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## Fungal strains associated with post-harvest deterioration of dried walnut fruits in Kashmir, India

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### Abstract

Kashmiri walnuts are better than most of the nuts because of the amazing benefits. They are rich in antioxidants, high in protein, helpful in inflammatory diseases, promotes gut health, have high fibre contents, improves immunity, good for skin and what not. However, the major factors that contribute quality losses of these walnuts are post-harvest diseases. Fungal pathogens can damage fruit and reduce its antioxidant content, other nutritional values and in addition limits the storage period and marketing life. So, the present work is designed to study intensively, the fungi associated with dry fruits of walnut that are causing rotting or decaying. Present study was undertaken to isolate and identify fungal pathogens associated with dry fruits of walnut and our results indicate that many pathogens are responsible for decaying of dried walnut fruits. The isolated fungal strains were identified by pure microbiological methods viz. cultural and microscopic characteristics. The fungal species that were isolated and identified are *Fusarium solani* (Mart) Sacc, *Penicillium citrinum* Thom, *Cladosporium herbarum* (Pers.) Link and *Fusarium oxysporum* (Schl.) emend. Snyder & Hansen.

**Keywords:** Walnut, fungal pathogens, isolation, identification, post-harvest diseases

### Introduction

The Kashmir valley harbours a rich diversity of valuable medicinal plants and the age-old traditional values attached with the various plants especially dry fruits have gained tremendous importance in the present times. Dry fruits of walnut, apricot, almond etc. are best source of income for the inhabitants of Kashmir and are considered as best source of essential nutrients as they provide protein, omega-3-fatty acids, dietary fibres, antioxidants and bioactive compounds. They are also rich source of essential minerals and vitamins like phosphorus, potassium, copper, manganese, Vitamin B6, Vitamin E (gamma-tocopherol), and Folic acid. They also provide benefits to human health by reducing the chances of obesity, cardiovascular illnesses as well as overcome the possibilities of diabetes. Lowering LDL (bad) cholesterol, reducing inflammation, and improving blood vessel function. Dried fruits have higher shelf life as compared to fresh fruits, so they are best substitute to fresh fruits for long time usage but unfortunately these fruits are more prone to post-harvest diseases.

Walnut (*Juglans regia* L.) is an important tree nut, which belongs to family Juglandaceae and is one of the most nutritious nuts available. Among other nuts like almond, cashew, and hazelnut it is considered as one the major nut as it is distributed worldwide. (Li *et al.*, 2023)<sup>[9]</sup>. Traditionally walnuts have been a huge part of the diet in India, particularly in the areas where they are grown. Recent studies have shown that walnut can reduce the risk of cognitive decline and supplying various macro and micronutrients and thus improves memory (Zaini *et al.*, 2020; Bishop & Zuniga, 2021)<sup>[16, 4]</sup>.

From India especially in Kashmir, the studies on post-harvest fungi associated with dried fruits of walnut (*Juglans regia* L.) are scanty so there is a need of more careful laboratory studies on fungi infesting dry fruits during storage. The area needs further study in the development of latent and quiescent infection of various types of fruits by pathogens which initiate infection in the field long before harvest. A proportion of these infections are expressed as disease at harvest, and fruits can be easily removed. However, another portion of these infections will be expressed as disease symptoms during the postharvest life of the fruits. It is this latter portion of infections that caused huge losses to growers and reduce its

market value. It thus needs to be studied further and possibly develop procedures to quantify it. The incidence of latent infection before harvest could be used as a biological predictor of the amount of decay at pre harvest and post-harvest stages. This information will be very useful in helping growers and packing house operators in decision making for pre- and post- harvest treatments, longevity of storage, and time of marketing of the produce. The isolation and identification of fungi from the dry fruits will help in developing the proper management strategy in order to prevent the loss and damage caused by infections. The main post-harvest processing method of walnut is drying, as it lowers moisture content and making walnut safe to storage and transport.

The metabolites in walnut will undergo different changes during drying and thus alter nutritional value of walnuts. (He *et al.*, 2021; Li *et al.*, 2023) [7, 9]. The antioxidant activity and phenolic metabolites during drying was found to increase significantly, among which polyunsaturated fatty acids have significant effects on their antioxidant activity. The most abundant class of metabolites in walnuts are lipids that tend to undergo significant changes during drying. In previous studies, identification of lipids in seven main walnut varieties In Xinjiang it was revealed that identification of lipids in seven main walnut varieties had significantly higher contents of lipid metabolites than fresh walnuts. The conversion of glycerophospholipids to lysophospholipids, was found to promote during drying which is primarily related to the moisture content in walnuts (Wang *et al.*, 2022) [15].

