



Isolation and identification of fungi associated with the selected spices

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Abstract

Spices are commonly stored in dried form and may become contaminated with fungi during processing, handling, transportation, and storage. The present study was conducted to isolate and identify fungi associated with selected market-available spices and to assess their moisture content and pH. Cinnamon (*Cinnamomum verum*), black cardamom (*Elettaria cardamomum*), and cumin (*Cuminum cyminum*) were collected from a local market in Jaipur, Rajasthan. Moisture content was determined by oven-drying, while pH was measured from aqueous spice suspensions. Fungi were isolated by the direct plating method on Potato Dextrose Agar and identified based on cultural and microscopic characteristics. Moisture content ranged from 5.15% to 9.11%, with the highest value recorded in cumin (9.11%), followed by cinnamon (6.36%) and black cardamom (5.15%). The pH ranged from 4.23 to 7.45, with black cardamom showing the highest pH (7.45) and cumin the lowest (4.23). A total of nine fungal isolates were obtained from the three spice samples. *Aspergillus* sp. was the predominant genus, accounting for 6 isolates (66.67%), and was detected in all three spices. *Rhizopus* sp. accounted for 2 isolates (22.22%) and was detected in cinnamon and cumin, while *Penicillium* sp. was detected only in black cardamom, representing 1 isolate (11.11%). The results demonstrate that commercially available dried spices can harbour diverse fungal flora. The predominance of *Aspergillus* sp. highlights the importance of proper drying, hygienic handling, and suitable storage conditions to maintain the microbiological quality of spices.

Keywords: Spices, fungal contamination, *Aspergillus*, *Rhizopus*, *Penicillium*, moisture content, pH, direct plating, Potato Dextrose Agar, food storage

Introduction

Spices are important food commodities used worldwide for their flavour, aroma, colour, and other culinary properties. Cinnamon (*Cinnamomum verum*), black cardamom (*Elettaria cardamomum*), and cumin (*Cuminum cyminum*) are commonly used spices and are generally marketed in dried form. During harvesting, drying, processing, transportation, and storage, spices can become contaminated with microorganisms, particularly fungi. Fungal contamination may reduce the quality of spices and can also be associated with food safety concerns when toxigenic fungi are present (Mandeel, 2005; Jeswal & Kumar, 2015) [3, 4].

Dried spices can provide suitable conditions for the survival of storage fungi. Moisture content is one of the important factors affecting fungal development and storage stability. Variations in moisture content among spice products may result from differences in drying methods, processing, packaging, and storage conditions. Mandeel (2005) [4] examined 17 imported spice samples and reported considerable variation in moisture content and fungal contamination. *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium*, and *Trichoderma* were among the major fungal genera recovered from the tested spices. The study also showed that packaging and storage conditions could influence fungal contamination.

Several studies have reported the presence of fungi in commercially available spices. In a study of seven spices, including cumin, cinnamon, and cardamom, Elshafie *et al.*

(2002) [1] isolated 20 fungal species. *Aspergillus flavus*, *A. niger*, *Penicillium*, *Rhizopus*, and *Syncephalastrum racemosum* were among the dominant fungi. Cumin showed the highest fungal contamination among the spices examined in that study. The authors also reported that fungal contamination varied among different spice types and commercial sources.

Aspergillus is one of the most frequently reported fungal genera associated with dried spices. Studies of commercially available spices have commonly detected *Aspergillus*, *Penicillium*, and *Rhizopus*. A large-scale study of spices also reported *Aspergillus* as the most common genus, with black pepper showing high fungal contamination (Perrone *et al.*, 2018) [6]. The ability of several *Aspergillus* species to survive under relatively dry storage conditions may contribute to their frequent occurrence in dried food commodities.

The occurrence of *Penicillium* and *Rhizopus* in spices has also been documented. Mandeel (2005) [4] reported both genera among the fungal flora associated with imported spices. Similarly, studies of spice samples from different markets have identified *Aspergillus*, *Penicillium*, and *Rhizopus* as important fungal contaminants. The composition and frequency of fungal flora may differ according to spice type, geographical origin, moisture conditions, processing methods, packaging, and storage practices.

The presence of fungi in spices is also relevant to food safety because some species may have the ability to produce

mycotoxins. Jeswal and Kumar (2015) [3] investigated nine commonly consumed Indian spices, including cumin, and reported *A. flavus* and *A. niger* as dominant fungal species. They also detected aflatoxins, ochratoxin A, and citrinin in some spice samples. Their findings demonstrated that spices can serve as substrates for the growth of potentially mycotoxigenic fungi and subsequent mycotoxin production. However, the presence of a fungal genus alone does not confirm mycotoxin production, and species-level identification and toxin analysis are required to establish such risks.

The physicochemical properties of spices may also influence their microbiological quality. Moisture content provides information about the drying and storage condition of a product, while pH can influence the growth and survival of microorganisms. Assessment of these parameters along with fungal isolation can provide useful preliminary information about the quality of dried spices. Such basic analyses are particularly useful for market samples because contamination can occur at different stages between production and consumption.

Although fungal contamination of spices has been investigated in several countries, the fungal flora associated with market-available spices can vary with geographical location and local storage and handling practices. Studies focusing on commonly consumed spices from local markets can therefore provide useful information about their microbiological quality. Cultural and microscopic examination remains a useful preliminary approach for isolating and characterizing fungal contaminants before species confirmation using molecular methods.

