

## Research Article



## Fungal Contamination of Powdered Samples of Different Plant Parts of Two Important Medicinal Plants: *Momordica charantia* and *Syzygium cumini*

Madhu Gupta<sup>1</sup>, Sushil Sharma<sup>1,2\*</sup>, Rekha Bhadauria<sup>1</sup>

<sup>1</sup>School of Studies in Botany, Jiwaji University, Gwalior (M.P.), India.

<sup>2</sup>Department of Environmental Studies, Amity University, Madhya Pradesh, Gwalior, M.P., India.

\*Corresponding author's E-mail: [sharmasushil07@gmail.com](mailto:sharmasushil07@gmail.com)

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### ABSTRACT

Various parts (leaves, stem, fruit, and root) of *Momordica charantia* and leaves, stem bark, seed, and fruit of *Syzygium cumini* have been investigated for their fungal diversity at the time of collection and storage. A total of 20 fungal species were found to be associated with the various parts of the tested plants. Results revealed that fresh and stored samples of various parts of *M. charantia* and *S. cumini* were contaminated with various types of fungal species with varying degree of total incidence. Out of 20 isolated fungal species, 14 species were associated with fresh and stored powder samples of various parts of *M. charantia* and 15 fungal species were isolated from *S. cumini*. All isolates belongs to nine different genera, namely *Aspergillus*, *Alternaria*, *Cladosporium*, *Penicillium*, *Pestalotia*, *Rhizopus*, *Fusarium*, *Fusicoccum* and *Helminthosporium*. *Aspergillus niger* was the most prevalent fungi recorded in fresh and stored samples of various parts of *M. charantia* and *S. cumini*.

**Keywords:** Fungal contamination, Fresh and stored, Medicinal plants, *Momordica charantia*, *Syzygium cumini*.

### INTRODUCTION

Since time immemorial plants are used throughout the world to alleviate various ailments of human body. Most of the rural population of the world relies on plants and their parts for health care<sup>1</sup>. The plant based formulations are readily available in rural areas as they are cheaper than modern medicine. *Momordica charantia* (Karela) and *Syzygium cumini* (Jamun) have been used in various traditional systems of medicine including Ayurveda for a long period of time for prevention and treatment of various diseases. The fruits of *M. charantia* (karela) are used in asthma, burning sensation, colic, rheumatism, constipation, cough, diabetes, fever (malaria), gout, inflammation, leprosy, skin diseases, ulcer, wound and disease of liver and spleen<sup>2-4</sup>. Leaves are used for the treatment of menstrual troubles, burning sensation, constipation, fever (malaria), colic infections, worms and parasites, as an emmenagogue, measles, hepatitis and helminthiasis. Seeds are used in the treatment of ulcers, liver and spleen problems, diabetes, intestinal parasites, high cholesterol, and intestinal gas, heal wounds and stomachache etc. Roots are used in the treatment of syphilis, rheumatism, boils, ulcer and septic swellings<sup>2</sup>. Similarly most of the parts of *S. cumini* such as leaves, bark, seed, and fruit have been used in traditional medicine.

The plant bark is astringent, sweet, refrigerant, stomachic, carminative, diuretic, digestive, antihelminthic, constipating and antibacterial<sup>5</sup>. The leaves have been extensively used for the treatment of diabetes, constipation, leucorrhoea, stomachalgia, fever, gastropathy and dermopathy<sup>6</sup>. Fruit is useful in various

diseases such as diabetes, diarrhea, dysentery, liver disorders, bleeding piles, female sterility, polyuria and seeds are used to cure diabetes, pharyngitis, spleenopathy and ringworm infection<sup>7</sup>.

Beside these properties, medicinal plant materials normally carry a large number of microbes originating from the soil. Various kinds of microorganisms are normally adhered to leaves, stems, flowers, roots, fruits, and seeds. Fungi are widely distributed in every ecosystem and also known to be responsible for the off-flavor formation and production of toxic and allergenic compounds. Some fungi are responsible for severe losses of many crops in agriculture production and also reported to produce a number of fungal metabolites that are toxic to man and animals<sup>8</sup>.

