PRODUCTION OF AFLATOXINS ON MEDICINAL HERBS

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Keywords: phytotherapic products, toxicogenic capacity, aflatoxins, safety, control

Abstract

For the National Health Authority it is very important to ensure the harmlessness of phytotherapeutic products. The aflatoxins are potent carcinogenic contaminants present on materials of vegetable origin. They should be investigated on vegetable drugs.

In this survey 41 species of medicinal and aromatic herbs have been studied to determine the susceptibility to aflatoxin contamination by artificial inoculation with *Aspergillus parasiticus* NRRL 2999. From all the species infested with this toxicogenic fungus, 17 were positive for aflatoxins. Natural aflatoxin contamination must be checked on these species whenever regulated industries use them as phytotherapeutic medicine components.

1. Introduction

The National Health Authority wants to have a proper legal background that insures the quality and harmlessness of phytotherapeutic products. The Authority is active to obtain especially, the best way to control the safety of them.

Considering aflatoxins, natural carcinogenic contaminants, as an hazard to human and animal health the WHO (1992) has recommended their control on any material of vegetable origin.

Medicinal herbs can contain, either antimycotic and antimycotoxicogenic inhibitory substances or components that do not affect the fungal growth and toxin production (Llewellyn, 1981). Records on aflatoxins contamination in medicinal herbs has been previously registered in our country (Rizzo, 1995). It is important to continue with these studies to establish a list of different herbs able to contain natural aflatoxin contamination with the purpose to reduce the number of species to be examined for a future control. For this reason, the aim of this survey was to determine the susceptibility to aflatoxin contamination of medicinal herbs artificially inoculated with *Aspergillus parasiticus* NRRL 2999.

2. Material and Methods

2.1. Sampling

Forty-one native and imported species of vegetable drugs used as raw materials for phytotherapeutic products (medicinal teas) have been studied (List I). Samples of 250 g were taken out from regulated industries qualified by the ANMAT (Administración Nacional de Medicamentos, Alimentos y Tecnología Médica: Drug, Food and Medicinal Devices Administration).

Proc. WOCMAP-2
Ind Processing, Standards & Regulations,
Quality Control, Supply & Marketing, Economics
Eds. V. Martino *et al*
Acta Hort 503, ISHS 1999

2.2. Organism

Aspergillus parasiticus Speare (strain NRRL 2999) was used to inoculate the samples. The culture was maintained on Potato-Dextrose Agar medium (PDA) and stored at 4°.

2.3. Artificial contamination

Approximately 3 g of sterile rice moistened with 50% water was used as positive control for toxins production (Shotwell, 1966).

Samples (3g), after UV light treatment during 30 minutes, were added with different volumes of sterile water, according to each herb. Then, they were inoculated with a homogeneous heavy suspension of *A. parasiticus* NRRL 2999 and incubated at 27° for 30 days.

2.4. Aflatoxin analysis

After the incubation, inoculated samples and control were treated at 60° overnight to destroy the fungus and were then ground to perform the chemical analysis of aflatoxins (Shotwell *et. al.*, 1966), modified at the detection stage using either Uni-dimensional TLC with two developing solvents in the same direction (anhydrous ethyl ether:hexane (3:2) for clean-up and chloroform:acetone (9:1) for detection) or two-dimensional TLC for extracts with interfering subtonics (first direction: chloroform: acetone (9:1), second direction: anhydrous ethyl ether: methanol: water (95:4.5:1.5)). For confirmation of identity 30% sulfuric acid solution was used.

3. Results

Noteworthy growth of *A. parasiticus* NRRL 2999 was observed on all the species assayed except on *Lavanda officinalis*.

From the 40 species infested with this toxicogenic fungus, 17 (42.5%) were positive for aflatoxins B_1 and B_2 (List 2). Aflatoxins B_1, B_2, G_1 and G_2 were detected only on *Crataegus oxyacantha* L. The 23 remaining species were negative.

4. Discussion

Fungal counts on *Lavanda officinalis* samples were only poor or negative (<10 propagules/g). These results and the absence of *A.parasiticus* NRRL 2999 growth could suggest that this species has inhibitory components that prevent the fungal contamination (Le Bars, 1992; Rizzo, 1996).

