Research Article

Natural Occurence of Fungal and Aflatoxins Contamination in Some Genuine and Market Herbal Drugs

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Accepted on: 13-11-2012; **Finalized on:** 31-12-2012.

ABSTRACT

This study was carried out to determine the percentage incidences of associated mycoflora, presence and level of aflatoxins in fresh and market root drug. Five root herbal drugs belong to Dashmoola have been selected for present investigation. Isolation and identification of associated fungi were adopted using standard methods. Total 43 and 37 fungi were isolated from genuine and market samples. The genus *Fusarium* sp. from fresh samples and genus *Aspergillus* sp. from market samples recorded as predominant fungi. Qualitative and quantitative estimation of aflatoxins (B1, B2, G1 and G2) was determined by BGFY test and HPTLC method. The results indicated the fresh and market samples were contaminated with different quantities of aflatoxins especially AFB_1 and AFB_2 but natural occurrence of AF in analyzed herbal drugs was very less and it was below of tolerance level (20 µg kg) fixed by the World Health Organization.

Keywords: Mycoflora, aflatoxins, medicinal plants, percentage incidence.

INTRODUCTION

Most drugs are prone to bio deterioration by moulds and other fungi during post-harvest processing, transport, and storage; thus they are responsible for considerable economic loss. The shelf-life of crude drugs are influenced by many factors which include not only the quality of storage conditions but also the stability of the secondary metabolites present there in. Aflatoxins are very powerful hepta carcinogen classified as class[1] human carcinogens. The four major aflatoxins are B_1, B_2, G_1 and G_2. Approximately 40% of the productivity lost to diseases in developing countries is due to diseases exacerbated by aflatoxins. Many of people in these countries are not aware of the effect of consuming moulds products. Due to the poor education levels and other socio-economic factors. These countries also have poorly developed infrastructures such as processing facilities, storage, transportation and skilled human resources.[4]

A large number of reports are available on fungal contamination of agricultural and their products[1, 6, 7, 9], However a few published accounts are available on fungal and their mycotoxins in herbal plants. The present study was carried out on some herbal plants belongs to Dashmoola such as *Aegle marmelos*, *Clerodendrum phlomoides*, *Desmodium gangeticum*, *Gmelina arborea* and *Oroxylum indicum,* the roots of these plants are very important and they use in medicine alone or combination. In Ayurvedic texts, roots of 10 perennial medicinal plants are used together, 6 herbs and 4 tree species as Dashmoola. The ten root drugs together, also, are used in remittent fever, puerperal, fever inflammatory affections within the chest, affections of the brain and many other diseases supposed to be caused by derangement of vata, pitta and kopha. In this present investigation 5 roots of Dashmoola plants are selected for experiment.

MATERIALS AND METHODS

Isolation and identification of mycoflora

The fresh roots of herbal drugs were collected in healthy, flowering and fruiting conditions from different places of Maharashtra, India. Market drug samples were also collected from various Shopkeepers in Pune and Mumbai. Samples were brought to the laboratory in polyethylene bags and were cut into small pieces and soaked for 2 minutes in 2% sodium hypochlorite solution then thoroughly washed with sterilized distilled water. Ten pieces of roots placed in each Petri plate. Agar plate method (Potato Dextrose Agar, Water Agar, Czapek Dox Agar and Carnation leaf Agar) and Blotter test as recommended by International Seed Testing Association[15] were done for isolation and identification of mycoflora associated with roots. The root samples thoroughly washed with distilled water and plated in Petri plates and they were incubated at 25°C and after 3-4 days, developed colonies of fungi observed and the percentage incidence of mycoflora was recorded. Fungal were identified up to genera and species level by using previously published methods[5, 8, 18, 20, 25].

