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Research Article

# DETECTION OF TOXIGENIC FUNGI AND MYCOTOXINS IN SOME STORED MEDICINAL PLANT SAMPLES

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## Abstract

A total forty samples of eight different medicinal plants were taken for the detection of toxigenic fungi and their mycotoxins. The fungal mycroflora comprises of six different fungal species belonging to three genera. *A. niger, Mucor species, A. flavus and Rhizopus species* dominate other fungal species isolated. Among ten samples of different medicinal plants which were contaminated with *A. flavus* was further analysed for mycotoxins potential. Four of them shows positive results for mytoxins potential. Although the presence of toxigenic fungi in a product did not imply the presence of mycotoxins in the product, their presence represents a potential risks of contamination with mycotoxins. Therefore, these medicinal plants should be carefully stored and the growth of the naturally found toxic fungi should be inhibited. Besides that, these medicinal plants must be tested for the presence of mycotoxins present prior to their use.

**Keywords:** Medicinal plants; Mycroflora; Toxigenic fungi; Mycotoxins.

## Introduction

The use of Ayurveda is one of the oldest, richest, and most diverse tradition, associated with the use of medicinal plants in India (Tandon *et al.*, 2004). In this system of medicine, different parts of the medicinal plants like (bark, stem, leaves, root, fruit etc.) are used in crude as well as powered form. Over 8000 plants species have been reported to prepare some 25,000 formulation to treats the various ailments (Dubey, 2004). A large section of the Indian population unquestionably believes in the efficacy of herbal medicinal plants, and this belief has gained impetus in other countries also in the recent years. The interest in herbal medicine is due to its miraculous efficacy in curing several human ailments. Despite in the advancement in the synthetic drugs, traditional medicine system are still viable and keeping pace with the modern synthetic medicine. Popularity of this indigenous system is due to the low production cost, easy availability and fewest side effects as compare to the modern synthetic medicine.

The increasing popularity of herbal medicinal drugs made their use a Public Health problem due to lack of effective surveillance of the use, efficacy, and toxicity and quality of their natural products. The premises that traditional use of these medicinal products for generations establishes their safety does not necessarily attest to their safety and efficacy. Indeed the adverse effects of long term herbal use, adulteration with toxic compounds and contamination by pathogenic microbial or natural toxins like mycotoxins have been reported for herbal products and medicinal plants. (Roy *et al.*, 1988; Roy and Chourasia, 1989; Efuntoye, 1999; Roy and Chourasia, 1990; Roy and Kumari, 1991; Reif, 1995; Aziz *et al*., 1998; Halt, 1998; Abou- Arab *et al*., 1999; Elshafie *et al*., 1999; Freire *et al.*, 2000; Elshafie *et al*., 2002; Rizzo *et al.*, 2004; Tassaneeyakul *et al.*, 2004 and Mandeel, 2005;).

Fungal contamination of stored herbal medicinal plants not only linked to discoloration, quality deterioration, reduction in commercial value as well in therapeutic potential but the mycotoxin produced by them in these herbal medicinal plants can also cause several ailments of liver, kidney, nervous system muscular, skin, respiratory organ, digestive tract, genital organs etc. (Purchase, 1974; Muntanola, 1987; Durakovic *et al*., 1989; Rai and Mehrotra, 2005; Truckesses and Scott, 2008).

Quality control to prevent growth of toxigenic fungi and mycotoxins contamination in medicinal plants is essential. Keeping this in view, in the present investigation a attempt has been made to examine some stored medicinal plants samples for the association of toxigenic fungi and mycotoxins contamination under stored condition.

## Materials and Methods

***Source of samples***

A total forty samples of 1. *Saraca Indica* (Ashoka) (n=5), 2. *Terminalia arjuna (*Arjuna*)* (n=5), 3. *Withania somnifera* (Ashwagandha) (n=5), 4. *Bacopa monnieri* (Brahmi) (n=5), 5. *Evolvulus alsinoides* (Shankhpushpi) (n=5) 6. *Zingiber officinale* (Ginger) (n=5),7. *Tribulus terrestris* (Gokharu) (n=5) 8. *Hemidesmus indicus* (Anantmula) (n=5) were collected form the different markets of Agra and nearby regions during the year 2009 – 10. The samples were packed in air–tight polythene bags and were transported to the Department of Botany, School of Life Science, Khandari Campus, Agra, immediately from their they were stored at room temperature till further analysis.

