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SURFACE MYCOFLORA OF STORED PART OF HERBAL MEDICINE

***DHALE D. A.**

Post-Graduate Department of Botany, SSVPS's, L.K.Dr.P.R.Ghogrey Science College, Dhule -424005 (M.S.) India.

ABSTRACT

This investigation was designed to throw light on the mycoflora of some crude herbal materials. Randomly 15 different stored herbal medicines were brought to the laboratory from three different shops in Dhule City (M.S., India). The surface mycoflora associated with these samples was studied by standard methods of incubation - Blotter test, Agar plate test and Surface washing method. During the present investigations on herbal medicines, the high percentage of mycoflora observed in blotter test. Almost all the samples screened for the study were found to be contaminated with 44 species belonging to 15 genera of fungi. The samples were mixed with dust and debris, while contaminated contained maximum amount of soil particles. Their effect on patients consuming such contaminated medicines also calls for urgent attention. General cleanliness and hygienic habits in handling of herbal stocks, awareness of necessity for sterility will decreases risks of proliferating sickness will be minimized.

KEY WORDS: **Mycoflora, Herbal Medicine, *Penicillium* spp., *Awalakanthi, Vaidus***

DHALE D. A.
Post-Graduate Department of Botany, SSVPS's, L.K.Dr.P.R.Ghogrey Science College, Dhule -424005 (M.S.) India.

Corresponding author

INTRODUCTION

In India, the references to the curative properties of some herbs in the Rigveda seem to be the earliest records of use of plants in medicine. Ayurveda, the ancient science of medicine is a branch of Indian culture. Right from Rigveda (3000 - 2000 B.C.) down to the present day it has remained a part and parcel of our daily activities. Organs of plants like roots, stems, leaves, flowers, fruits, seeds, etc., are used in Ayurvedic medicines.The increasing popularity of natural drugs made their use a Public Health problem due to the lack of effective surveillance of the use, efficacy, toxicity and quality of these natural products. The premise 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 microbials or natural toxins like mycotoxins have been reported for herbal products and medicinal plants. The concern over quality of these products is mainly due to their potential contamination, considering their natural origin. Practices used in harvesting, handling, storage, production and distribution make medicinal plants subject to contamination by various fungi, which may be responsible for spoilage and production of mycotoxins[1-15].Considering this situation the present studies on the mycoflora of stored herbal parts used in Ayurvedic medicines, started as a first step to fill up this major lacuna in the field of Ayurvedic Pharmacopia. The main objective is to study the surface mycoflora of stored herbal samples used in Ayurvedic therapy and to prepare a comparative floristic account of fungi associated with them.

MATERIALS AND METHODS

Collection of Samples

The various parts of stored herbal medicines were brought to the laboratory from different shops in Dhule selling herbal medicines. These samples were collected from 3 different shops. In the laboratory, they were stored in clean plastic containers with screw caps. The medicinal importance of randomly 15 selected herbal medicinal part are described here along with their botanical names, families, etc. (Table No. 1). The information about authentic identification of plants, drugs obtained, their uses, etc. are compiled by many authors like[16-18].

Biological Analysis

The surface mycoflora associated with these samples was detected following standard incubation methods by Blotter test, Agar plate test and Surface washing method. Depending upon the size and weight of the sample, the quantity used for each sample varied e.g. 100 seeds of Isabgol or 10 gm of Kudasal, Ritha, Ashwagandha (in pieces) were used.

Blotter Test

The samples were incubated for 8-10 days at room temperature in sterile petriplates lined sterile distilled water soaked, three Layered blotting paper circles.

Agar Plate Test

The samples were incubated at room temperature on potato dextrose agar (PDA) without any treatment. The culture plates were incubated at room temperature for 7-9 days and observations were recorded.