Qualitative and quantitative estimation of aflatoxins

BGFY test under UV light for showing presence of aflatoxins in fresh and market sample were done. One sample of fresh and one sample of market were selected, then, presence of aflatoxins (B1, B2, G1 and G2) in all samples observed. AFB_1 and AFB_2 produce a blue fluorescence, whereas AFG_1 and AFG_2 produce a green fluorescence[16]. The BGYE fluorescence under UV light (365 nm) is regarded as first hand confirmatory test for

Aflatoxin contaminated samples. HPTLC technique was employed for qualitative and quantitative analysis and the confirmation tests of aflatoxins present in the drugs. Fresh and market root samples used as a drug were analyzed chemically for natural contamination of aflatoxins using the method of Thomas *et al.*[26]

Preparation of Standard solution: Aflatoxin standards B1, B2, G1, and G2 were purchased from Himedia Chemical Co. in crystal form and weight of 1.0 mg. Data were collected and represented in graphs 1-5 and table 1.

RESULTS AN DISCUSSION

Results of percentage incidence of mycoflora

From roots of drug *Aegle marmelos* in fresh and storage samples, total 20 and 15 fungi isolated, followed by *Credendum phlomoides*, 18 and 13 fungi. From fresh and market roots of *Desmodium gangeticum* and *Gmelina arborea* 17, 18; 15 and 15 fungi respectively. From fresh and market samples of drug *Oroxylum indicum* 14 and 13 fungal species were isolated. In the fresh sample of selected drugs, different species of genus *Fusarium* specially *F. solani* and *Trichoderma* sp. and in market samples of these drugs the fungi *A. niger* recorded in high percentage incidence (Graphs 1, 2, 3, 4 and 5).

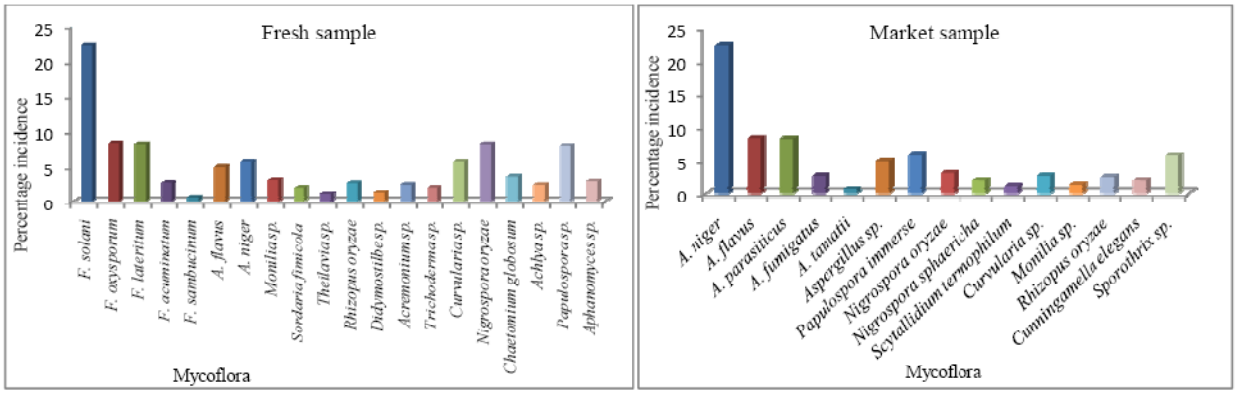
Results of aflatoxins contamination

Fresh and market sample of *A. marmelos* show positive response to BGYF test and after analysis by HPTLC; aflatoxin B_2 with 0.054970 (fresh samples) and 0.057366 (market samples) µg/g detect in sample (Table 1), in case of *C. phlomoides* fresh and market samples of this drug, after showing positive response to BGYF test, shows aflatoxin B_2 with 0.00104 µg/g amount in fresh samples and aflatoxins G_1 with 0.000622 and aflatoxin B_2 0.001965 µg/ g in market samples (Table 1). Fresh and market samples of *D. gangeticum* show negative response to BGYF test and in HPTLC analysis any aflatoxins not detect (Table 1). In *G. arborea*, fresh and market samples shows positive response to BGYF test and aflatoxin which detect in HPTLC analysis is B_2 with 0.000493 and 0.000627 µg/g in fresh and market samples, respectively (Table 1). Fresh and market samples of *O. indicum* show positive response to BGYF test but in HPTLC analysis no aflatoxins detect in fresh samples, market samples of this drug show aflatoxin B_2 in 0.001073 µg/g (Table 1).