Various reports related to fungal contamination of powdered samples are available from India and other countries. The presence of *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, *Cladosporium* and *Aureobasidium* with dominance of *Aspergillus* and *Penicillium* species from the forty-nine samples of powder herbal drugs marketed in Tokyo (Japan) is well documented<sup>9</sup>. The high contamination of medicinal plants with *Aspergillus flavus* and the presence of species of *Aspergillus* and *Fusarium* in crude samples of twelve drug plants collected randomly from different store houses of Southern Bihar is well documented<sup>10-11</sup>. Kumar<sup>12</sup> isolated the 906 fungal strains from the raw materials of six medicinal plants viz. *Terminalia arjuna*, *Acorus calamus*, *Rauvolfia serpentina*, *Holarrhena antidysenterica*, *Withania somnifera* and *Boerhaavia diffusa*. Fungal contamination results in to significant variation in active constituents of raw material



as well as processed herbal formulations during storage. Raw materials and formulations in powder forms are more prone to this menace. This always results in to decreased active constituents in final products as compared to the raw material. Therefore, this experiment was carried out to study the associated mycobiota of fresh and powdered stored plant samples of *M. charantia* and *S. cumini*.

## MATERIALS AND METHODS

### Collection of plant materials

Fresh and healthy sample of leaves, stem, and roots of *M. charantia* were collected from the plants grown in the botanical garden of the University while fruits were obtained from the market. Fresh leaves, and stem bark of *S. cumini* were collected from the trees, growing at various location in the city including the university campus. Fresh fruits of *S. cumini* were obtained from the local fruit vendors and the seeds were separated from the fruits.

### Preparation of powdered samples

Samples free from diseases were surface sterilized with Sodium Hypochlorite and dried in hot air oven at 60°C. After drying, plant samples were grounded to fine powder and stored in airtight containers for further use.

### Isolation of Mycoflora from fresh and stored samples

Various fungi were isolated from the *M. charantia* and *S. cumini* samples by pour plate technique, using Potato Dextrose Agar media (CDA) supplemented with chloramphenicol. Collected fresh plant materials were washed with running tap water followed by sterilized distilled water. Under aseptic condition three to four small pieces of fresh samples cut with sterilized blade were inoculated on petri-plates containing potato dextrose agar media (PDA) in triplicates along with a control (without samples). After inoculation, plates were incubated for seven days at 25 ± 2 °C. The plates were examined after 7 days of incubation period.

For stored sample, 1gm sample was suspended in 9 ml of sterilized distilled water and serial dilutions were made up to 10<sup>-5</sup>.

One ml aliquot of each sample from appropriate dilution was poured in sterilized petri-plates (in triplicate) and appropriate amount of media was added in petri-plates and mixed well. After solidification, plates were incubated at 25 ± 2 °C and growth of fungal colonies was recorded at various time intervals.

After five or seven days of incubation period (depending upon the types of fungal species appearing on plates), pure culture of each fungal isolate was prepared by using PDA media for identification purpose.

Colony forming units per gram (CFU/g) were calculated using following formula:

CFU =  $N \times 10^n$  where N= Total number of colonies, n= dilution

### Fungal Identification

Isolated fungal species were identified on the basis of morphology (shape, size, growth rate and color of the colonies) and microscopic characteristics (characteristics of mycelium, size, shape, color and arrangement of conidia, spore, conidiophores, sporangiophores, vesicle, sterigmata etc.) as described by Thom and Raper (1945)<sup>13</sup>, Gilman (1971)<sup>14</sup>, Barnett (1969)<sup>15</sup>, Jamaluddin (2004)<sup>16</sup>, Samson (2007a, b)<sup>17,18</sup>.