Surface Washing Method

1 gm sample was placed aseptically in 150 ml flask containing 25 ml sterile distilled water. The flask was mechanically shaken for 15-20 minutes to wash out surface mycoflora. The solution was centrifuged at 300-400 RPM for 15-20 minutes, using hand operated centrifuge. The residual matter was taken in 2 ml sterile distilled water. This residual solution was further diluted 3-4 times and placed on PDA medium. The culture plates were incubated at room temperature for 8-10 days and observations were recorded.

Identification of fungi

After 6-7 days of inoculation, fungi growing on fruit and powdered samples were isolated and identified primarily on the basis of their morphological and cultural characteristics and then stained with lactophenol cotton blue and identified microscopically with reference to standard texts[19,20]. The percent relative density of different fungal species isolated in each sample was calculated[21, 22].

Table 1
List of Herbal Medicine studied for surface mycoflora

Sr. No.	Drug Name	Botanical Name	Family	Part used
1.	Ajowan	*Carum copticum* Benth	Umbelifereae	Fruits
2.	Ashwgandha	*Withania somnifera* (L.)Dunal.	Solanaceae	Roots
3.	Bahava	*Cassia fistula* Linn.	Caesalpinaceae	Pods
4.	Bavachi	*Psoralia corylifolia* Linn.	Fabaceae	Seeds
5.	Chandan	*Santalum album*Linn.	Santalaceae	Wood
6.	Awalkathi	*Phyllanthus emblica* Linn.	Amaranthaceae	Fruits
7.	Isabgol	*Plantago ovata* Forsk.	Plantaginaceae	Seeds
8.	Jatamansi	*Nardostachys jatamansi* DC.	Valerinaceae	Rhizome
9.	Kalmegh	*Andrographis paniculata* Ness.	Acantaceae	Whole plant
10.	Kudasal	*Holarrhena pubescens* (Buch. Ham.)Wall.ex G.Don	Apocynaceae	Bark
11.	Murudseng	*Helicteres isora* Linn.	Sretculaceae	Fruits
12.	Nagarmotha	*Cyprus rotindus* Linn.	Cyperaceae	Rhizome
13.	Pimpali	*Piper longum* Linn.	Pipaeraceae	Fruits
14.	Ritha	*Sapinndus laurifolius* Vahl.	Sapindaceae	Fruits
15.	Shatavari	*Asperagus racemosus* Willd	Liliaceae	Tuberous Roots

RESULTS

Usually, many herbs are used as home remedies without consulting Ayurvedic practitioners. If by any chance, heavily contaminated or infected material is used, the effect is likely to be harmful, rather than curative.Hence some microbial standardization work should be carried out to check sterility of the Indian medicinal drugs[23]. The present investigation deals with studies on surface mycoflora of stored herbal parts, mostly used in Ayurvedic medicines. 15 different stored herbal medicines were brought to the laboratory from shops in Dhule. The samples were found to be stored in either gunny bags or open or closed tin containers. The samples of *Kudasal* were mixed with dust and debris, while *Kalmegh* and *Nagarmotha* contained maximum amount of soil particles. The samples of *Chandan* were fully covered with bluish green spore mass of to *Penicillium* spp. This may be due to improper drying of material, unhygienic preservation technique and lack of cleanliness. The blotter and agar plate methods are the two important procedures commonly applied in routine seed health tests for detection of seed-borne fungi. During the present investigations on material of herbal medicines, the blotter method proved to be more suitable than the agar plate method. The high percentage of mycoflora in blotter test as observed during present studies were in agreement with the earlier findings[24]. The blotter test provided a large number of fungal population but it included mostly common forms. Blotter test gave an opportunity to observe sporulation *in-situ* a helpful clue in the identification of many fungi. However, slow growing forms and those which do not sporulate easily escaped the attention during observations. Moreover, there are limitations on incubation period due to drying up of blotters. Thus, in agreement with Dickinson, it was noticed that for obtaining reliable data in totality, suitable combination of several techniques should be employed[25]. Almost all the samples screened for the study were found to be contaminated. 44 species belonging to 15