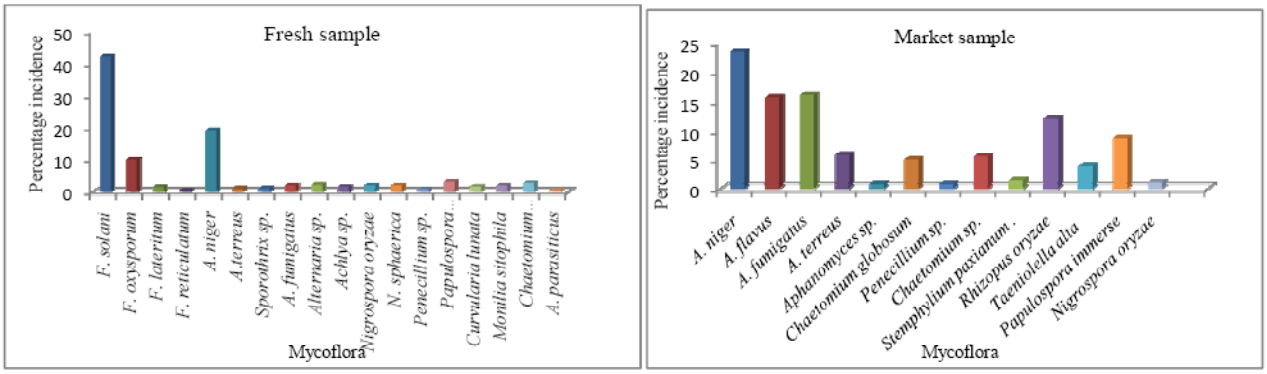
Christensen[10] grouped fungi into two ecological categories: field fungi and storage fungi. This division is not taxonomically valid but is based primarily upon moisture requirements. The major field fungi are species of *Alternaria*, *Cladosporium*, *Fusarium* and *Helminthosporium.* Field fungi attack developing and mature tissue of herbal drugs which contain at least 20% moisture. In the other hand, storage fungi are those encountered on plants at moisture condition routinely found in stored plant products. Storage fungi are predominantly species of *Aspergillus* and *Penicillium*.

Table 1: Qualitative and quantitative estimation of Aflatoxins in fresh and market drug samples

