



Mycoflora associated with the fruit of *Trichosanthes tricuspidata* (Lour.) during storage

Gaikwad Deepmala A¹ and Anil N. Korpenwar²

¹Shri Shivaji Science and Arts College Chikhli, Dist. Buldana 443201 (MS) India.

²Rashtrapita Mahatma Gandhi Science and Arts College, Nagbhid, Dist. Chandrapur 441205 (MS) India.

*dipalirgaikwad82@gmail.com

Article Info

Received: 27-02-2017,

Revised: 22-03-2017,

Accepted: 24-03-2017

Keywords:

Trichosanthes tricuspidata,
Mycoflora, Isolation.

Abstract

The fruits of *Trichosanthes tricuspidata* Lour. is used in the treatment of asthma, earache, leprosy and for rheumatism. During unscientific methods of storage of plant a part causing fungal contamination. The fungal contamination affects the chemical composition of raw materials and thereby decreases the 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. paraceticus*, *A. terreus*, *A. ustus*, *Curvularia lunata*, *Colletotrichum sp.*, *Cladosporium sp.*, *Drechslera sp.*, *Fusarium oxysporum*, *F. equiseti*, *F. moniliforme*, *Helminthosporium sp.*, *Mucor globosus*, *Phoma .sp.*, *Penicillium citrinum*, *Rhizopus stolonifer*, *Trichoderma viride*, *Verticellium sp.* were isolated from one year old authentic stored fruit sample and 16 from fresh fruit sample on Potato Dextrose Agar (PDA) method. Similarly, minimum 17 fungal species were observed from authentic store fruit sample and 08 from fresh sample on Standard Moist Blotter (SMB) Method.

INTRODUCTION

Trichosanthes tricuspidata Lour (family: Cucurbitaceae) commonly known as Lal Indrayan. It grows as a large climber, often attaining a height of 9-10 meters. *T. tricuspidata* has been widely used for curing asthma, migraine, fever, diabetic and carbuncles (Snehlata *et al.*, 2008). The seeds are emetic and a good purgative. In the Thai traditional system of medicine, the plant is used as an anti-fever remedy, a laxative, an anthelmintic as well as in migraine treatments (Kanchanapoom *et al.*, 2002). Masoumeh and Deokule (2013) concluded that medicinal plants may be associated with a broad variety of microbial contaminants, represented by bacteria, fungi and viruses. Inevitably, 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 conditions make them victims to fungal contamination. Kumar *et al.* (2009) studied and concluded that the moulds are responsible for the biodeterioration of a number of substrates including raw materials of some medicinal plants. These moulds reduce the raw herbal drugs' shelf life and market value. Similar (Muntanola, 1987 and Durakovic *et al.*, 1989) studies that the fungal contamination 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 cause several ailments of liver, kidney, nervous system, muscular, skin, respiratory organs, digestive tract, genital organs etc. Pinkey (2014) recorded that 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. The presence of potential contaminants in herbal preparations viz. (Martins *et al.*, 2001; Czech *et al.*, 2001; Kulshrestha *et al.*, 2008; Alwakeel 2008, Kosalec *et al.*, 2009 and Idu *et al.*, 2011). 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).

60% of the population use herbal medicines prescribed by traditional practitioners due to non availability of medical health facilities in rural areas (Hamayun *et al.*, 2004). 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. So present investigation is an attempt to identify the mycoflora associated with the fruit of *Trichosanthes tricuspidata* Lour.

MATERIALS AND METHODS

Collection of plant material.

Trichosanthes tricuspidata fruits were collected from different locations and Authentic Stores of Jalna district. Samples were brought to the laboratory in pre-sterilized polyethylene bags to

avoid aerial contamination. samples were identified using the Flora of Marathwada (Naik, 1998) at Department of Botany, Dr. Babasaheb Ambedkar Marathwada University. The plant material was first cleaned by washing several times under running tap water and Surface sterilization was performed by sequentially rinsing the plant material with 70% ethanol for 30 seconds, then with 0.01% mercuric chloride for 5 minutes and finally with sterile distilled water for 2-3 times, then dried in between folds of sterile filter papers, placed at equal distance on moist blotters on the sterilized petriplates similarly material inoculated aseptically on the sterilized petriplates containing Potato Dextrose Agar (PDA) medium and Czapek Dox Agar (CZA) medium and incubated at 25±2°C temperature for 7 days.

Isolation of mycoflora.

Mycoflora was isolated by using Czapek Dox Agar (CZA) medium, Standard Moist Blotter (SMB) 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; Alexopoulos, 1996 and Barnett, 1970) Pure cultures of these fungi were prepared and maintained on potato dextrose agar (PDA) slants.



Trichosanthes tricuspidata Lour.



Isolation of Mycoflora on CZA Medium.



Isolation of Mycoflora on SBM Method



Isolation of Mycoflora on PDA Medium.

Table. 1: Incidence of mycoflora from fruits of *Trichosanthes tricuspidata*.