***Measurement of moisture content***

Moisture content was measured at the beginning. For moisture content, weighted amount of individual sample were dried at 100ºC for 24 hours and the difference in weight before and after drying the sample was calculated according to Essono *et al*., (2007).

$$MC = [(W_1 - Wf)/W_1] \times 100$$

where MC = moisture content,

$W_1$ = Initial weight and W*f* = final weight.

***Isolation, purification and maintenance of fungal culture:***

Mycoflora isolation was done by serial dilution method. The different fungi from different samples of medicinal plants was isolated and purified using standard techniques and maintenance was done on Czapek Dox Agar medium and Sabouraud's Dextrose Agar medium.

***Identification of mycoflora:***

Identification of fungi was also done on the basis of morphological and cultural characteristics as described by (Barnet, 1960; Smith, 1969; Subrahmanian, 1971 and Gilman, 1975)

***Frequency of fungal species:***

The Frequency of different fungal species was assessed of calculated the frequency percentage. There values were obtained according to Girridher and Ready (1997).

$$Frequency\ \ percentage\ \ = \frac{No.\ of\ observation\ in\ which\ a\ species\ appeared}{Total\ no.\ of\ observations} \times 100$$

***Preliminary screening of fungal isolate for toxigenic potential and their mycotoxins:***

The different isolated fungus species obtained from the different samples of medicinal plants were tested for their Toxigenic nature by "orange, yellow" pigmentation method (Lin and Dianese, 1976).

## Results and Discussion

***Moisture content of different medicinal plant samples:***

Moisture content was observed in all medicinal plants ranges form of 0.2% to 8.9%. However, it was noted that there was significant difference between the mean moisture content of different medicinal plants samples. Highest mean moisture content was observed in *E. alsinodies* (8.9%) followed by *B. monnieri* (8.7%), *Z. officinale* (7.7%), *S. indica* (3.3%), *T. arjuna* (3.2%), *W. somnifera* (2.7%), *T. terrestris* (0.3%) and the lowest mean moisture content was observed in *H. indicus* (0.2%) are shown in Table 1.

The presence of moisture content in medicinal plant samples justify favorable impact of fungal growth in stored medicinal plants (Roy, 1989; Halt, 1998). Diverse abiotic factors operating in the processing and storage conditions as well as chemicals constituents of the medicinal plants might have resulted in variation in mycopopulation in different substrates (Chourasia *et al*., 2008). During the survey for samples collection, it was found that necessary precautions were not taken during processing and storage of these medicinal plant samples, at some places these medicinal plant samples were found under open environmental condition and some ware under dark store rooms under unhygienic conditions. All these practices may contaminate these medicinal plants by exposing them to microbial infections.

**Table 1**: Showing mean moisture content percentage in different medicinal plant samples

| S.No. | Name of Plants | Mean Initial Weight $W_1$ in gm. | Mean Final Weight Wf in gm. | Mean Moisture Content percentage |
|---|---|---|---|---|
| 1. | *Saraca indica* | 20 | 19.34 | 3.3 |
| 2. | *Terminalia arjuna* | 20 | 19.36 | 3.2 |
| 3. | *Withania somnifera* | 20 | 19.45 | 2.7 |
| 4. | *Bacopa monnieri* | 20 | 18.25 | 8.7 |
| 5. | *Evolvulus alsinoides* | 20 | 18.21 | 8.9 |
| 6. | *Zingiber officinale* | 20 | 18.46 | 7.7 |
| 7. | *Tribulus terrestris* | 20 | 19.94 | 0.3 |
| 8. | *Hemidesmus indicus* | 20 | 19.96 | 0.2 |

***Frequency of fungal contamination:***

The mycological examination of all 40 samples of 8 different medicinal plants revealed that, 37 (92.5%) of the total analyzed samples was found to be contaminated with one or more fungal species.

All (100%) samples of *S. indica, T. arjuna, W. sominifera, B. monnieri, E. alsinoides* and *Z. officinale* were found to be contaminated with one or more fungal species.

While in *T. terrestris* 80% of the total samples analyzed was found to be contaminated with one or more fungal species.

