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Fungal contamination of medicinal herbs during commercial storage in Punjab

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Abstract

Pakistan has a variety of herbs and medicinal plant resources, due to its varied ecological conditions. Large number of physicians of south Asia (locally called Hakeems and Tib-e-Unani or Ayurvedic medicines) use herbs, plant parts and extracts for treating human diseases. Source of medicinal plants are hilly areas, deserts and coastal zones of the country. Major supply of medicinal herbs is wild plant collection rather than cultivation. Due to improper storage of medicinal plants in the shops a wide variety of mycoflora gets activated. Usually plant parts of different material and their products are stored under unhygienic conditions. So they are exposed to a wide variety of biological and chemical contaminations. Interviews were conducted from markets and shops of southern, central and northern districts of province Punjab, Pakistan to investigate the prevalence and nature of contamination. Dried mycelium and spores of *Alternaria species*, *Fusarium oxysporum*, *Penicilliuim* sp. *Botrytis cinerea* and *Aspergillus niger* were recorded.
Key words: Medicinal plants, storage, marketing, mycoflora,

Introduction

Medicinal herbs (plant or plant part) are used to prevent and treat diseases as a drug or ingredients. Use of herbal medicines in Indo-Pak subcontinent is many centuries old. Peoples of this region believe herbal products are more close to nature and free of any side effect. A wide variety of herbs is found in Pakistan due to varied ecological conditions. But because of lack of coordination between stakeholders of the herbal trade, this sector has not been exploited in accordance with emerging needs and development in discipline. Therefore it is not playing its due role in the economics of the country. It is estimated that 60% of the population use herbal medicines prescribed by traditional practitioners due to non-availability of medical health facilities in rural areas (Nasir & Ali, 1972; Hussain, 1993; Hamayun *et al.*, 2004*)*.

Few registered and many unregistered institutions are involved countrywide in the preparation of medicinal products. Number of drugs and medicinal products are processed, packed and distributed in the country and abroad based on Tibb-e-Unani (Desi treatment).Large number of physicians (locally called Hakeems and Sunyasis) use herbs and plant parts for treating human diseases. Most of them are not qualified and their business is family based (locally called as Saddri Nuskhajat). Major source of medicinal plants are hilly areas, deserts and coastal zones of the country from where medicinal herbs are collected rather than cultivated. These are transported without any proper packing. Mostly jute and polythene bags are used for packaging during the transportation. Enormous amount of plant and their parts are either spoiled or contaminated by microorganisms especially fungi due to improper packing.

Quality of the herbs is influenced by the storage conditions (Snowden, 1992; Lisiewska *et al*., 1997). Due to improper storage of medicinal herbs, wide variety of mycoflora gets activated. Usually plant and plant products are stored in tin, plastic, jute and wooden buckets, boxes and sacs. The main objective of this study was to collect information about the marketing and possible fungal constraints of medicinal plants for promotion of herbal trade in the country.

Materials and Methods

On the basis of preliminary surveys conducted during October and November 2004, Punjab Province was divided in southern, central and northern Punjab following the marketing chain and business flow for medicinal herbs. Detailed investigative survey was carried out during spring and Monsoon seasons 2005. Data about the storage of herbs was collected from Lahore (Central Punjab), Multan (Southern Punjab, and Rawalpindi (Northern Punjab) districts (Plate 3B). A structured

questionnaire was used to collect maximum information regarding the storage.

In this survey 140 questionnaires were distributed among whole sellers and retailers (Pansaries) and herbal physicians. The physicians were classified as qualified hakims, quacks and hakim cum pansaries on the basis of their certification from "Tybbia council Punjab"

Fifty seven traders of herbal medicines including, wholesaler, and Punsari from Lahore, 40, 10 Okara, 10 Sahiwal from Multan, and 23 from Rawalpindi districts were randomly selected and interviewed. Protocol for the storage, transportation and marketing of herbs by different hakeems and traders was analyzed and pictorially documented (Plate 2).

