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ISOLATION OF POST-HARVEST SPOILAGE FUNGI ASSOCIATED WITH MEDICINAL PLANT PARTS

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ABSTRACT

In India the use of herbal medicines for the cure of human diseases has been foundincreased all over the world. In addition to this the collection of raw materials from naturally grown cultivated forest will be carried out. The raw materials are being handled by farmers and labors without scientific knowledge and unhygienic way. So present investigation is an attempt to isolate the mycoflora associated with the medicinal plants from local market under environmental conditions. The fungal contamination affects on the chemical composition of raw materials and thereby decreases potency of drugs. Regarding the above fact the present experiment was conducted and observed the maximum fungi were isolated from stored samples of medicinal plant parts from weekly market as compared with local market in Mantha.

INTRODUCTION

Medicinal plants are valued for their distinctive flavours, colors and aromas and are among the most versatile and widely used ingredient in food preparation and processing throughout the world. Spices have been defined as a natural compound, or a mixture of natural compounds that is extracted from the seeds, fruits, flowers leaves of several plants that indigenous or exotic origin, aromatic or with strong taste, used in minute quantities, and added to food preparation and processing throughout the world in order to provide colour, taste, smell, or flavor (Ayres *et al*., 1980).They are widely used as raw materials for pharmaceutical preparations and as a supplement for dietetic products, especially for “self medications” in public. As with many other agricultural products, spices and herbs may be exposed to a wide range of microbial contamination during pre- and post-harvest. The potential for spoilage and mycotoxins production depends upon the types of fungi present, the composition of the food and the conditions of handling and storage. For example, dried foods are susceptible to spoilage and toxin production if storage temperature is suitable for fungal growth (Misra, 1981). Moreover, spices are collected in tropical areas by simple methodsand are commonly exposed to many contaminants before, being dry enough to prevent microbial growth. They are also stored in conditions favouring contamination by insects, rodents and other vermin. The mycological quality of some spices on the market, especially of pepper, is quite poor, bearing many genera and species of fungi. Most fungi are present on pepper of the post-harvest and storage type, which develop after harvest if relative humidity is not controlled during storage (Aziz *et al*., 1998). Fungi are the predominant contaminants of spices, but most such microbial populations are probably regarded as commensals residents on the plant that survived drying and storage. Soil and air is the main inoculums source for causing contamination in crude spices in field (Kneifel and Berger, 1994). Spices are commonly heavily contaminated with xerophilic storage moulds and bacteria (Romagnoli*et al*., 2007). The fungal contamination affects on the chemical composition of raw materials and thereby decreases potency of drugs. The recent literature found that in order to search ecofriendly and safe control measure for plant diseases. Numbers of researcher and co-workers have worked on evaluating extracts of different plants against pathogenic fungi and bacteria. Among such reports it is reported by Chakravarty and Pariya (1977) that extracts of certain Indian medicinal plants proved to be significantly antifungal in relation to *Sclerotiumrolfsii, Alternariatenuis*and *Aspergillus niger.* Dhale, (2013) worked on surface mycoflora of stored part of herbal medicine. The surface mycoflora associated with these samples was studied by standard