While in *H. indicus* 60% of the total samples analyzed was found to be contaminated with one or another fungal species (Table 2)

Table : 2 Showing all the samples of different medicinal plants contaminated with one or more fungal species.

| Name of Plants | Sample No. | Name of Fungus | | | | | | Fungal contamination per sample |
|---|---|---|---|---|---|---|---|---|
| | | ***A. flavus*** | ***A. nidulan*** | ***A. niger*** | ***M. thermophila*** | ***Mucor*** | ***Rhizopus*** | |
| *S. indica* | 1 | + | - | + | - | - | + | 3 |
| | 2 * | + | + | - | - | - | - | 2 |
| | 3 | - | - | - | - | + | - | 1 |
| | 4 | - | - | + | - | + | - | 2 |
| | 5 | - | - | - | - | + | - | 1 |
| *T. arjuna* | 6 | + | - | + | - | - | - | 2 |
| | 7 | + | - | + | - | - | - | 2 |
| | 8 * | + | - | + | - | - | - | 2 |
| | 9 | - | - | + | + | - | - | 2 |
| | 10 | + | - | + | - | + | - | 3 |
| *W. somniera* | 11 | - | - | + | - | + | - | 2 |
| | 12 | - | - | + | - | + | - | 2 |
| | 13 | - | - | + | - | + | - | 2 |
| | 14 | - | - | + | - | - | - | 1 |
| | 15 | - | - | + | - | + | - | 1 |
| *B.monnieri* | 16* | + | - | + | - | - | + | 3 |
| | 17 | + | - | + | - | + | + | 4 |
| | 18 | - | - | + | - | + | + | 3 |
| | 19 | - | - | + | - | + | - | 2 |
| | 20 | - | - | + | - | - | + | 2 |
| *E.alsinoides* | 21* | + | + | + | - | + | + | 5 |
| | 22 | - | - | + | - | - | - | 1 |
| | 23 | - | - | + | - | + | + | 3 |
| | 24 | - | - | + | - | + | + | 3 |
| | 25 | - | - | + | - | + | + | 3 |
| *Z. officinate* | 26 | - | - | + | - | + | - | 2 |
| | 27 | - | - | + | - | + | - | 2 |
| | 28 | - | - | + | - | - | + | 2 |
| | 29 | + | - | + | - | + | - | 3 |
| | 30 | - | - | + | - | + | - | 2 |
| *T. terrestris* | 31 | - | - | + | - | + | - | 2 |
| | 32 | - | - | + | - | - | - | 1 |
| | 33 | - | - | - | - | - | - | - |
| | 34 | - | - | - | - | + | - | 1 |
| | 35 | - | - | - | - | + | - | 1 |
| *H. indicus* | 36 | - | - | + | - | + | - | 2 |
| | 37 | - | - | - | - | - | - | - |
| | 38 | - | - | - | - | - | - | - |
| | 39 | - | - | - | - | + | - | 1 |
| | 40 | - | - | + | - | + | - | 2 |
| Total contamination in all samples | | 10 | 2 | 30 | 1 | 25 | 10 | 78 |

- Positive for mycotoxins potential.

***Distribution of different fungal species in all samples of different medicinal plants***

Analysing of different sample of all medicinal plants revealed that the frequency of contamination of *A. niger* is maximum (38.46%) followed by Mucor (32.05%), *Rihzopus* (12.82%), *A. flavus* (12.82%), *A. nidulans* (2.56%), and *Myceliophthora* (1.28%) as shown in Table 3.

**Table 3**: Percentage Distribution of the fungal species detected in medicinal plants.

| S. No. | Fungi isolated | Number of isolates | Percentage |
|---|---|---|---|
| 1. | *A. flavus* | 10 | 12.82% |
| 2. | *A. nidulans* | 2 | 2.56% |
| 3. | *A. niger* | 30 | 38.46% |
| 4. | *M. thermophila* | 1 | 1.28% |
| 5. | *Mucor* species | 25 | 32.05% |
| 6. | *Rhizopus* species | 10 | 12.82% |

Species of *Aspergillus* dominate the mycoflora of collected samples of medicinal plants of *S. indica, T. arjuna, W. somnifera, B. monnieri, E. alsinoides, G. officinale, T. terrestris* and *H. indicus* were it was already reported that *Aspergillus* species dominating mycoflora of stored medicinal plants (Hitokoto *et al*., 1978; Ayres *et al*., 1980; Aziz *et al*., 1998; Arab *et al*., 1999; Elshafie *et al*., 1999; Mandeel, 2005). Hence, the presence of wide range of fungi in these medicinally important medicinal plants showed that there was is a potential risk for mycotoxins contamination, especially during prolonged storage in poor storing conditions without temperature and moisture control (Efuntoyc 2004; Bugno *et al*., 2006; Singh *et al*., 2008).