Investigations for seed / tissue, specimens collected during survey were carried in cellulose paper bags. Bags were labeled with all necessary information regarding identity of the subject, storage conditions, source, marketing chain. In laboratory specimens were investigated under aseptic conditions for presence of mycoflora. Ten infected seeds were placed in each Petri plate containing moist filter paper and incubated at 25 ^{o}C. These Petri plates were placed at room temperature for seven days. The infected seeds were examined under the microscope at (10X and 40X) in order to record the incidence of different fungi borne on seeds. Same set of Petri plates was adopted for vegetative and reproductive parts. Each plant part was divided into five pieces of 2 centimeter each before keeping in sterilized Petri Plates with three replicates. Fungal species were identified by using the keys (Nelson *et. al.*, 1983; Morth, 1971; Ellis, 1971, 1976)

Results and Discussion

Fungi can produce mycotoxins. Presence of certain fungi like *Fusarium oxysporum* and *Aspergillus* species can add such toxins in the final products of the herbal medicines. Survey of Lahore, Okara, Sahiwal, Multan, and Rawalpindi districts indicates (Table 2) stored rose petals of *Tagete erecta* carry spores of *Alternaria species* from petals, *Fusarium oxysporum* from the seeds of *Cucurbita* seeds *Penicillium species from bark of Terminalia arjuna, Aspergillus niger* from root of *Polygonum viavpurum* and *Botrytis cinerea* from *Allium* species have been observed (Plate 3A).

The stake holders have the awareness for hygienic storage of herbs but due to poor legislative control they ignore it under normal practice. This ignorance was reflected in the form of refusal to the information asked in the questionnaires. Few hakeems and shopkeepers maintain and clean the plant parts for display, otherwise majority of them do not care about the cleaning and storage to protect the plants from dust and molds. Quarantine rules also favor mycofloral growth. As imported herbs are checked for any objectionable material and not for pathological constraint.

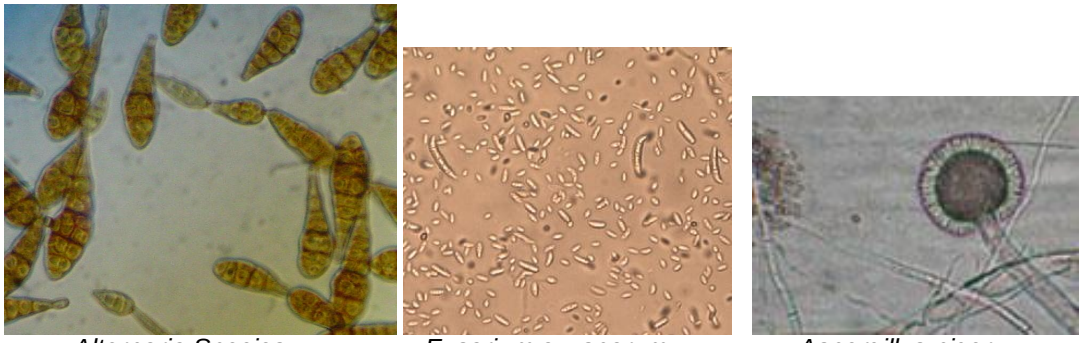
A wide range of variations in the response of the correspondent to the questionnaire was noticed (Table 1). The approach of qualified and non-qualified hakeems and traders is classified on the basis of behavior. Especially, 37% quacks, 14% suppliers and 12% Punsaris refused to answer the questionnaire. Whereas supportive behavior varies from herbal organization (40%) to Supplier (5%) (Fig. 1).

Table: 1 Categorization of response of the respondents in the herbal trade in Punjab (Pakistan) %.

Category	Refused	Reluctant	Reserve	Supportive	Obliged
Qualified Hakeem	5	10	13	40	32
Quack	37	35	20	10	10
Punsari cum Hakeem	10	20	30	25	15
Punsari	12	18	30	25	25
Wholesaler	10	15	55	12	8
Supplier	14	26	45	10	5
Herbal Organizations	0	5	5	50	40

Table 2: Fungal species observed from the medicinal Herbs from the shops and traders.

No.	Fungi observed	Plant part	Plant	Local Name	% age	Packing material
1	*Alternaria species*	Petals	*Tagetes erecta*	Gainda	30	Polythene bag
2	*Fusarium oxysporum*	Seeds	*Cucurbita* species	Khiarian	20	Glass jar
3	*Botrytis cinerea*	Seeds	*Allium cepa*	Piaz	10	Glass jar
4	*Penicillium* species	Bark	*Terminalia arjuna*	Arjun	80	Polythene bag
5	*Aspergillus niger*	Roots	*Polygonum viavpurum*	Anjbar	56	Jute sac



Alternaria Species *Fusarium oxysporum* *Aspergillus niger*

Plate 1: Fungi isolated from medicinal herbs under conventional storage conditions.