### Calculations

Total incidence/abundance and frequency of occurrence of fungi isolated from the different parts of *M. charantia* and *Sygium cumini* was calculated by following formula:

$$\text{Frequency of occurrence} = \frac{\text{Number of samples containing a genera/fungal species}}{\text{Total number of sample evaluated}} \times 100$$

$$\text{Total incidence} = \frac{\text{Number of isolates of the species}}{\text{Total number of isolates}} \times 100$$

## RESULTS

A total of 20 fungal species were found to be associated with the various parts of both the plants. Results revealed that fresh and stored plant parts of *M. charantia* and *S. cumini* were contaminated with various types of fungal species with varying degree of total incidence as shown in table 1.

Isolates belonging to nine different genera, namely *Aspergillus*, *Alternaria*, *Cladosporium*, *Penicillium*, *Pestalotia*, *Rhizopus*, *Fusarium*, *Fusicoccum*, *Helminthosporium* and two unidentified species were isolated during the study.

*Aspergillus niger* was the most prevalent fungi recorded in fresh and stored samples of various parts of *M. charantia* and *S. cumini*. Out of 20 fungal species, 14 species were associated with fresh and stored powder samples of various parts of *M. charantia* and 15 fungal species were isolated from *S. cumini*.

### Mycoflora associated with *Momordica charantia*

Leaves, stem, fruit and roots of *M. charantia* were enumerated for the associated mycoflora at the time of collection (fresh) and during storage (dried stored powder samples). Total 14 fungal species were isolated from fresh and stored powder samples of *M. charantia*. Among these, *Aspergillus* was the most dominant genera with 10 species (Table 1). It was observed that maximum fungal species were found to be associated with stored powder samples in comparison to fresh samples.

Result summarized in table-1 revealed that total 14 fungal species viz; *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *A. versicolor*, *A. nidulans*, *A. nipens*, *A. sydowi*, *A. tamari*, *A. ustus*, unidentified *Aspergillus* spp., and species of



*Alternaria*, *Cladosporium*, *Penicillium* and *Rhizopus* were isolated from the stored plant parts of *M. charantia*. Only five fungal species namely *A. niger*, *A. fumigatus*, *A. flavus*, *A. versicolor* and *A. nidulans* were recorded from fresh samples. *A. niger*, *A. fumigatus*, *A. flavus*, *A. versicolor* and *A. nidulans* have been recorded as commonly occurring fungal species in fresh and stored powdered samples of various plant parts of *M. charantia*.

Variability in the occurrence and total incidence of mycoflora was observed in fresh and stored powder samples. Highest incidence of fungal species was recorded from the stored powder sample of stem followed by leaves, fruits and roots (Table 1).

Among the isolated fungi *A. niger* has been recorded as most commonly occurring fungal species found to be associated with fresh and stored powder samples. Except roots, fresh samples showed higher values of total incidence of *A. niger* in comparison to stored samples i.e. 42.85 & 85.44% (in roots), 69.23 & 4.87% (stem), 63.04 & 11.76% (fruits) and 50.0 & 10.71% (leaves) from fresh and stored samples respectively.

Among aspergilli, *A. versicolor* was the second most dominant fungal species recorded with total incidence of 67.64% and 32.14% from stored fruit and leaves while it was 46.34 & 30.76% in stored and fresh stem respectively. Total absence of *A. versicolor* was observed in fresh samples of fruit, leaves and fresh as well as stored powdered sample of roots. *A. fumigatus* was associated with fresh and stored sample of fruits (36.95 & 8.82%), leaves (30.0 & 7.14%) and fresh root (34.28%) respectively while it was not observed from stored root, fresh and stored powder sample of stem. Presence of *A. flavus* was recorded from stored root and stem with total incidence of 22.85% and 4.87% respectively, while its total absence was observed from fresh and stored leaves, fruit, and fresh stem.