Name Of Fungi	Name of Media					
	CZA Medium		SMB Method		PDA Medium	
	Tt1	Tt2	Tt1	Tt2	Tt1	Tt2
<i>Alternaria alternata</i>	+	+	+	+	+	+
<i>Alternaria solani</i>	-	+	-	-	+	+
<i>Aspergillus flavus</i>	+	+	+	+	+	+
<i>Aspergillus niger</i>	+	+	+	+	+	+
<i>Aspergillus fumigates</i>	+	+	-	+	-	+
<i>Aspergillus nidulance</i>	-	+	-	+	+	-
<i>Aspergillus paraciticus</i>	-	-	-	-	-	+
<i>Aspergillus terreus</i>	+	+	+	+	+	+
<i>Aspergillus ustus</i>	-	+	-	+	-	+
<i>Curvularia lunata</i>	+	-	-	-	-	+
<i>Colletotrichum sp.</i>	+	+	-	-	+	+
<i>Cladosporium sp.</i>	-	+	-	+	+	+
<i>Drechslera sp.</i>	-	-	-	-	-	+
<i>Fusarium oxysporum</i>	+	+	+	+	+	+
<i>Fusarium equiseti</i>	-	+	-	+	+	+
<i>Fusarium moniliforme</i>	+	+	-	+	+	+
<i>Helminthosporium sp.</i>	-	-	-	-	-	+
<i>Mucor globsus</i>	+	+	+	+	+	+
<i>Phoma .sp</i>	+	+	-	-	+	+
<i>Penicillium notatum</i>	-	+	-	+	-	-
<i>Penicillium citrinum</i>	+	+	-	+	+	+
<i>Rhizopus stolonifer</i>	+	+	+	+	+	+
<i>Rhizoctonia solani</i>	-	-	-	+	-	-
<i>Trichoderma viride</i>	+	+	-	+	+	+
<i>Trichothecium sp.</i>	-	+	+	-	-	-
<i>Verticellium sp.</i>	+	-	-	-	-	+
Total no. of isolates	15	20	08	17	16	22

+ = fungi present. - = fungi absent.

Tt1= Fresh fruit material. Tt2 = One year old Authentic Stores fruit material.

RESULTS AND DISCUSSION

This study revealed that the maximum fungal species were associated with one year old authentic stores fruit sample of *Trichosanthes tricuspidata* as compared with fresh fruit sample. The data presented indicate that maximum 22 fungal species viz. *Alternaria alternate*, *A. solani*, *A. flavus*, *A. niger*, *A. fumigates*, *A. paraciticus*, *A. terreus*, *A. ustus*, *Curvularia lunata*, *Colletotrichum sp.*, *Cladosporium sp.*, *Drechslera sp.*, *Fusarium oxysporum*, *F. equiseti*, *F. moniliforme*, *Helminthosporium sp.* *Mucor globsus*, *Phoma sp.*, *Penicillium citrinum*, *Rhizopus stolonifer*, *Trichoderma viride*, *Verticellium sp.* were isolated from one year old authentic stored fruit material and 16 from fresh fruit sample on Potato Dextrose Agar (PDA) method. Similarly in case of Czapek Dox Agar (CZA) method maximum 20 fungal incidence were isolated viz. *Alternaria alternate*, *A. solani*, *A. flavus*, *A. niger*, *A. fumigates*, *Aspergillus nidulance*, *A. terreus*, *A. ustus*, *Colletotrichum sp.*, *Cladosporium sp.*, *Fusarium oxysporum*, *F. equiseti*, *F. moniliforme*, *Mucor globsus*, *nPhoma sp.*, *Penicillium citrinum*, *Penicillium notatum*, *Rhizopus stolonifer*, *Trichoderma viride*, *Trichothecium sp.* and 15 fungi on fresh sample as shown in table no. 1. It is clear from table that 17 fungal species were observed from authentic stores fruit sample and 08 from fresh sample on Standard Moist Blotter (SMB) Method. All fungi were identified on the basis of their cultural and morphological characteristics. The frequent occurrence of *Aspergillus*, *Fusarium* and *Penicillium* species on different crude herbal drugs (Roy, 2003). 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 (Sumanth *et al.*, 2010). Dhale (2013) studied and concluded that 45 fungi were recorded on the blotter and agar plate methods. All samples of plant material showed maximum infestation of *A. niger* and *Aspergillus* spp. The some herbs are good substrate for *Aspergillus flavus* infestation and production of aflatoxins with potential hazard to the health of consumers (Sharma *et al.*, 2013). The herbal preparations had the presence of fungal contaminants with predominance of *Aspergillus* spp. and *Penicillium* spp., also found (Kumar *et al.*, 2009). The fungal deterioration adversely affects the chemical composition of the raw materials and

thereby decreases the medicinal potency of herbal drugs .respectively, supporting findings of present investigations. In general, fresh fruit material showed decrease in the growth and incidence of fungi as compared with one year old authentic Stores fruit material of *Trichosanthes tricuspidata*. It was found both the Czapek Dox Agar (CZA) method and Potato Dextrose Agar (PDA) methods of fungal isolation are effective, routinely and consistently applicable and provide reliable results. Therefore, this study suggests that the methods of harvesting, collection, preparing and storage of medicinal plants must be improved for reducing percentage incidence of mycoflora and mycotoxins contaminations.