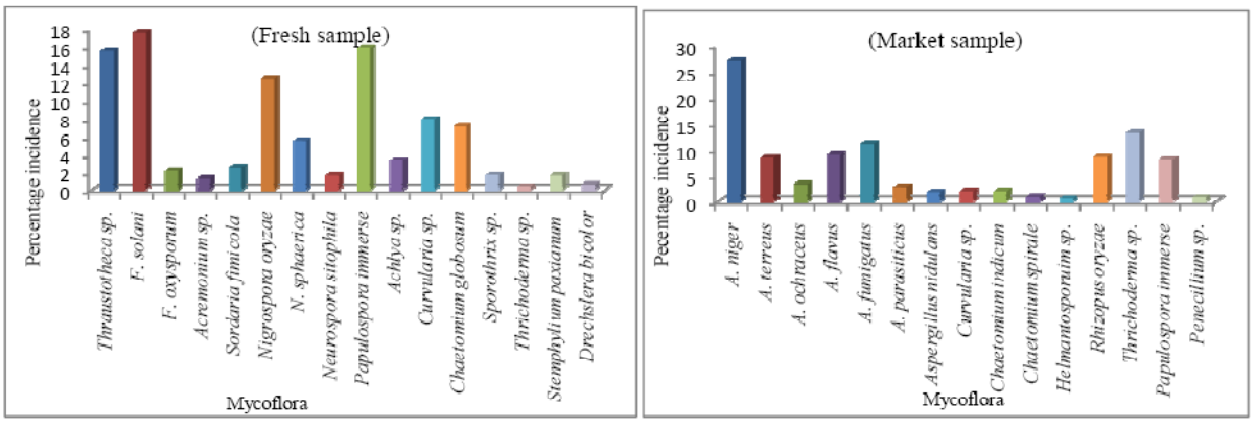
No.	Name of the drugs	Fresh/market sample	Positive/ negative to BGFY test	Specific Aflatoxin B_1 B_2 G_1 G_2	Conc. of Aflatoxin (microgram/garam)
1	*Aegle marmelos*	Fresh	+	B_2	0.054970
2	*A. marmelos*	Market	+	B_2	0.057366
3	*Clerodendrum phlomoides*	Fresh	+	B_2	0.001042
4	*C. phlomoides*	Market	+	G_1 B_2	0.000622 0.001965
5	*Desmodium gangeticum*	Fresh	-	-	-
6	*D. gangeticum*	Market	-	-	-
7	*Gmelina arborea*	Fresh	+	B_2	0.000493
8	*Gmelina arborea*	Market	+	B_2	0.000627
9	*Oroxylum indicum*	Fresh	+	-	-
10	*O. indicum*	Market	-	B_2	0.001073



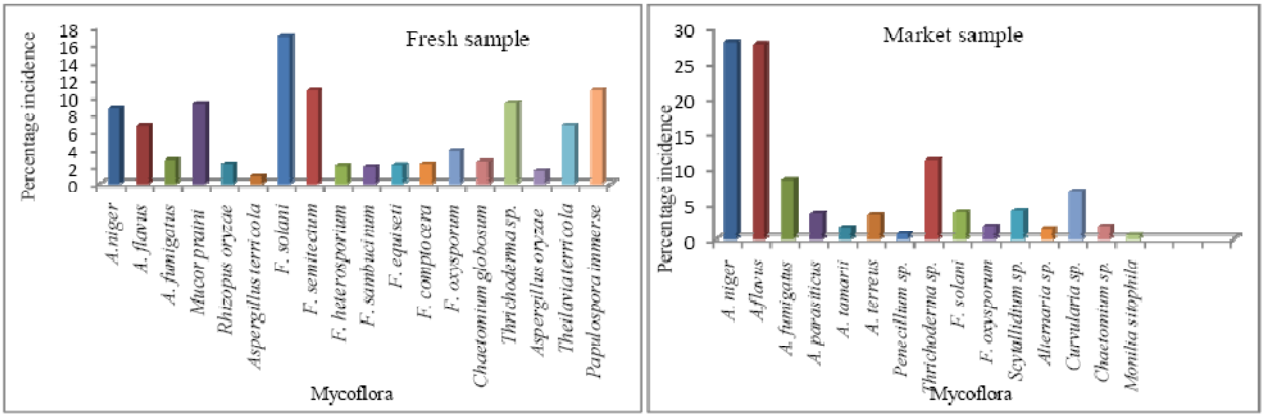
Graph 1: Percentage incidence of mycoflora associated with the root of *Aegle marmelos* (Fresh and market samples)