*A. nidulans* was isolated from fresh and stored powder sample of leaves and stored fruit with total incidence of 20.0 & 14.28% and 5.88% respectively. It was not recorded from fresh fruit, fresh and stored stem and root. Only stored samples of leaves, stem and fruit showed presence of *Cladosporium* sp. with total incidence of 21.42%, 7.31% and 2.94% respectively, its absence was recorded in fresh samples of leaves, stem, fruit and root and stored powder sample of root.

Stored powdered samples of stem were found to be associated with *A. ustus*, (19.51%), *A. nipsei*, (2.43%), unidentified *Aspergillus* sp. (12.19%) and *Rhizopus* sp. (2.43%).

These fungal species were totally absent in remaining fresh and stored plant parts samples. Presence of *A. sydowi* and *Penicillium* sp. was recorded only from stored leaves with the total incidence of 7.14%. *A. tamari* and *Alternaria* sp. were isolated only from stored powdered

samples of root and fruit with the total incidence of 6.10% and 2.94% respectively. During study it was observed that among all the samples highest number of fungal spp. were found to be associated with stored samples of stem followed by leaves, fruits, and roots then the fresh samples.

### Mycoflora associated with *Syzygium cumini*

A total of 15 fungal species were found to be associated with fresh and stored powder samples of various parts viz. leaves, stem bark, seeds and fruits of *S. cumini*. Results revealed the presence of much more number of fungal species in fresh samples as compared to stored powder samples (except stem bark).

Total eleven fungal species mainly *A. niger*, *A. fumigatus*, *A. flavus*, and species of *Alternaria*, *Penicillium*, *Pestalotia*, *Rhizopus*, *Fusarium*, *Fusicoccum* and unidentified-1&2 were recorded from fresh samples. Whereas eight fungal species viz *A. niger*, *A. versicolor*, *A. nidulans*, species of *Alternaria*, *Cladosporium*, *Penicillium*, *Fusarium* and *Helminthosporium* were isolated from stored powder samples. Most commonly occurring fungal species isolated from fresh and stored powder samples of various parts of *S. cumini* were *A. niger*, species of *Alternaria*, *Penicillium* and *Fusarium*. Variability in the occurrence and total incidence of fungal species were observed in fresh and stored powder samples. Highest number of fungal species was found to be associated with fresh and stored powdered sample of stem bark followed by fruit, leaves, and seeds (Table 1).

*A. niger*, one of the predominant fungal species, has been recorded from all fresh and stored powdered samples of *S. cumini*. *A. niger* was the single species associated with stored powder samples of leaves and fruits with the hundred percent total incidence followed by 90.69% in seeds. In fresh samples, total incidence of *A. niger* was 72.0%, 53.33% and 20.0% in seeds, leaves and fruits respectively. Whereas minimum 9.52 & 21.05% incidence was observed in fresh and stored powder sample of stem bark respectively. *A. fumigatus* was isolated only from fresh seed and leaves with the total incidence of 28.8 and 26.66% respectively.

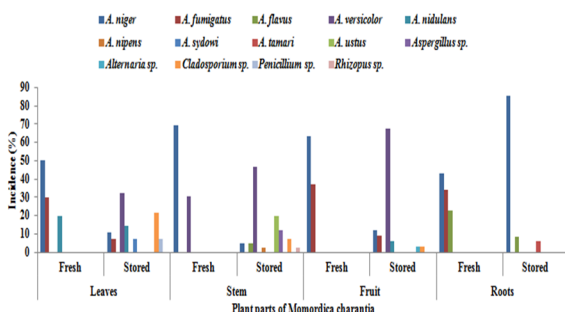
Presence and the total incidence of *Alternaria* sp. was recorded in fresh fruit (70.0%) and stem bark (28.57%); while lowest (2.63%) was observed in stored powder sample of stem bark. *A. flavus* (20.0%) and *Fusicoccum* sp. (10.0%) were isolated from fresh samples leaves and fruits respectively. Remaining isolates of *Pestalotia* sp., *Rhizopus* sp. and two unidentified isolates were isolated only from fresh stem bark with the total incidence of 19.04%, 9.52%, 14.29% and 4.76% respectively.

