International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:153-156
UGC Approved Journal No 48951

Original Article Open Access

Fungi associated with the flowers of *Spilanthes acmella* (l.) Murr during storage.

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Gaikwad Deepmala A and Korpenwar Anil N (2018) Fungi associated with the flowers of *Spilanthes acmella* (l.) Murr during storage, *Int. J. of. Life Sciences*, Special Issue, A12: 153-156.

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ABSTRACT

Spilanthes acmella (L.) Murr. Flowers are used as a raw material for the preparation of some important drugs for curing various human diseases. During unscientific methods of storage causing fungal contamination. The fungal contamination effect on the chemical composition of raw materials and thereby decreases potency of drugs. Regarding the above fact the present experiment was conducted and observed that the young flowers were showed no incidence of fungi on Blotter Paper Method except *Aspergillus flavus* on PDA. In case of mature flowers the Blotter Paper Method showed incidence of four fungi like *Alternaria alternata*, *A. niger*, *Aspergillus terreus*, *F. oxysporum*, *Fusarium roseum* whereas PDA Method showed nine fungi. The maximum incidences of fungi were reported in stored flowers as compared to young and mature flowers.

Key words : *Spilanthes acmella* , flowers, fungal contamination.

INTRODUCTION

Spilanthes acmella (L.) Murr belongs to the family Asteraceae. Commonly it is known as Toothache plant grown as an ornamental and as a medicinal plant in various parts of the India. The active constituent spilanthol chiefly present in leaves and flower heads, and produce analgesic activity used to numb toothache. The whole plants can be used in the treatment of dysentery and rheumatism. The flower heads of *S. acmella* can be chewed to relieve toothache and also as a haemostatic and analgesic Leng *et al*. (2011). Ayurvedic system of medicine, flower heads and roots are used in treatment of scabies, psoriasis, scurvy, infections of gums Pandey *et al.* (2004). The flower heads and roots have been used for treatment of toothache, scabies, scurvy, infections of throat and gums, paralysis of tongue. The leaves and flower heads contain analgesic, antifungal, anthelminthic, antimalarial, antibacterial, diuretic Ratnasooriya *et al*. (2004), Rani and Murty, (2006), Barman *et al.*(2009), Prachayasittikul *et al.* (2013).

Medicinal plants may be associated with a microbial contaminants, represented by bacteria, fungi and viruses. This microbiological background depends on several environmental factors and exerts an important impact on the overall quality of herbal products and preparations. The traditional methods of collection, storage and marketing coupled with humid climatic condition make them victim to the fungal contamination. (Masoumeh and Deokule, 2013., Muntanola, 1987) and Durakovic *et al*. (1989) studied that the fungal contaminates has been reported to affect the chemical composition of the raw materials and thereby, decreases the medicinal potency of the plant material whereas mycotoxins produced by these fungal contaminants causes several effects on liver organs, , kidney, genital digestive tract, respiratory organs nervous system, skin etc. The unscientific methods of harvesting, collection, storage of raw materials, post harvest processing, transport and storage of herbal drugs in unhygienic conditions, are the main causes considered to make both, raw materials as well as herbal drugs prone to microbial infections leading to deterioration in safety and quality and can also cause health hazard to consumer in spite to cure the disease Pinkey.(2014), Many researchers have reported that the presence of potential contaminants in herbal preparations viz. Czech *et al*.(2001), Idu *et al*. (2011), Kulshrestha *et al.* (2008), Alwakeel (2008).The manufacturers should ensure the lowest possible level of microorganisms in the raw material, finished dosage forms and the packaging components to maintain appropriate quality, safety and efficacy of the natural products. Okunlola *et al*. (2007).

According to the WHO, about 80% of the population of the world depends on traditional medicine, mostly herbal remedies, for their primary health care needs. Moerman (1996) . Various pathogens adversely affect the medicinal plant parts and decrease the medicinal value of the part. It may be harmful to the human body while using these infected parts as a medicine. Hamayun *et al.(*2004) So present investigation is an attempt to identify the mycoflora associated with the flowers sample of *Spilanthes acmella* (L.) Murr.

METHODOLOGY

Collection of plant material:

Spilanthes acmella (L.) Murr. flowers were collected from different authentic stores of Jalna district 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. Stored flowers were inoculated aseptically on the sterilized petriplates containing Potato Dextrose Agar (PDA) Medium and Blotter Method incubated at25±2°C temperature for 7 days.