In some developing countries post-harvest losses of fruits and vegetables are very high and sometimes reach 50% and more due to pathological and physiological deterioration (Smilanick *et al.*, 2006) [14]. This loss usually occurs because of inappropriate handling and lack of right methods and means to prevent diseases. Dried fruits are more susceptible to severe losses caused by several fungal pathogens which include *Alternaria alternata*, *Botrytis cinerea*, *Penicillium digitatum*, *Penicillium italicum*, *Rhizopus stolonifer*, *Monilia laxa*, *M. fructicola*, *Colletotrichum gloeosporioides*, *Alternaria solani* (Hashim *et al.*, 2019) [6]. Dry fruits particularly of walnut are economically important and are considered as best source of essential nutrients as they provide amino acids, vitamins, fibres, minerals and more.

## Materials and Methods

### Collection of diseased samples and isolation of fungi

Different samples of dried Walnut (*Juglans regia* L.) were

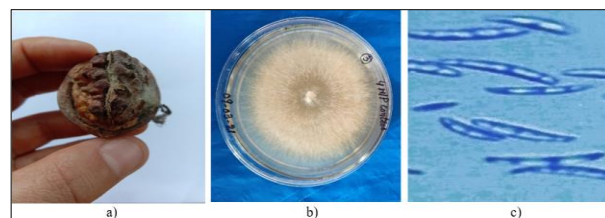
collected from commercial markets, go downs and storage houses in clean polyethene bags from different areas of Kashmir division with proper labelling. The collected fruits were transferred to Plant Pathology, Mycology, Microbiology Research laboratory for isolation and identification of fungi. For isolation of the fungi the required glassware was first washed with tap water and detergent solution and then rinsed with distilled water and air dried. The Petri plates were autoclaved at 121 °C / 15 psi for 15 minutes. The direct plating technique was employed for isolation of the fungi. The dried fruit sterilized samples, were directly inoculated on PDA plates with three replicates for each sample. The fungal colonies were isolated by inoculating a single spore on PDA Petri plate and the pure cultures were maintained on PDA slants for further studies.

### Identification of isolated fungi

The isolated fungi were identified following the relevant keys, references, monographs, etc. (Raper and Fennell, 1965; Barnett and Hunter, 1972; Pitt, 1979; Klich, 2002) [12, 3, 11, 8]. The fungal morphology was observed with regards to colony features (colour, shape, size, spore, and hyphae), and identification of fungi was based on comparison of morphology, colour, shapes of spores and colony characteristics as described by Coony and Emerson (1964) [5].

### Results

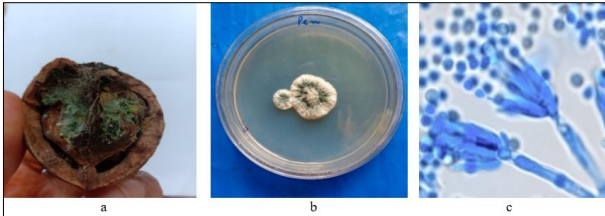
It was revealed from the results that four fungal pathogens were responsible for spoilage or decay of dried walnut fruits under storage, viz. *Fusarium solani* (Mart) Sacc, *Penicillium citrinum* Thom, *Cladosporium herbarum* (Pers.) Link and *Fusarium oxysporum* (Schl.) emend. Snyder & Hansen. The cultural and microscopic characteristics of the identified fungi associated with dried walnuts are described in table 1 and Figs. 1-4.



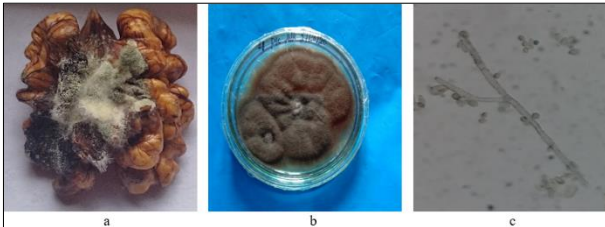
**Fig 1:** (a) Infected sample of dried walnut showing decay and discoloration (b) Culture of *Fusarium solani* (Mart) Sacc on PDA (c) Sickle shaped conidia of *Fusarium solani* (400X)