genera of fungi (Table 2) were recorded after examining 45 samples. Maximum number of fungi belonged to Deutroomycetes. All samples of plant material showed maximum infestation of *A. niger* and *Aspergillus* spp. It was followed by *Penicillium* spp. (Table 3). It was previously recorded on *amala* fruits in storage rooms and market places of Banaras[26]. A large number of fungal species were isolated from *Isabgol* (21 spp.), *Murudsheng* and *Ajwan* (18 spp.), and *Murudseng* (17 spp.). Minimum numbers of fungi were isolated from *Chandan* and *Kalmegh* (05 spp.), then by *Jatamansi* and *Kudasal* (07 spp.).

Table 2
List of Associated Mycoflora on Medicinal plant part

Sr. No.	Name of the Fungi spp.	Sr. No.	Name of the Fungi spp.
1.	*Alternaria alternata*	23.	*Fusarium moniliforme*
2.	*Alternaria* spp.	24.	*Fusarium oxysporum*
3.	*Alternaria tenuissima*	25.	*Fusarium roseum*
4.	*Aspergillus canididus*	26.	*Fusarium* spp.
5.	*Aspergillus flavipes*	27.	*Memnoniella* spp.
6.	*Aspergillus fumigatus*	28.	*Mucor* spp.
7.	*Aspergillus glaucus*	29.	*Myrothecium* spp.
8.	*Aspergillus niger*	30.	*Nigrospora sphaerica*
9.	*Aspergillus ochraceous*	31.	*Nigrospora*
10.	*Aspergillus* spp.	32.	*Penicillium chrysogenum*
11.	*Aspergillus terreus*	33.	*Penicillium citrinum*
12.	*Aspergillus ustus*	34.	*Penicillium* spp.
13.	*Chaetomium globosum*	35.	*Penicillium stiloniferum*
14.	*Chaetomium* spp.	36.	*Phoma spp.*
15.	*Cercospora* spp.	37.	*Phytopthora* spp.
16.	*Cladosporium oxysporum*	38.	*Pithomyces spp.*
17.	*Cladosporium* spp.	39.	*Pythium spp.*
18.	*Curvularia lunata*	40.	*Rhizopus nigricans*
19.	*Curvularia pallescens*	41.	*Rhizopus* spp.
20.	*Curvularia prasadii*	42.	*Trichoderma* spp.
21.	*Curvularia* spp.,	43.	*Uncinulla* spp.
22.	*Fusarium roseum*	44.	*Uromyces* spp.

Table 3
Total Frequency of Fungi found on Medicinal Plant parts

Sr. No.	Name of the Fungi spp.	Out of 15 Medicinal parts
1.	*Aspergillus* spp.	15
2.	*Fussarium* spp.	15
3.	*Alternaria* spp.	15
4.	*Curvularia* spp.	11
5.	*Penicillium* spp.	5
6.	*Helminthospore* spp	4
7.	*Cladospora* spp.	3
8.	*Phytopthora* spp.	3
9.	*Pythium* spp	2
10.	*Chaetomium* spp.	2
11.	*Collatotricum* spp.	2
12.	*Phyllactinia* spp.	2
13.	*Uromyces* spp.	2
14.	*Cercospora* spp.	2
15.	*Uncinulla and* other spp.	Each 1

Table 4
Fungi species found on Medicinal plant part

Sr. No.	Drug Name	Botanical Name	No. of Species found
1.	Ajowan	*Carum copticum* Benth	18
2.	Ashwgandha	*Withania somnifera* (L.)Dunal.	09
3.	Bahava	*Cassia fistula* Linn.	08
4.	Bavachi	*Psoralia corylifolia* Linn.	08
5.	Chandan	*Santalum album*Linn.	05
6.	Awalkathi	*Phyllanthus emblica* Linn.	06
7.	Isabgol	*Plantago ovata* Forsk.	21
8.	Jatamansi	*Nardostachys jatamansi* DC.	07
9.	Kalmegh	*Andrographis paniculata* Ness.	05
10.	Kudasal	*Holarrhena pubescens* (Buch. Ham.)Wall.ex G.Don	07
11.	Murudseng	*Helicteres isora* Linn.	17
12.	Nagarmotha	*Cyprus rotindus* Linn.	08
13.	Pimpali	*Piper longum* Linn.	15
14.	Ritha	*Sapinndus laurifolius* Vahl.	09
15.	Shatavari	*Asperagus racemosus* Willd	11