Isolation of mycoflora:

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

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), Similarly confirmation of identification was made at 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

In order to study changes occurring during storage period on flowers, different parameters such as appearance, color, odor and texture was observed after 6,12,18 and 24 months intervals during storage and results are given in the table 1. It is clear from table no.1 that no black spotted cone shape yellow appearance, black pale yellow color, foul odor and britter texture was found in 6 and 12 months storage period. But after the 18 and 24 months of storage periods, severe type of infection of fungi with black spotted cone shape yellow appearance, black pale yellow in color, foul in odor and briter in texture were observed.

In order to study the percent incidence of fungi on flowers (young, mature and stored) were studied with Blotter Paper Method and PDA and the result are given in table 2. It is clear from result that the young flowers were showed no incidence of fungi on Blotter Paper Method and except *Aspergillus flavus* (05) on PDA.

Table 1. Physical changes in flowers of *Spilanthes acmella* under different storage period.

Parameters	Storage period (months)				
	Fresh	6	12	18	24
Appearance	Cone shape Yellow	Cone shape Yellow	Cone shape Yellow	Black spotted Cone shape Yellow	Black spotted Cone shape Yellow
Color	Dark Yellow	Yellow	Pale yellow	Pale yellow	Black Pale yellow
Odor	Odorless	Odorless	Odorless	Foul odor	Foul Odor
Texture	Normal	Normal	Breakable	Briter	Briter

Table 2. Incidence of fungi on flowers of *Spilanthes acmella* from different age.

Fungi	Young		Mature		Stored	
	Blotter	PDA	Blotter	PDA	Blotter	PDA
Alternaria alternata	-	-	05	05	10	10
Aspergillus flavus	-	05	-	15	15	35
Aspergillus niger	-	-	05	10	10	55
Aspergillus fumigatus	-	-	-	-	05	20
Aspergillus nidulance	-	-	-	-	05	20
Aspergillus terreus	-	-	05	10	10	20
Cladosporium species	-	-	-	-	-	30
Fusarium oxysporum	-	-	05	15	-	50
Fusarium roseum	-	-	05	10	10	10
Mucor globsus	-	-	-	-	10	25
Phoma species	-	-	-	-	-	10
Penicillium notatum	-	-	-	15	10	20
Rhizopus stolonifer	-	-	-	-	-	30
Trichoderma viride	-	-	-	15	05	10

In case of mature flowers the Blotter Paper Method showed incidence of four fungi like *Alternaria alternata* (05), *A. niger* (05), *Aspergillus terreus* (05), *F. oxysporum* (05), *Fusarium roseum* (05) whereas PDA method showed nine fungi viz. *Alternaria alternate* (05), *Aspergillus flavus* (15), *Aspergillus niger* (10), *Aspergillus terreus* (10), *F. oxysporum* (15), *Fusarium roseum* (10), *Penicillium notatum* (15) and *Trichoderma viride* (15).

In case of stored flowers, the maximum incidences of fungi were reported as compared to young and mature flowers. In stored flowers, fourteen fungi were reported viz *Alternaria alternata* (10), *Aspergillus flavus* (35), *Aspergillus niger* (55), *Aspergillus fumigatus* (20), *Aspergillus nidulance* (20), *Aspergillus terreus* (20), *Cladosporium sp.* (30), *Fusarium oxysporum* (50), *Fusarium roseum* (10), *Mucor globsus* (25), *Phoma sp.* (10), *Penicillium notatum* (20), *Rhizopus stolonifer* (30) and *Trichoderma viride* (10) on PDA. whereas in case of Blotter Paper Method only ten fungi were observed. Roy, (2003) studied that the frequent occurrence of *Aspergillus, Fusarium* and *Penicillium* species on different crude herbal drugs. Santhosh *et al.* (2011) observed 41 endophytic fungi from 195 samples of healthy leaves and stem of a red listed endangered medicinal plant *Coscinium fenestratum.* The herbal preparations had the presence of fungal contaminants with predominance of *Aspergillus* spp. and *Penicillium* spp. Sumanth *et al*.(2010) who isolated fungal genera from tested spices, found that the most common fungi isolated were *Aspergillus* spp. followed by *Alternaria alternata*, *Cladosporium*, *Curvularia*, *Fusarium* spp., *Helminthosporium* and *Trichoderma* show maximum incidence on Agar plate method.

The fungal deterioration adversely affects the chemical composition of the raw materials and thereby decreases the medicinal potency of herbal drugs,respectively, supporting the findings of present investigations. In general, flowers (young, mature) material showed decrease in the growth and incidence of fungi as compared with stored flowers material of *Spilanthes acmella*. It was found that both the Potato Dextrose Agar (PDA) method and Blotter Paper Method are effective,

routinely and consistently applicable and provide reliable results.

CONCLUSION

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

Acknowledgement:
The authors express their sincere thanks to principal, Shri Shivaji Science And Arts College, Chikhli for providing the necessary laboratory facilities.

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