methods of incubation i.e. 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.Gaikwad and Korpenwar (2017) worked on mycoflora associated with the fruit of *Trichosanthes tricuspidata* (Lour.) during storage. The unscientific method of storage of plant a part causing fungal contamination .The fungal contamination affect on the chemical composition of raw materials and thereby decreases potency of drugs. Regarding the above fact the present experiment was studied and concluded that maximum 22 fungal species viz. *Alternaria alternate, A. solani , A. flavus, A. niger, A. fumigates, A. paraciticus, A. terreus, A. ustus, Curvularialunata, Colletotrichum sp., Cladosporium sp., Drechslera sp., Fusarium oxysporum, F. equiseti, F. moniliforme, Helminthosporium sp., Mucorglobsus, Phoma*sp*, Penicillium citrinum, Rhizopus stolonifer, Trichodermaviride, Verticelliumsp.*were isolated from one year old authentic stored fruit sample and16 from fresh fruit sample on Potato Dextrose Agar (PDA) method similarly minimum 17 fungal species were observed from authentic Stores fruit sample and 08 from fresh sample on Standard Moist Blotter (SMB) Method.Korpenwar and Gaikwad (2017) reported that isolatation of fungi from *Enicostemma axillare* (Lam.) Poir ex Lam.*Enicostemmaaxillare*Poir ex Lam is commonly known as *Katvinaye.* The leaves used as a raw material for the preparation of some important drugs for curing various human diseases. The fungal contamination affects on the chemical composition of raw materials and thereby decreases potency of drugs. Regarding the above fact the present study was conducted that the maximum 21 fungal species viz. *Alternariaalternate, A. solani, Aspergillus flavus, A. niger, A. fumigat , A. nidulance, A. terreus, A. ustus,Curvularialunata, Colletotrichum*species, *Cladosporium* species, *Drechslera*species, *Fusarium oxysporum, F. equiseti, Helminthosporium*species, *Mucorglobsus, Phoma*species, *Penicillium notatum, P. citrinum, Rhizopus stolonifer, Trichoderma viride* were isolated from six months old authentic stored leaf sample and 12 from fresh fruit sample on Potato Dextrose Agar (PDA) method as compared with Czapek Dox Agar (CZA) medium and Moist Blotter method.The fungal contamination affects on the chemical composition of raw materials and thereby decreases potency of drugs. The present study aimed to throw light on the investigation a detailed survey of mycoflora of medicinal plant partsfrom local market under environmental conditions.

MATERIAL AND METHODS

- **Collection of plant material.**
- Medicinal plant partswere collected from differentauthentic stores of Mantha market viz.*Ellettariacardamomum,Syzygiumaromaticum,Piper nigrum, Allium sativum,Zingiberofficinale*in pre-sterilized polythene bags and brought to the laboratory. Samples were identified using the Flora of Marathwada Naik, (1998) at Department of Botany, Dr. Babasaheb Ambedkar Marathwada University Aurangabad.Medicinal plant partswere inoculated aseptically on the sterilized petriplates containing Potato Dextrose Agar (PDA) Medium incubated at25±2°C temperature for 7 days.
- **Isolation of mycoflora.**

Mycoflora was isolated by using Potato Dextrose Agar (PDA) Medium.

3) Identification of fungi

The fungi occurring on plant material in the plates were identified preliminary on the basis of sporulation characters like sexual or asexual spores with the help of stereoscopic binocular microscope. The identification and further confirmation of fungi was made by preparing slides of the fungal growth and observing them under compound microscope. The identification was made with the help of manuals Mukadam *et al*, (2006), (Alexopoulous, 1996; Barnett,1970). Similarly confirmationof identification was madeat Department of Plant Pathology Laboratory, Dr. Babasaheb Ambedkar Marathwada University Aurangabad. Pure cultures of these fungi were prepared and maintained on potato dextrose agar (PDA) slants.

RESULTS AND DISCUSSION

Table 1: Isolation of fungi associated with medicinal plant parts collected from Mantha weekly market.

Fungi	Medicinal plant parts				
	Ellettaria Cardamomum	*Syzygium Aromaticum*	*Piper nigrum*	*Allium sativum*	*Zingiberofficina*
Alternariaalternata	-	-	-	-	+
Aspergillus flavus	+	-	-	+	+
Aspergillus fumigatus	+	-	-	-	+
Aspergillus niger	+	+	+	+	+
Aspergillus terreus	-	-	+	+	+
Curvularia lunata	+	-	-	+	+
Colletotrichum sp.	+	-	-	+	+
Cladosporium sp.	+	-	-	+	+
Fusarium moniliforme	+	-	-	-	+
Fusarium oxysporum	+	-	+	-	+
Fusarium roseum	-	-	-	-	+
Mucorglobsus	+	+	+	-	+
Penicillium notatum	-	-	-	+	+
Rhizopus stolonifer	+	-	-	+	+
Trichoderma viride	+	-	-	-	+
Total no. of isolates	11	02	04	08	15

+ = fungi present. - = fungi absent.