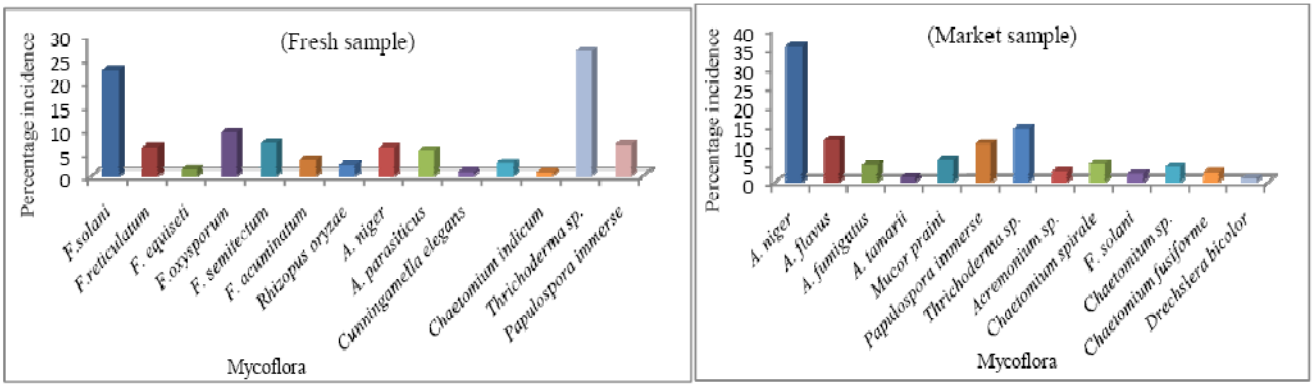
Graph 2: Percentage incidence of mycoflora associated with the root of *Clerodendrum phlomoides* (Fresh and market samples)



Graph 3: Percentage incidence of mycoflora associated with the root of *Desmodium gangeticum* (Fresh and market sample)



Graph 4: Percentage incidence of mycoflora associated with the root of *Gmelina arborea* (Fresh and market sample)



Graph 5: Percentage incidence of mycoflora associated with the root o f *Oroxylum indicum* (Fresh and market sample)

For the most part, the above categories are accurate; however, exceptions exist, *A. flavus* can invade in the field, and *Fusarium* can continue to decay of plant tissues in storage if the moisture is high enough. Our results are agreement with these two categories. Contamination of crude herbal drugs and medicinal plants have been reported earlier[3, 2, 11, 13, 14, 17-24]. Roy and Chourasia[21] expressed that contamination of herbal drugs is due to unscientific and traditional storage methods of medicinal plants.

Fungi can invade, colonize and produce mycotoxins either during preharvest (at the field level) or postharvest stages (storage, transport, and processing).These fungi colonize and utilize solid substrates by penetrating deep into their matrices by secreting enzymes to break down complex products. In most of the cases, the colonizing fungi produce and secrete low molecular-weight compounds (with confirmed toxic properties), generally referred to as "secondary metabolites" or "mycotoxins," which are usually not required for normal growth and survival. Mycotoxins are produced by some of the specific strains of filamentous fungi belonging to species of the genera *Aspergillus*, *Penicillium*, and *Fusarium* that invade crops at the field level and may grow during storage under favourable conditions.

The nature and quantity of mycotoxins produced is entirely influenced by interactions of several factors: types of substrate, moisture content, available nutrition, temperature, humidity in the surrounding environment, maturity of the fungal colony, occurrence with other fungi, competition from other microorganisms, stress factors, physical damage of the substrate due to insect activity and other associated factors. Mycotoxins might be present on all parts of the fungal colony, including the hyphae, mycelia on spores, and on or in the substrate on which the colony grows.

The results indicate that although the natural occurrence of AF in commercial traditional herbal medicines analyzed in this study was very less than tolerance level (20 µg kg) fixed by the World Health Organization, the contamination may be significant due to their frequent and prolonged consumption. It was concluded that herbal plants may be potential risk of fungal and mycotoxins contamination in suitable places with favourable conditions and also methods of harvesting, collecting, drying, transporting and storage of medicinal plant have to be improved.

Acknowledgment: Authors would like to express a sincere thank to Head, Department of Botany, University of Pune, Pune-411 007 for encouragement and necessary laboratory facilities.

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Source of Support: Nil, **Conflict of Interest:** None.