A positive artificial contamination indicates the herb could be naturally contaminated with aflatoxins. If this assay is negative it would be necessary to repeat it, at least 10 times, with the same species from different periods of recollection and from different geographic zones (statistical validity) to conclude that the herb contains antimycotoxicogenic substances. Therefore this survey will go on with the 23 remaining species to determine their real susceptibility.

As conclusion, according to our results, aflatoxin checking wouldn't be necessary on *Lavanda officinalis* but it must be performed on medicinal herbs included in List 2 whenever regulated industries use them as phytotherapeutic medicine components.

5. References

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List 2: Medicinal and aromatic herbs susceptible to aflatoxin contamination

Argentinean name	Scientific name
Ambay	*Cecropia adenopus* Mart.
Belladona	*Atropa belladonna* L.
Cardo mariano	*Cardus marianus* L.
Carqueja	*Baccharis articulata* (Lam.)
Cascara de naranja amarga	*Citrus aurantium* Linneo
Cascara sagrada	*Rhamnus purshiana* DC.
Cedrón	*Aloysia triphylla* (L'Hérit.)
Cola de Caballo	*Equisetum giganteum* L.
Crataegus	*Crataegus oxyacantha* L.
Gramilla	*Cynodon dactylon* (L.) Peerson
Marrubio	*Marrubium vulgaris* L.
Melisa	*Melissa officinalis* L.
Pasionaria	*Passiflora coerulea* L.
Peperina	*Minthostachys mollis* (Kunth) Gris.
Sen	*Cassia angustifolia* Vahl.
Tilo	*Tilia spp.*
Valeriana	*Valeriana officinalis* L.

List 1: Species of medicinal and aromatic herbs analyzed

Argentinean name	Scientific name
Ambay	*Cecropia adenopus* Mart.
Anacachuita	*Blepharocalyx tweediei* (Hook.te Arn.)
Baila Bien	*Haplopappus rigidus* Phil.
Belladona	*Atropa belladonna* L.
Boldo	*Boldea boldus*(Mol.)
Borraja de Campo	*Echium plantagineum* L.
Canchalagua	*Schkuhria pinnata* (Lam.)
Cardo mariano	*Carduus marianus* L.
Cardo santo	*Cnicus benedictus* (L.)
Carqueja	*Baccharis articulata*(Lam.)
Cascara de naranja amarga	*Citrus aurantium* Linneo
Cascara sagrada	*Rhamnus purshiana* DC.
Cedrón	*Aloysia triphylla* (L'Hérit.)
Ceibo	*Erythrina crista-galli* L.
Cepa Caballo	*Xanthium spinosum* L.
Chañar	*Geoffroea decorticans* (Gill.ex H.et A.)
Celidonia	*Chelidonium majus* L.
Cola de Caballo	*Equisetum giganteum* L.
Crataegus	*Crataegus oxyacantha* L.
Fumaria	*Fumaria officinalis* L.
Gramilla	*Cynodon dactylon* (L.)Peerson
Lavanda	*Lavandula officinalis* Cnaix
Manzanilla romana	*Anthemis nobilis* L.
Marrubio	*Marrubium vulgaris* L.
Mático	*Piper angustifolium* R.et P.
Melisa	*Melissa officinalis* L.
Menta piperita	*Mentha piperita* L.
Muña Muña	*Satureja parvifolia* (Phil.)
Paico	*Chenopodium ambrosioides* L.
Pasionaria	*Passiflora coerulea* L.
Peperina	*Minthostachys mollis* (Kunth) Gris.
Poleo	*Lippia turbinata* Griseb.
Retamilla	*Bulnesia retama*
Sen	*Cassia angustifolia* Vahl.
Tilo	*Tilia spp.*
Tramontana	*Ephedra triandra* (Tul.) J.H. Hunziker
Valeriana	*Valeriana officinalis* L.
Yerba de la Piedra	*Usnea barbata* (L.) Vigg.
Yerba del Pollo	*Alternanthera pungens* H.B.K.
Yerba Meona	*Euphorbia serpens* H.B.K.
Zarzaparrilla	*Krameria iluca* Phil