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Mycotoxin problems in some common medicinal plants of flood prone areas of Bihar

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ABSTRACT

The climate of Bihar remains warm and humid for most part of the year. Regular visit of flood and drought along with fluch factors as high level of illiteracy, socioeconomic backwardness and use of primitive agronomic practices in field and storage enhance mycotoxin risk in Bihar drastically. 75 samples of 15 common drug plants were collected from different flood prone districts of Bihar. Vital parts, used for medicinal purposes, of these plants were chemically analysed for the natural occurrence of mycotoxins in them. Most samples contained mycotoxin as a natural contaminant. Among the mycotoxins, aflatoxins occurred most frequently. Other mycotoxins reported to be present were ochratoxin, citrinin, zearalenone and fumonisin. These mycotoxins are very harmful to both man and animals. Some of these mycotoxins are carcinogenic, mutagenic and/or teratogenic. This is a matter of great concern as these plants are used for the preparation of traditional medicines. Treatment of one disease will be the unintentelyded cause of another which is still more dangerous. The problem needs our serious attention.

Introduction

Poor water management creates such stress conditions as flood and draught which provide conducive conditions for mycotoxin synthesis. Six worst flood affected districts were selected in the present investigation to study the incidence and level of mycotoxins in drug plants. The menace is further aggravated with high level of illiteracy, socio-economic backwardness and primitive methods of cultivation and storage that enhance mycotoxin risk in this state (Varma, 2004). Moisture along with temperature is the critical factor in the incidence of fungal species and subsequent production of mycotoxins in the substrate (Atanda et. al., 2013).

Mycotoxins are a diverse group of low molecular weight secondary metabolites of moulds. They comprise a wide variety of chemical types and have been implicated in causing diseases and death of man and animals in many parts of the world (Majid et. al. 2013). Toxigenic species mainly belong to the genus Aspergillus, Penicillium and Fusarium and they occur most frequently on drug plants both in field and in storage. Efficacy of herbal drugs in curing diseases is a well established fact (Evans et. al., 2002). Drug yielding plants have been extensively examined for their active principles and protective efficacy the world over (Pal and Shukla, 2003).. They are used either in crude forms or as medicines prepared from them. The traditional, unscientific methods of growing, harvesting, storing, and marketing herbal drug plants provide several opportunities for their association with fungal species. This exposes them to twin risks of biodegradation and mycotoxin contamination. Plant products as potential treatment candidates seemed to be more pragmatic, non-toxic, immunogenic and cost effective.

Mycotoxin problems in agricultural commodities have been studied by several workers (1, 3, 8); however, the occurrence of mycotoxins in plant drug yielding plants has not been studied properly so far (Roy et. el.1988) .So in the present investigation an attempt has been made to assess mycotoxin problems in some common drug yielding plants collected from the six flood prone districts of Bihar.

Materials and Methods

Altogether 80 samples comprising vital parts of drug yielding plants were obtained in clean labelled packets, each containing 50 g. from the six worst flood affected districts of Bihar. The samples were brought to the laboratory as soon as they were collected, finely ground and either tested on arrival or stored at 4°C to arrest any mycotoxin formation before analysis. The moisture content of each sample was determined by "OSAW Universal Moisture Meter". For isolation of the associated mycoflora, samples were subjected to standard blotter (ISTA, 1966) or agar plate test. Samples of plant parts were extracted chemically for natural occurrence of mycotoxins following the methods of Thomas et al., (1975) for aflatoxin and zearalenone and that of Roberts and Patterson (1975) for other mycotoxins. Qualitative estimation of mycotoxins was done on Thin Layer chromatography (TLC) plates (Reddy et. al., 1970). Quantitation of mycotoxin was done by uv-spectrophotometer (Nabney and Nesbitt, 1965) and also by visual methods by comparing spot areas with a graded series of reference standards.