Table no 01 showed that the maximum 15 fungi viz.*Alternaria alternata,Aspergillus flavus,Aspergillus fumigatus Aspergillus niger, Aspergillus terreus, Curvularia lunata,Colletotrichum sp. Cladosporium sp.Fusarium moniliforme, Fusarium oxysporum,Fusariumroseum,Mucorglobsus, Penicilliumnotatum, Rhizopus stolonifer*,*Trichoderma viride*were associated with store sample of *Zingiberofficinale*and minimum fungi were associated with *syzygiumaromaticum* viz. Aspergillusniger, Mucorglobsus, (02).Similarly,*Ellettariacardamomum*(11),*Piper nigrum*(04), *Allium sativum*(04) fungi were isolated from selected medicinal plants.Mahajan *et al*., (2014) studied that isolation and identification of fungal contamination in stored medicinal plants. As a result of Mycological examination total of 13 different fungal species was isolated from all the three medicinal plant samples. The predominant mycoflora obtained was distributed in five different genera comprised of *Aspergillus, Penicillium, Alternaria, Rhizopus*and *Syncephalastrum.* The *Aspergillus* (71.95%) was observed as the most dominant genera recovered, followed by *Penicillium* (15.44%), *Rhizopus* (9.51%), *Alternaria*(1.67%) and *Syncephalastrum*(1.41%). Most of the identified fungal species like *Aspergillus, Penicillium* and *Alternaria* are reported to have the ability to produce mycotoxins like aflatoxins, ochratoxins, citrinin and *Alternaria* toxins. The presence of a wide range of storage fungi indicates that the mould probably infects the crude herbal drugs during harvesting and post harvesting, processing i.e. mainly during drying, storing, transportation and processing.

Table 02: Isolation of fungi associated with medicinal plant parts collected from Mantha local market.

Fungi	Medicinal plant parts				
	Ellettaria Cardamomum	*Syzygium Aromaticum*	*Piper nigrum*	*Alliumsativum*	*Zingiberofficina*
Alternariaalternata	+	-	-	-	+
Aspergillus flavus	+	-	+	+	+
Aspergillus niger	+	+	+	+	+

Aspergillus terreus	-	-	-	-	+
Fusarium oxysporum	+	+	-	+	+
Fusarium roseum	+	-	-	-	+
Mucorglobsus	+	+	+	+	+
Penicillium notatum	+	-	-	+	+
Rhizopus stolonifer	+	-	+	+	+
Total no. of isolates	08	03	04	06	09

+ = fungi present. - = fungi absent.

Table no 02 showed that the maximum 09fungi viz. *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Aspergillus terreus,Fusarium oxysporum, Fusariumroseum, Mucorglobsus, Penicilliumnotatum, Rhizopus stolonifer*were associated with the stored sample of *Zingiberofficinale.***S**imilarly,eight fungi *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Fusariumroseum, Mucorglobsus, Penicilliumnotatum, Rhizopus stolonifer* were isolated from*Ellettariacardamomum*while (03) fungi from *Syzygiumaromaticum,* (04) fungi from *Piper nigrum*and (06)fungi from *Allium sativum.*Pereira *et al*., (2015) worked on isolation and identification of toxigenic and non-toxigenic fungi in samples of medicinal plants from the market. Total 12 samples of medicinal plants were analyzed in relation to the level of fungal contamination, and the presence of producers of ochratoxin A and aflatoxins was assessed by visualization of fungi using a cromatovisor in coconut milk. Most of the species found belong to the genus *Cladosporium, Fusarium, Aspergillus* and *Penicillium*. Species producing ochratoxin A were present in 2 samples (16.7%), Melissa and Hibiscus. Species producing aflatoxin were found in samples of *Jacaranda decurrens*(8.33%). This study suggests that herbs, if stored improperly, can provide the growth of fungi and should be examined before consumption.

CONCLUSION

The present study suggests that the methods of harvesting, collection, preparing and storage of medicinal plants part must be improved for reducing percent incidence of mycoflora and mycotoxins contaminations.

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