of plants and herbal shops. The experiment was performed on 36 samples of vegetable and medicinal species, samples that were tested by first screening test in LUV and samples which showed fluorescence were studied further by high pressure chromatography. Most plant products from manufacturers freelancers do not correspond with the organoleptic rules and shows mycotoxins load.*

Key words: medicinal species, active principles, vegetal products, mycotoxin, quantitative and qualitative mycological test, mycotoxicological exam, *Tiliaeflos*, *Hypericiherba*, *Maydis stigmata*, ochratoxin, sterigmatocistin

Rezumat*. Species (specii medicinale) reprezintă o formă farmaceutică alcătuită din diferite amestecuri de organe de plante (produse vegetale) uscate, care se utilizează în tearapeutică sub formă de infuzii, decocturi, macerate, siropuri, tincturi, extracte glicerinate, extracte uleioase, vinuri etc. În zona temperat continentală în care se situează ţara noastră miceţii trăiesc saprofit pe plantele medicinale,astfel încât în speciile medicinale se decelează adesea diferite micotoxice, produşi metabolici ai miceţilor. Incidenţa cea mai crescută în acest tip de fitopreparate o au micotoxinele difuranice (aflatoxinele şi sterigmatocistinele), ochratoxina şi patulina. Obiectivul acestei lucrări constă in determinarea calitativă şi cantitativă a micotoxinelor in probe de specii medicinale provenite din farmacii, producători liber profesionişti lipsiţi de cunoştinţe legate de toxicocinetica şi toxicodinamia principiilor active din plante şi de la magazine naturiste. Experimentul s-a efectuat pe 30 probe de produse vegetale şi specii medicinale, probe care au fost testate mai întâi prin testul screening in LUV, iar probele ce au prezentat flourescenţă au fost studiate în continuare prin cromatografie de înaltă presiune. Majoritatea produselor vegetale provenite de la producători liber profesionişti nu corespund organoleptic normelor în vigoare şi prezinta incărcătura micotoxinică.*

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Cuvinte-cheie: specii medicinale, principii active, produse vegetale, micotoxină, test micologic calitativ şi cantitativ, examen micotoxicologic, *Tiliaeflos, Hypericiherba, Maydis stigmata*, ochratoxina, sterigmatocistina

INTRODUCTION

Medicinal species (*Species, FR X)* represent an old pharmaceutical formula obtained from the mixture of vegetal products (plant organs with a certain pharmacological action used in therapy). Depending of the therapeutic and physicochemical features of their active principles, medicinal species are administered as aqueous, alcoholic, hydro-alcoholic, glycerinate, hydro-glycerinated extract solutions, oils, aromatic waters, syrups, tinctures, wines, dry extracts etc. Due to its high bioavailability and good tolerance, phytomedicinesoccupy an important place among the modern therapeutic methods. Medicinal and aromatic plants can be the ideal substrate for numerous mycetes that can parasite the plant in both vegetative and storage phase, especially in continental climate areas where the environmental factors ease their proliferation and the biosynthesis of mycotoxins. Mycetes possess a remarkable capacity to synthesise certain secondary metabolites: pigments, antibiotics, chemotherapics, phytotoxins and mycotoxins. (Butler, 1974, Coman *et al*., 1985). Equally stunning is their capacity to adapt to the most different environments (Davis, 1987; Feng, 1998). Their hyphal structure facilitates their access and their development on solid life-hostile surfaces and the synthesis of mycotoxins (Vining, 1992; Moss, 1996). Mycotoxins are metabolic products with different chemical structures and highly stabile physicochemical features which make the detoxification of contaminated food products by means of chemical, physical and biological proceduresaccessible to the food industry to be impossible. Mycotoxins manifest the so called "relay toxicity" for humans and all the inconvenients of expressing toxicity through DL_{50},an indicator that excludes the "cumulative toxicity" which includes chronic toxicity, carcinogenesis, teratogenesis and immunosuppression. The real dimension of mycotoxin pathogenicity is not yet considered to be clearly established, as the presence of mycetes and their metabolites in diseases with occult pathologies is suspected (Reye syndrome, acute respiratory distress syndrome, congenital malformations etc.) (Prisăcaru, 1998; Moretti, 2010).