***Distribution of different fungal species with in samples of different medicinal plants:***

By Analyzing the samples of different medicinal plants it was observed that the samples of *S. indica* medicinal plant samples were frequently contaminated with *Aspergillus flavus* (40%) *A. nidulans* (20%), *A. niger* (40%), *Mucor* (60%) and *Rhizopus* (20%), in *T. arjuna* medicinal plant samples *A. flavus* (80%), *A. niger* (100%), *M. thermophilia* (20%), *Mucor* (20%), in *W. sominifera* medicinal plant samples *A. niger* (80%), *Mucor* (80%), in *B. monnieri* samples *A. flavous* (40%), *A. niger* (100%), *Mucor* (60%), *Rhizopus* (80%), in *E. alsinoides* samples *A. flavus* (20%) *A. nidulans* (20%), *A. niger* (100%), *Mucor* (80%), *Rhizopus* (80%), in *Z. officinate* samples *A. flavus* (20%), *A. niger* (100%), *Mucor* (80%), *Rhizopus* (20%), in *T. terrestris* samples *A. niger* (40%), *Mucor* (60%) and in *H. indicus* samples *A. niger* (40%), *Mucor* (60%) were found (Table 4.)

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***Determination of toxigenic contamination of samples***

It is known that, some fungi can synthesize orally toxic metabolites – mycotoxins. So, in our present study we paid our attention to study the potential of *A. flavus* fungal species to produced mycotoxin. The isolate of *A. flavus* was subcultured on mycotoxin activator media (Coconut Agar Media). According to the results out of total ten samples of different medicinal plants which was contaminated with *A. flavus* were subcultured on mycotoxin activating media, four samples are found as mycotoxins producing fungi (Table 5).

Fungal contamination of raw materials of medicinal plants is a major impediment preventing India from becoming a herbal giant. Therefore, fungal contamination in medicinal plants, especially raw materials, should be prevented during storage. Plant materials used for medical purposes should be carefully stored and the growth of toxigenic fungi should be inhibited. In the present study 40 samples of eight different medicinal plants were collected from different markets places of Agra and nearby regions were taken to evaluate the presence of toxigenic fungi. Out of 40 samples of different medicinal plants 37 (92.57%) samples are found to be contaminated with one or more fungal species. All (100%) the samples of *S. indica, T. arjuna, W. sominifera, B. monnieri, E. alsinoides* and *Z. officinale* were found to be contaminated with one or more fungal species, while 80% in the case of *T. terrestris* and 60% in case of *H. indicus* was found to be contaminated with one or more fungal species

**Table 4**: Percentage of samples contamination by different fungal species isolated from different medicinal plants.

| Fungal Species | % of samples yielding different species of fungi | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *S. indica* | *T. arjuna* | *W. sominifera* | *B. monnieri* | *E.alsinoides* | *Z.officinale* | *T. terrestris* | *H. indicus* |
| *A. flavus* | 40% | 80% | - | 40% | 20% | 20% | - | - |
| *A. nidulans* | 20% | - | - | - | 20% | - | - | - |
| *A. niger* | 40% | 100% | 80% | 100% | 100% | 100% | 40% | 40% |
| *M. thermophila* | - | 20% | - | - | - | - | - | - |
| *Mucor* species | 60% | 20% | 80% | 60% | 80% | 80% | 60% | 60% |
| *Rhizopus* species | 20% | - | - | 80% | 80% | 20% | - | - |

**Table 5**: Distribution of mycotoxin producing *A. flavus* among different medicinal plant samples

| S. No. | Medicinal plant sample | Total number of fungi examined | Positive strains of mycotoxin production |
|---|---|---|---|
| 1. | *S. indica* | 2 | 1 |
| 2. | *T. arjuna* | 4 | 1 |
| 3. | *W. somnifera* | - | - |
| 4. | *B. monnieri* | 2 | 1 |
| 5. | *E. alsinoides* | 1 | 1 |
| 6. | *Z. officinate* | 1 | - |
| 7. | *T. terrestris* | - | - |
| 8. | *H. indicus* | - | - |
| | Total | 10 | 4 |

(-) not detected

The mycoflora comprise of six different fungal species belonging to three genera viz. The maximum fungal species are of *A. niger* (38.46%), followed by *Mucor* species (32.05%), *Rhizopus* species (12.82%), *A. flavus* (12.82%), *A. nidulans* (2.56%) and *M. thermophila* (1.82%). Among fungi isolated the presence of *A. niger* 38.46%, Mucor 32.05%, *A. flavus* 12.82% and *Rhizopus* species 12.82% dominate other fungal species isolated. Among total 10 samples of different medicinal plants which were found to be contaminated with *A. flavus* were further analyzed for mycotoxin potential by subcultured on mycotoxin activating media (coconut agar media) 4 out of 10 shows positive toxigenic potential for mycotoxins.

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