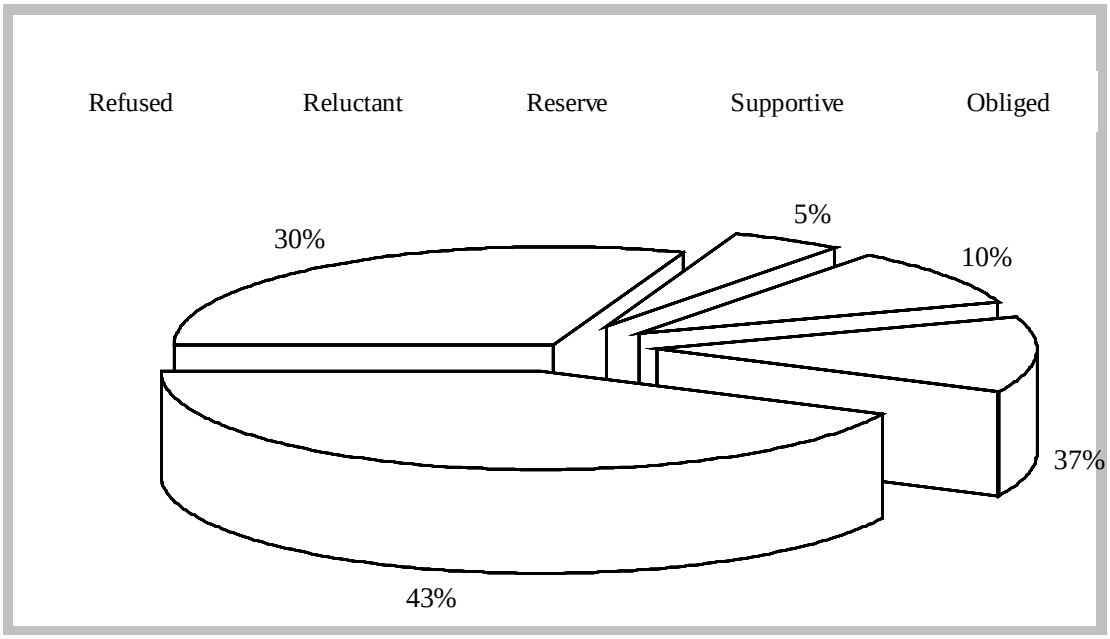


Fig. 1: Refusal percentage of herbal stakeholders towards the information on following authentic hygienic storage conditions for raw herbs.

Plate 2: Conventional methods of herbal storage

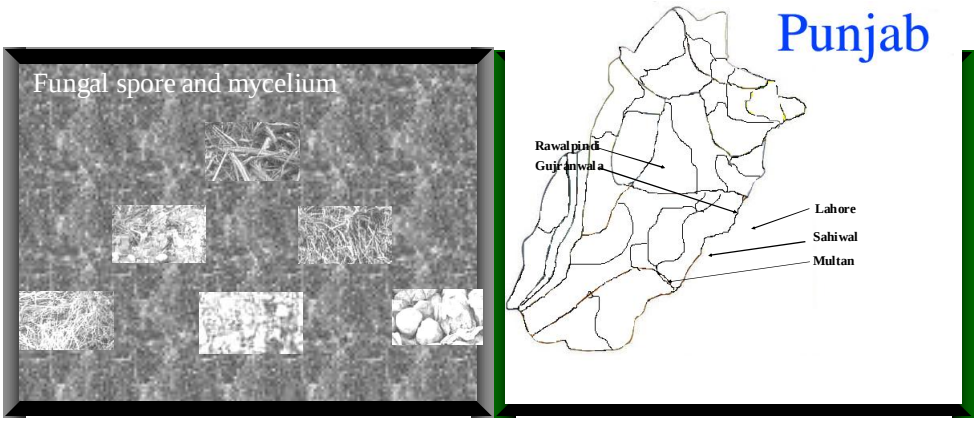


Plate 3: A: Mycelial growth of fungi on various herbs.
B: Areas surveyed in Punjab for investigation of fungi.

Most of the Punsari and Punsari cum Hakeems remained reluctant to answer the interview.

In Punjab, trade of medicinal plants remains active throughout the year. Plants and plant parts are collected from the hilly areas of the country and are imported from India, China, Afghanistan, Iran and many other European countries. Usually jute and polythene sacs are used during transportation. In markets they are kept in sacs but at shop level they are kept in glass jars, tin pack of Vanaspati Ghee and Vegetable oil with lids. Some sellers put them in plastic buckets or wooden boxes. Quality of packing material was not constant and especially packing material used by quacks was sub-standard. Their sacs were dusty and were placed without any suitable storage conditions. As the local collectors supply most of the medicinal plants directly from the fields, they need cleanness before sale and consumptions. Except few qualified and herbal organizations, Punsaris, traders and suppliers do not take care of mycoflora. Many quacks and Punsaris did not reply about the treatment of spoiled herbs if attacked by the mycoflora. However, qualified hakeems and herbal organizations claimed they discard the infected plant or plant parts.

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