Species of *Cladosporium*, *A. versicolor* and *Helminthosporium* were found to be associated with stored powdered sample of stem bark with total incidence of 47.36%, 7.89 % and 2.63% respectively. *A.*





*nidulans* (9.30%) was isolated from stored seeds only. 9.52 (fresh) & 15.78% (stored) of *Penicillium* sp. and 4.76 (fresh) & 2.63% (stored) incidence of *Fusarium* sp. respectively was recorded from the samples of stem bark. Results of the present study revealed that fresh and stored powder samples of stem bark of *S. cumini* were found to be most contaminated samples in comparison to the other samples investigated.



**Figure 1:** Total incidence of mycoflora isolated from various parts of *Momordica charantia*

## DISCUSSION

A total of nine different genera belonging to *Aspergillus*, *Alternaria*, *Cladosporium*, *Penicillium*, *Pestalotia*, *Rhizopus*, *Fusarium*, *Fusicoccum*, *Helminthosporium* and two unidentified species were isolated from different parts of *M. charantia* and *S. cumini* during the study. Similar observation were made by Shakoor<sup>19</sup> where they isolated six fungal species from *M. charantia* out of which *A. flavus* showed highest percentage i.e. 27.3% followed by *R. stolonifer* 17.98%, *A. alternata* 13.34%, *A. niger* 5.23%, *Myrothecium roridum* 7.37% and *F. solani* 6.69%. *Aspergillus* comprised the highest percentage (50%) of the fungal isolates recovered from seven vegetables including *M. charantia*<sup>20</sup>. This is in accordance with our findings where *Aspergillus* was also recorded as most dominant fungal species.

During present investigation, *A. niger* was reported as most prevalent fungi in fresh and stored samples of various parts of *M. charantia* and *S. cumini*. Santos<sup>21</sup> also reported high frequencies of *A. niger* and *A. flavus* on bitter melon seeds. Fungi like *Aspergillus*, *Chaetomium*, *Cladosporium*, *Rhizopus* and *Fusarium semitectum* has also been reported as most frequently occurring fungi with bitter melon seed<sup>22</sup>. Storage time is also reported as one of the important factor responsible for fungal contamination of seeds of *M. charantia*<sup>19</sup>.

Avinash and Rai (2013)<sup>23</sup> reported two major fungal pathogens *F. oxysporum* and *Cladosporium cucumerinum* from pumpkin seeds and *A. cucumerina* and *A. alternata* were from bitter melon seeds.

This study is related to the present study. Both *A. cucumerina* and *A. alternata* infect almost all cucumbers worldwide, spores are transported by wind over long

distances through rain, warm and 60-80% humid conditions which are favorable for infection development<sup>24</sup>.

The *S. cumini* is of wider interest for its medicinal applications than for its edible fruit, commonly called jambolan. The purpose of the study was to get an overview of the mycoflora of this medicinally important plant. In present investigation, sp. of *Pestalotia*, *Rhizopus* and two unidentified species were isolated only from fresh stem bark of *S. cumini* with total incidence of 19.04%, 9.52%, 14.29%, and 4.76% respectively. A *Pestalotiopsis* sp. has already been identified as the major pathogen, causing leaf blight disease of *S. cumini* in naturally infected leaf samples of *S. cumini*<sup>25</sup>.

Most commonly occurring fungal species isolated from fresh and stored powder samples of various parts of *S. cumini* were *A. niger*, species of *Alternaria*, *Penicillium* and *Fusarium*. Bashir and Mushtaq (2012)<sup>26</sup> also reported a total of five fungal species belonging to three different genera namely *Alternaria*, *Aspergillus* and *Fusarium* from leaves of *S. cumini*. Whereas Abbas and Mushtaq (2008)<sup>27</sup> reported the presence of *Bidenticula cannaea*, *Monodictys paradoxa* and *Torula terrestris*, from different parts of *S. cumini*. This finding is in contrary to our results where no such fungal species were isolated.