DISCUSSION

The above elucidated studies carried out during 2011-12, resulted in the detection of several fungi from raw medicines proving thereby heavy contamination with fungal spores. This opens up a new field for further studies. Not much work has been done in the past dealing with mycoflora of herbs used in Ayurvedic medicines, although a few diseases of medicinally important plants under cultivation such as leaf spots, wilts, die backs etc. were known. However, some more extended studies on mycoflora associated with these samples especially obtained from different cities as well as with some more herbs are necessary, as they are likely to have been collected from different forests. There are reputed factories in cities like Ahmednagar, Bombay manufacturing patented processed Ayurvedic preparations from raw herbs. Survey of such herbal samples from these areas will surely help in the detection, and in perfecting our observations, of more varied mycoflora having the toxigenic or allergic properties associated with such samples.The deleterious effects of these fungi on the active medicinal compounds of the substrates need to be studied. Their effect on patients consuming such contaminated medicines also calls for urgent attention. There is a two-fold danger in consuming such medicines. The harmful effect of such fungi due to their mycelium, spores or metabolites is one way of inducing disorders in patients while loss in curative properties of active principles, present in herbs due to fungal invasion is an another way attributing to the endanger. By the examination of the phyllosphere and some important fungi which recorded are *Alternaria* spp., *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium* spp., *Fusarium* spp., *Helminthosporium* spp., *Phytopthora* spp., *Pythium* spp., *Phyllactinia* spp, *Uncinulla* spp., *Urosystis* spp., *Uromyces* spp., etc.[27].Filamentous fungi and moulds are able to produce an enormous number of secondary metabolites, including antibiotics and mycotoxins. The term mycotoxin refers to those secondary metabolites which, at a low concentration, are toxic to humans and animals[28].Mycotoxins have been implicated as causative agents of human foodborne intoxication, as well as human hepatic and extra-hepatic carcinogenesis[29]; clinical symptoms include diarrhoea, liver and kidney damage, pulmonary oedema, vomiting, haemorrhaging and tumours[30]. The most frequent toxigenic fungi are *Aspergillus*, *Penicillium* and *Fusarium* species[28]. The foodborne mycotoxins of greatest significance in

Africa and other tropical developing countries are the fumonisins (FB), aflatoxins (AFs) and trichothecenes[31]. The some herbs are good substrate for *Aspergillus flavus* infestation and production of aflatoxins with potential hazard to the health of consumers[32].

CONCLUSION

During the screening of 15 herbal medicines (total 45 samples) for associated mycoflora, 44 species belonging to 15 genera of fungi were recorded, amongst which maximum belonged to Deutromycetes group. Thus majority of fungi belonged to the class Deuteromycetes, which is a general observation of all workers in the field of mycofloristic studies. A large number of fungal species were isolated from *Isabgol* (21 spp.), *Murudsheng* and *Ajwan* (18 spp.), and *Murudseng* (17 spp.). Minimum numbers of fungi were isolated from *Chandan* and *Kalmegh* (05 spp.), then by *Jatamansi* and *Kudasal* (07 spp.). The identification of fungi was carried out with the help of standard books and monographs, review papers on taxonomy or by using taxonomic keys, review of literature, screening of general and regional lists of fungi etc. General cleanliness and hygienic habits in handling of herbal stocks, awareness of necessity for sterility of such plant-parts etc among layman or untrained *Vaidus* will help in avoiding consumption of contaminated or spoiled stuff and also the risks of proliferating sickness will be minimized.

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