**Table 1:** Cultural and microscopic characteristics of fungi isolated from dried walnut fruits

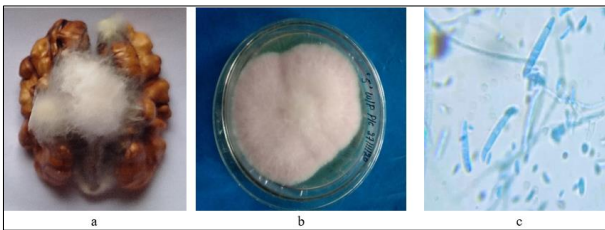
Pathogen Name	Macroscopic Morphology	Microscopic Morphology
<i>Fusarium solani</i>	Colonies are white and cottony with dark brown zonation. Some isolates become blue-green or ink-blue on the underside.	Microscopic observations of the fungus revealed that mycelium is septate, branched, conidiophores arise from the hyphae. Conidiophores give rise to long sickle shaped conidia.
<i>Penicillium citrinum</i>	Colonies are rapid growing, velvety, woolly, or cottony in texture. Initially colonies are white and become blue green, grey green, olive green in time.	Hyphae are septate, hyaline. Smooth walled conidiophores and is biverticillate. Metulae are 12-15 µm in length which are found in whorls.
<i>Cladosporium herbarum</i>	Colonies are slow growing velvety, olivaceous-brown to blackish- brown. The reverse is olivaceous-black.	Septate dark vegetative hyphae. Conidia are produced in branches acropetal chains.
<i>Fusarium oxysporum</i>	Fast-growing colonies with white cottony and dark-purple undersurface. The colour changes from pale to yellow over time.	Septate hyaline hyphae that typically branch to acute angles. Spores were oval to ellipsoid and septate in three cells.



**Fig 2:** (a) Infected sample of dried walnut showing decay and discoloration (b) Culture of *Penicillium citrinum* Thom on PDA (c) Conidiophore & conidia of *Penicillium citrinum* (400X)



**Fig 3:** (a) Infected sample of dried walnut showing decay and discoloration (b) Culture of *Cladosporium herbarum* (Pers.) Link on PDA (c) Mycelium of *Cladosporium herbarum* (100X)



**Fig 4:** (a) Infected sample of dried walnut showing decay and discoloration (b) Culture of *Fusarium oxysporum* (Schl.) emend. Snyder & Hansen on PDA (c) Conidia of *Fusarium oxysporum* (100X)

## Discussion

This study showed that fungal pathogens viz. *Fusarium solani* (Mart) Sacc, *Penicillium citrinum* Thom, *Cladosporium herbarum* (Pers.) Link and *Fusarium oxysporum* (Schl.) emend. Snyder & Hansen are associated with the spoilage and loss of dried walnut fruits during storage. Past studies have shown similar contamination profiles of these commodities. Our results agreement with Abbas, *et al.* (2019) <sup>[1]</sup>, which reported *Penicillium* and *Fusarium* spp. as the predominant fungi. Our results are in accordance with (Alhussaini, 2012; Saadullah and Abdullah 2014) <sup>[2, 13]</sup> who reported similar mycobiota associated with nuts and fruits from Saudia Arabia and Iraq. Similar to our study, Mirabile *et al.* (2021) <sup>[10]</sup> reports some main fungal taxa and their mycotoxins associated with dried fruits. A recent study conducted in Brazil has also revealed the presence of mycoflora in dried fruits. They isolated *A. niger*, *A. carbonarius*, *A. ochraceus*, *Aspergillus flavus*, *Eurotium* sp., *Fusarium* sp., *Penicillium* sp., and yeasts from dried fruit samples. Fungi being an important component cause contamination in many food stuffs including dried fruits. This fungal strains not only leads to spoilage of these nutritive substances but also liable for mycoses and mycotoxicosis. The isolation and identification of fungi from the dry fruits will help in developing the proper management strategy in order to prevent the loss and damage caused by infections. The main purpose of this work is to isolate and identify the most prevalent postharvest fungal pathogens from dry walnuts of Kashmir valley.

## Conclusion

Fungal infection of warehoused walnuts randomly collected in Kashmir valley; India was investigated. The dominant species were *F. solani* (Mart) Sacc, *P. citrinum* Thom, *Cladosporium herbarum* (Pers.) Link and *Fusarium oxysporum* (Schl.) emend. Snyder & Hansen. As the fungi caused post-harvest losses during storage to dried fruits and warrants effective management. The best way to control decay is the use eco-friendly approaches such as use of biological control agents and botanicals for the management of fungi responsible for post- harvest losses to dried walnut fruits.

## Conflict of Interests

Authors do not have any conflicts of interest.

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