## CONCLUSION

Presence of large number of fungal species in medicinally important plants is a serious issue. Large number of these fungal species has capability to produce mycotoxins which is one of the most potent carcinogenic compounds.

Simultaneously the fungi can even deteriorate the quality of the product by consuming the active compounds of the plants.

This may further results in to quality deterioration. This high contamination may also hit the global market of herbal product, which will be a setback for Indian herbal Industry which is facing strong competition from Chinese herbal Industries.

Therefore, Indian herbal industries need to strengthen their quality control measures, especially during storage and processing with controlled moisture content, so that microbial contamination can be reduced to globally expectable limits.

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**Table 1:** Mycoflora isolated from various parts of *M. charantia* and *S. cumini*

S. No.	Isolated Fungi	Total Incidence (%)															
		<i>Momordica charantia</i>								<i>Syzygium cumini</i>							
		Leaves		Stem		Fruits		Roots		Leaves		Stem bark		Seeds		Fruits	
		Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
1.	<i>Aspergillus niger</i>	50.0	10.71	69.23	4.87	63.04	11.76	42.85	85.44	53.33	100.0	9.52	21.05	72.00	90.69	20.0	100.0
2.	<i>A. fumigatus</i>	30.0	7.14	-	-	36.95	8.82	34.28	-	26.66	-	-	-	28.00	-	-	-
3.	<i>A. flavus</i>	-	-	-	4.87	-	-	22.85	8.45	20.00	-	-	-	-	-	-	-
4.	<i>A. versicolor</i>	-	32.14	30.76	46.34	-	67.64	-	-	-	-	-	7.89	-	-	-	-
5.	<i>A. nidulans</i>	20.0	14.28	-	-	-	5.88	-	-	-	-	-	-	-	9.30	-	-
6.	<i>A. nipers</i>	-	-	-	2.43	-	-	-	-	-	-	-	-	-	-	-	-
7.	<i>A. sydowi</i>	-	7.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8.	<i>A. tamari</i>	-	-	-	-	-	-	-	6.10	-	-	-	-	-	-	-	-
9.	<i>A. ustus</i>	-	-	-	19.51	-	-	-	-	-	-	-	-	-	-	-	-
10.	<i>Aspergillus</i> sp.	-	-	-	12.19	-	-	-	-	-	-	-	-	-	-	-	-
11.	<i>Alternaria</i> sp.	-	-	-	-	-	2.94	-	-	-	-	28.57	2.63	-	-	70.00	-
12.	<i>Cladosporium</i> sp.	-	21.42	-	7.31	-	2.94	-	-	-	-	-	47.36	-	-	-	-
13.	<i>Penicillium</i> sp.	-	7.14	-	-	-	-	-	-	-	-	9.52	15.78	-	-	-	-
14.	<i>Pestalotia</i> sp.	-	-	-	-	-	-	-	-	-	-	19.04	-	-	-	-	-
15.	<i>Rhizopus</i> sp.	-	-	-	2.43	-	-	-	-	-	-	9.52	-	-	-	-	-
16.	<i>Fusarium</i> sp.	-	-	-	-	-	-	-	-	-	-	4.76	2.63	-	-	-	-
17.	<i>Fusicoccum</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.00	-
18.	<i>Helminthosporium</i> sp.	-	-	-	-	-	-	-	-	-	-	-	2.63	-	-	-	-
19.	Unidentified-1	-	-	-	-	-	-	-	-	-	-	14.29	-	-	-	-	-
20.	Unidentified-2	-	-	-	-	-	-	-	-	-	-	4.76	-	-	-	-	-



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