Results

Altogether. 80 samples of 15 common drug yielding plants were collected from different flood prone districts of Bihar (Table 1). Altogether, 14 fungal species were isolated. *Aspergillus flavus, A. parasiticus, A. candidus, A. niger, A. luchuensis, A. ochraceus, A. nidulans, Fusarium moniliforme*, and *Penicillium citrinum* were the most common fungi. *A. flavus / parasiticus* had the highest incidence (41%) of occurrence. Incidence of species belonging to the genus *Alternaria*,

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Curvularia and *Chaetomium* was low. Vital parts of these plants were analysed for the natural occurrence of mycotoxins. 11 out of 80 samples analysed (incidence of 14%) contained mycotoxin as a natural contaminant. Among the mycotoxins, aflatoxins occurred most frequently. Other mycotoxins reported to be present were aflatoxin, ochratoxin, citrinin, zearalenone and fumonisin. Concentration of these toxins in many cases was much above the safe limit (30 µg / kg) prescribed by WHO. Presence of toxins as additive in drug plants poses a serious threat to our health. Maximum amount recorded were 1650 µg / Kg for aflatoxin, 810 µg / Kg for zearalenone, 720 µg / Kg for citrinin, 650 µg / Kg for fumonisin and 560 µg / Kg for ochratoxin. The amount of aflatoxins ranged from 120 – 1650 µg / Kg, fuminosins from 110 – 650 µg / Kg, zearalenone 310 – 810 µg / Kg, ochratoxins 180 – 560 µg / Kg and citrinin120 – 720 µg / Kg. Black cumin, fennel, cinnamon, saffron and curcuma recorded highest amount of aflatoxin, fuminosin, citrinin, ochratoxin and zearalenone respectively

Discussion

Floods are the recurrent features of Bihar. Rivers that cause havoc are Ganga, Sone, Bagmati, Kosi, Budhi Gandak and Adhwara Samooh. Overflowing rivers inundate thousands of villages and render more than a million people homeless every year. Out of the 20 districts prone to flood, the worst affected are – Darbhanga, Madhubani, Sitamarhi, East Champaran, Samastipur and Muzaffarpur.

Though mycotoxin risk is a world wide problem it is more acute in the tropical and sub-tropical countries like India. Bihar is particularly very susceptible to the risk owing to the factors like frequent visits of flood and drought, high or fluctuating temperatures in most parts of the year, high relative humidity, poor socio-economic condition of farmers, illiteracy and use of unsafe agronomic practices in agriculture (Varma, 2004).

The occurrence of mycotoxin in medicinal plants has already been established (Wongwiwat, 2004; Rani and Singh, 1990; Roy and Chourasia, 1990). The present study revealed that most samples of medicinal plants collected from the six worst affected districts of Bihar contained different mycotoxins as a natural contaminant. Most of these samples contained toxins much above the safe limit. As these plant materials were used for the preparation of traditional medicines, the possibility of medicines getting contaminated with mycotoxins is quite natural (Krishanthi and Bean, 1992). This is a matter of great concern. Use of contaminated medicines will become unintended cause another disease. Mycotoxins have been reported to be carcinogenic, teratogenic, tremorogenic, haemorrhagic, and dermatitic to a wide range of organisms and cause hepatic carcinoma in man (Refai, 1988). Greater number of people are now seeking remedies and health approaches from herbal drugs as they are free from side effects. Plant-based products for the prevention and cure of different human diseases is preferable to synthetic chemicals. 80% of the world's population relies on traditional medicine, particularly herbal drugs for their primary healthcare (Kamboj, 2000). There is a general belief that herbal drugs are without any side effects besides being cheap and locally available (Gupta and Raina, 1998).However, the stored drug samples has been reported to harbour mycotoxin-producing fungi in high frequency (Sewram 2006; Horie, Y. et. al., 1979; Narita, N. et al., 1980; Roy and Chaurasia, 1989)9–11. Mycotoxin in herbal drugs though is a global problem, its incidence is higher in tropical and subtropical countries as the agronomic practices and high temperature and moisture contents are conducive to fungal invasion and mycotoxin elaboration (Roy, A. K., 2003, 1989,)12 Presence of mycotoxins above the safe limit as prescribed by WHO is a point of great concern. It will create health hazards and our products will not be acceptable in the global market. The problem is associated with improper storage and processing of herbal drugs by Indian farmers. This also lowers the efficacy of the herbal drugs in curing diseases. The situation is alarming and appropriate quality control measures have to be taken urgently. The season at which each drug is collected and the age of the plant is also a matter of great importance. Improper drying of the harvested medicinal herb also enhance the menace (Evans, W. C et. al. 2002; Horonok, l. et. al. 1992).21,23. Poor water management aggravates the problem as it produces such stress conditions as flood and droughts which provide favourable conditions for toxin elaboration. The problem is further accentuated with unseasonal rains and rains during the harvesting time. Slow drying at moderate temperatures is advisable as it does not affect the enzyme adversely. Plant parts need be stored under hygienic conditions. However datas related to suitable conditions for storage of most of our medicinal herbs, is not available. Careless processing without considering these points is associated with twin risk of biodegradation and mycotoxin contamination of traditional medicines. This may be a major reason for ineffectiveness of some of our traditional medicines. India is rich in medicinal flora and so has a potentiality to occupy a significant position in the world trade of botanical drugs (Dubey et. al. 2004).