MATERIAL AND METHOD

The experimental model (tab. 1) consisted in the mycological and mycotoxicological examination performed on 36 samples of *Tiliaeflos* (linden flowers with stigmata), *Hypericiherba* (the upper part of Saint John's wort in adult plants) and *Maydis stigmata* (corn silk). The samples were collected from the Copou – Iasi, Pietrarie – Iasi, Comanesti-Bacau areas and from herbal shops of Iasi city and afterwards dried.

A) The mycological examinationbegan with the *quantitative mycological exam* that was intended to establish the total number of mycetes per gram of vegetal product (NTM/g). The method used was the serial dilution method (Prior, 1981). The mycological exam continued with the*qualitative mycological exam* that was intended to identify the genders and eventually the species they belong to.

B) The mycotoxicological examination consisted of three phases, the results of each of them determining the passing to the next one. The three phases were: (I)the LUVexposure of 3 extracts from each sample, (II) analysis of the extracts with positive results in phase I using the HPLC test and (III) the TLC confirmation (thin layer chromatography) for the samples that had positive results in the previous phases (Badria, 1994; Prisăcaru, 1998; Radulović, 2013).

Table 1

The Experimental Model

Crt. no.	Vegetal product	No. of samples	Provenience areas	Sample abbreviation
1	*Tiliaeflos*	2	Iasi: Pharmacy I, Pharmacy II	F_I , F_{II}
		2	Copou area gardens	C_I , C_{II}
		3	Comăneşti (Lăloaia) locality	L_I , L_{II} , L_{III}
		3	Comăneşti (Leorda) locality	L_1 , L_2 , L_3
		1	Pietrărie: monastery	P_1
		1	Pietrărie: orchard	P_2
		1	Pietrărie: center	P_3
		5	Iaşi: healt stores	N_1 , N_2 , N_3, N_4 , N_5
2	*Hypericiherba*	2	Iasi:Pharmacy I, Pharmacy II	F_I , F_{II}
		2	Copou area gardens	C_I , C_{II}
		3	Comăneşti (Lăloaia) locality	L_I , L_{II} , L_{III}
		3	Comăneşti (Leorda) locality	L_1 , L_2 , L_3
		1	Pietrărie: monastery	P_1
		1	Pietrărie: orchard	P_2
		1	Pietrărie: center	P_3
		5	Iaşi: healt stores	N_1 , N_2 , N_3, N_4 , N_5
3	*Maydis stigmata*	1	Comăneşti (Leorda) locality	CL_1
		1	Comăneşti (Lăloaia) locality	CL_2
		2	Pietrărie: monastery	PM_1 , PM_2
		2	Pietrărie: orchard	PL_1 , PL_2

RESULTS AND DISCUSSIONS

The results obtained from the mycological and mycotoxicological analysis of the 36 samples were recorded in table 2. After studying the data it was noticed the presence of the *Fusarium* gender in one of the samples coming from aherbal shop. This gender includes highly toxicogenic mycetes that produce ochratoxins, fumonisins and trichothecenes. The corresponding extract, subjected to the mycotoxicological test indicated a relatively high mycotoxin load (Ochratoxin A, mycotoxin involved in the apparition of the acute respiratory distress syndrome in pigs NEF, Balkan endemic nephropathy). All the 15 samples of *Hypericiherba* are clean from both mycological and mycotoxicological point of view. Two of the six samples of *Maydis stigmata*, although collected and preserved according to the FR rules, 10th edition, presented a mycological load (CL_1 includes mycetes from the *Penicilium* gender, and CL_2 from the *Aspergillus* gender*).* As it results from table 2, from these two samples contaminated with toxigenic mycetes, only the CL_1 sample was characterised by the presence of furo-furanicmetabolite, sterigmatocystin.