ACKNOWLEDGEMENT

The authors are thankful to Principal, Shri Shivaji Science and Arts College, Chikhli. For providing the necessary laboratory facilities.

REFERENCES

- Alexopolous CJ, 1996.** Introductory To Mycology, John Wiley and Sons, Inc.Publication, New York Winchester, Brisbane, Toronto and Singapore.
- Alwakeel SS, 2008.** Microbial and heavy metals contamination of herbal medicines. *Research Journal of Microbiology*, **3**(12): 683-691.
- Barnett HC, 1970.** *Illustrated genera of Fungi . im Perfecti*, Burges Publication, Minn ,(USA).
- Czech E, Kneifel W, and Kopp B, 2001.** Microbiological status of commercially available medicinal herbal drugs- A screening study, *Planta Medica.*, **67**, 263-269.
- Dhale DA, 2013.** surface mycoflora of stored part of herbal medicine *Int. J. pharm Bio. Sci.*, **4**(3): (B) 568 – 574.
- Durakovic S, Galic J, and Pajnovic P, 1989.** Toxic and cancer metabolites of moulds in food and fodder. *Hrana Iishrana.*, **30**: 71-100.
- Idu, M, Erhabor JO and Idele SO, 2011.** Microbial load of some medicinal plants sold in local markets of Benin City, Nigeria. *International Journal of Medicinal and Aromatic Plants*, **1**(3): 272-277.
- Kanchanapoom T, Ryoji K and Yamasaki K. 2002.** Cucurbitane, hexanorcucurbitane and octanorcucurbitane glycosides from fruits of richosanthes tricuspidata. *Phytochemistry*, **59**:215-228.
- Kulshrestha R, Gupta CP, Shukla G, Kundu MG, Bhatnagar SP and Katiyar CK, 2008.** The effect of water activity and storage temperature on

the growth of *Aspergillus flavus* in medicinal herbs. *Planta Medica*, **74**:1308-1315.

Kumar A, Shukla R, Singh P and Dubey NK, 2009. Biodeterioration of some herbal raw materials by storage fungi and aflatoxin and assessment of *ymbopogon flexuosus* essential oil and its components as antifungal. *International Biodeterioration and Biodegradation.*, **63**: 712-716.

Kosalec I, Cvek J and Tomic S, 2009. Contaminants of medicinal herbs and herbal products. *Archives of Industrial Hygiene and Toxicology*, **60**: 485-501.

Martins HM, Martins ML, Dias MI and Bernardo F, 2001. Evaluation of microbiological quality of medicinal plants used in natural infusions. *International Journal of Food Microbiology*, **68**: 149-153.

Masoumeh Rashidi and Deokule SS, 2013. Associated fungal and aflatoxins contamination in some fresh and market herbal drugs. *J. Microbiol. Biotech. Res.*, **3**(1):23-31.

Mukadam DS, Patil MS, Chavan AM, Patil AR, 2006. *The Illustrations of Fungi*. Saraswati Printing Press Aurangabad. (M.S) India.1-254.

Muntanola M, 1987. *General mycology*, Beograd: NIRO. Knjez evne novine Pp 257-269

Naik VN, 1998. *Flora of Marathwada*, Amrut Prakashan, Aurangabad (M. S.) India.

Okunlola A, Adewoyin BA and Odeku AO 2007. Evaluation of pharmaceutical and microbial qualities of some herbal medicinal products in South Western Nigeria. *Tropical Journal of Pharmaceutical Research*, **6**: 661-670.

Pinkey Khati, 2014. Mycoflora And Aflatoxin Assessment Of Crude Herbal Drugs During Storage in Haridwar, Uttarakhand, *India Indian Phytopath.*, **67**(4): 407-411 .

Roy AK, 2003. Mycological problems of crude herbal drugs: Overview and challenges. *Indian Phytopath.*, **56**: 1-13.

Sharma Sumedha, Dimple Gupta and Sharma YP, 2013. Aflatoxin Contamination In Chilgoza Pine Nuts (*Pinus gerardiana* Wall.) Commercially Available In Retail Markets of Jammu, *India. Int. J. Pharm Bio. Sci. Apr*; **4**(2): (B) 751 – 759.

Sumanth GT, Waggmare BM and Shinde SR, 2010. Incidence of mycoflora from the seeds of Indian main spices. *Afr. J. Agric. Res.*, **5**(22): 3122-3125.

How to Cite this Article:

Gaikwad Deepmala A and Anil N Korpenwar, 2017. Mycoflora associated with the fruit of *Trichosanthes tricuspidata* (Lour.) during storage. *Bioscience Discovery*, **8**(2):280-284.