Table 1. Percent incidence of mycotoxin in medicinal plant samples

English name	Scientific name	Part of plant used	Total No. of samples	No. of samples contaminated with mycotoxin	% Incidence
Black cumin	Nigella sativa L.	Seeds	6	1	17
Fennel	Foeniculum vulgare	Dry fruit	5	1	20
Rauwolfia	Rauwolfia serpentina	Roots	5	-	0
Aconite	Aconitum napellus	Roots	5	-	0
Ginger	Zingiber officinale Rosoe	Rhizome	5	1	20
Cinnamon	Cinnamomum cassia	Bark	5	1	20
Pepper mint	Mentha spicata L.	Leaves	6	-	0
Coriander	Coriandrum sativum	Seeds	6	-	0
Quinine	Cinchona sp	Bark	5	-	0
Saffron	Crocus sativus L.	Dry parts of stylles and stigma	5	1	20
Curcuma	Curcuma longa L.	Rhizomes	6	2	33
Digitalis	Digitalis purpurea	Dry leaves	5	1	20
Rose	Rosa canina L.	Dry buds	6	2	33
Cloves	Eugenia aromatica	Buds	5	1	20
Pepper	Piper nigrum	Fruits	5	-	0
Total	15		80	11	14

Table 2. Level of mycotoxins in different drug plant samples

English name	No. of samples examined/contaminated	%Incidence	Amount of Mycotoxins (µg / Kg)				
			Aflatoxins	Fumonisins	Zearalenone	Ochratoxins	Citrinin
Black cumin	6 / 1	17	1650				120
Fennel	5 / 1	20	210	650	-	-	-
Rauwolfia	5 / 0	0					
Aconite	5 / 0	0					
Ginger	5 / 1	20	150	-	-	220	-
Cinnamon	5 / 1	20	-	-	-	-	720
Pepper mint	6 / 0	0	-	-	-	-	-
Coriander	6 / 0-	0	-	-	-	-	-
Quinine	5 / 0	0	-	-	-	-	-
Saffron	5 / 1	20	160	-	-	560	-
Curcuma	6 / 2	33	120 - 250	110	310 - 810	180	-
Digitalis	5 / 1	20	-	250	360	-	-
Rose	6 / 2	33	150 - 340	110 - 120	-	250	-
Cloves	5 / 1	20	850	220	-	-	-
Pepper	5 / 0	0	-	-	-	-	-
Total	80 / 11	14	120 - 1650	110 - 650	310 - 810	180 - 560	120 - 720

Table 3. List of mycotoxin producing fungi

MYCOTOXINS	PRODUCING FUNGI	Maximum amount recorded (µg / kg)
Aflatoxins (Carcinogen & mutagen)	Aspergillus flavus and *A. parasiticus*.	1650
Fumonisins	*Fusarium moniliforme,*	650
Zearalenone (teratogen)	*Fusarium roseum (F. graminearum) and F. moniliforme*	810
Ochratoxins (nephrotoxic)	*A. ochraceous & Penicillium sp*	560
Citrinin (nephrotoxic).	*P. citrinum*	720

Table 4. Ambient temperature and moisture conditions for the production of different toxins

Microorganism (mycotoxin)	Temp (◦C)	Available water
Aspergillus flavus, A. parasiticus (aflatoxin)	33	0.99
Aspergillus ochraceus (ochratoxin)	30	0.98
Penicillium verrucosum (ochratoxin)	25	0.90 – 0.98
Aspergillus carbonarius (ochratoxin)	15 to 20	0.85 – 0.90
Fusarium verticillioides, F. proliferatum (fumonisin)	10 to 30	0.93
Fusarium graminearum (zearalenone)	25 to 30	0.98

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