Table 2

Results of the mycological and mycotoxicological study of the samples

Crt. no.	Vegetal product	Sample	Mycological examination	Mycotoxicological examination
1	*Tiliaeflos*	F_I , F_{II}	-	-
		C_I , C_{II}	-	-
		L_I , L_{II} , L_{III}	-	-
		L_1 , L_2 , L_3	-	-
		P_1	-	-
		P_2	-	-
		P_3	-	-
		N_1 , N_2 , N_3, N_4 , N_5	N_4 –*Fusarium*	N_4 - ochratoxin A

2	*Hypericiherba*	F^*_I , F^*_{II}	-	-
		C^*_I , C^*_{II}	-	-
		L^*_I , L^*_{II} , L^*_{III}	-	-
		L^*_1 , L^*_2 , L^*_3	-	-
		P^*_1	-	-
		P^*_2	-	
		P^*_3	-	-
		N^*_1 , N^*_2 , N^*_3, N^*_4 , N^*_5	-	-
3	*Maydis stigmata*	CL_1	*Penicillium*	Sterigmatocistin
		CL_2	*Asperillus*	-
		PM_1 , PM_2	-	-
		PL_1 , PL_2	-	-

CONCLUSIONS

1. From the 15 samples of *Tiliaeflos* (linen flowers) only one sample presented the existence of mycological load (the presence of *Fusarium* gender). The sample came from anherbal shop.

2. In the extract of sample N_4 where a mycetic load was identified there was also Ochratoxin A, a mycotoxin whose target are the kidneys and the lung.

3. The 15 samples of *Hypericiherba*presented no mycological load, and no mycotoxins.

4. Two of the 6 analysed samples, belonging to the vegetal product *Maydisstgmata*, presenteda mycetic load (the presence of Penicilium and Aspergillus gendersin CL1 and CL_2 and the presence of mycotoxin, chemically related to aflatoxin (stericmatocystin) was discovered only in sample CL_2.

REFERENCES

1. Butler W.H., 1974 – *Aflatoxin, In: Micotoxins,* Purchase, I.F.H., Elsevier, 215

2. Badria F.A., Amer M.A., Agwa H.E., 1994 – *A comprehensive screening of Aflatoxins in Egyptian Animal Feeds*, ZagacZig, J. of Pharmaceutical Sciences, 92

3. Coman I., Popescu O., 1985 – *Micotoxine si micotoxicoze*, Ed. Ceres, Bucuresti

4. Davis N.D., Diener U.L., 1987 – *Mycotoxins In Food and Beverage Mycology*, 2nd ed. (ed. L.R. Beuchat), 517-570, Van Nostand Reinhold: New York.

5. Feng G.H., Leonard T.J., 1998 – *Culture conditions control expression of the genes for aflatoxin and sterigmatocistyn biosynthesis in Aspergillusparasiticus and A. nidulans*, Appl. Environ Microbiol., Jun.

6. Moretti A., Ferracane L., Somma S., Ricci V., Mulè G., Susca A., Ritieni A., Logrieco A.F., 2010 - *Identification, mycotoxin risk and pathogenicity of Fusarium species associated with fig endosepsis in Apulia, Italy, Food Additives & Contaminants: Part A*, Volume 27, 2010, Issue 5, 718-728

7. Moss M.O., 1996 – *Mycotoxins, Centenary Review*, Mycol. Res. 100, 513-523

8. Prisacaru C., Cotrau M., 1998 – *Un nou semnal de alarma pentru mileniul III: Fumonizinele*, Sesiunea Ştiinţifică Anuală de Medicină Veterinară, Iaşi, 7-8 mai 1998, p.12.

9. Prior M.G., 1981 – *Mycotoxins in animal feedstuff and tissues in Western Canada*, 116-119

10. Radulović S., Marković R., Milić D., Jakić Dimić D., Šefer D., 2013 - D*egree of mycotoxicological contamination of feed and complete feed mixtures for pigs and poultry during the period 2007–2012. on the territory of the Republic of Serbia,* Jour. Nat. Sci, Matica Srpska Novi Sad, No. 124, 153—169

11. Vining L.C., 1992 – *Role of secondary metabolites from microbes, In: Secondary matabolites: their function and evolution*, John Wiley: Chichester, 1122-1123.