Therefore, the present study was conducted to determine the moisture content and pH of selected cinnamon (*Cinnamomum verum*), black cardamom (*Elettaria cardamomum*), and cumin (*Cuminum cyminum*) samples collected from a local market in Jaipur, Rajasthan, and to isolate and identify their associated fungi using cultural and microscopic characteristics. The frequency of the isolated fungal genera was also determined to identify the predominant fungi associated with these commercially available spices.

Materials and Methods

Collection of Spices

Frequently utilized spices such as cinnamon, black cardamom, and cumin were acquired at the local market in Jaipur, Rajasthan. The spices were kept at ambient temperature in sterile polyethylene bags to avert additional contamination.

Determination of moisture content and pH of the obtained spices

To determine the moisture content of the spices, samples were weighed individually and then dried in oven at 70°C till getting a constant weight. The difference in weight was calculated using the method of Mandeel (2005) [4].

The moisture content was calculated from the following formula-

% Moisture content = (Fresh wt. of spices – dry wt. of spices/ fresh wt. of spices) *100

To determine pH of the samples, those were grounded into a fine powder using sterilized mixer grinder. Then in 1 g of sample, 10 ml of distilled water was added and kept on

shaker for over night in a flask. The pH of the suspension was measured by using pH meter (Mandeel, 2005) [4].

Isolation of mycoflora associated with the collected spices

Fungi linked to the gathered spices were extracted by the direct plating technique. All specimens underwent surface sterilization with 1% sodium hypochlorite for 40 seconds and were thereafter rinsed three times with distilled water. Subsequently, spice samples were sectioned using a sterile knife and inoculated onto petri dishes containing 25 ml of potato dextrose agar medium (PDA - Hi media). The plates were maintained at 30°C for a duration of three days. Three plates were utilized for each sample. The presence of fungal colonies was seen after a duration of 3 to 4 days. Following incubation, the plates were assessed both visually and through the utilization of a light microscope.

Cultural and Microscopic Identification of Isolated Fungi

Fungi with distinct morphological characteristics were separately sub-cultured on PDA media. Fungal species were recognized based on cultural and physical traits (Gillman, 1998). The percentage frequency of each fungus on spices was determined utilizing the methodology of Varma and Dubey (2001).

Results

Determination of moisture content and pH of the obtained spices

The moisture content and pH of the selected spice samples are given in Table 1. The moisture content varied between the different spices. It ranged from 5.15 % to 9.11%. Cumin had the highest moisture content (9.11%), cinnamon (6.36%), and black cardamom (5.15%).

The pH also varied among the spice samples. Black cardamom had the highest pH value (7.45), showing a slightly alkaline nature. Cumin had the lowest pH (4.23) and was more acidic. Cinnamon and red chili showed almost similar pH values of 6.14 and 6.15, respectively. These differences in moisture content and pH may affect the storage quality of spices. They may also influence the growth of fungi and other microorganisms during storage.

Table 1: Moisture content and pH of the selected spices samples.

S No	Sample name	% Moisture	pH
1	Cinnamon	6.36	6.14
2	Black cardamom	5.15	7.45
4	Cumin	9.11	4.23

Isolation of mycoflora associated with the collected spices

The selected spices were examined for the presence of fungal flora. The samples included cardamom (*Elettaria cardamomum*), cinnamon (*Cinnamomum verum*) and cumin (*Cuminum cyminum*). The samples were placed on Potato Dextrose Agar (PDA) medium for fungal growth. The plates were incubated under suitable conditions. This allowed the fungi present on or inside the spice samples to grow and be identified. Different fungal colonies were observed on the inoculated PDA plates. Based on the colony characteristics, the fungal isolates obtained from each spice were assigned codes for further identification and study, three fungal isolates from each spice were selected. The isolates from

cardamom were coded as EC1, EC2, and EC3. Cinnamon isolates were named CV1, CV2, and CV3. The cumin isolates were coded as CC1, CC2, and CC3.

Fungal growth was observed in the tested spice samples. This shows that commercially available dried spices can carry different types of fungi. The isolated cultures were maintained on PDA medium for further study. They were then examined using colony characteristics and microscopic features for fungal identification.

Cultural and Microscopic Identification of Isolated Fungi

The fungal isolates obtained from the selected spices were identified using their cultural and microscopic characteristics. The isolates were grown on PDA medium. Colony colour, texture, growth pattern, surface appearance, and sporulation were observed. Microscopic examination was also carried out after staining the fungal structures. This helped in observing important features such as hyphae, conidiophores, sporangia, and conidia.

Fungal growth was observed in all the tested spice samples. The isolates obtained from cardamom (*Elettaria cardamomum*) were identified as *Aspergillus* sp., *Rhizopus* sp., and *Penicillium* sp. The fungal isolates from red chilli (*Capsicum annum*) showed characteristics of *Aspergillus* sp.

The isolates obtained from cinnamon (*Cinnamomum verum*) were identified as *Aspergillus* sp., *Rhizopus* sp., and *Aspergillus* sp. The isolates from cumin (*Cuminum cyminum*) included two *Aspergillus* sp. isolates and one *Rhizopus* sp. isolate.

Among the tested spices, *Aspergillus* and *Rhizopus* were the most commonly observed fungal genera. *Penicillium* sp. was found only in cardamom. The cultural and microscopic features of the isolated fungi are shown in the respective photoplates. These results confirm the presence of different fungal contaminants in the market-collected spice samples. The presence of these fungi also indicates that dried spices can carry fungal flora during storage and marketing.



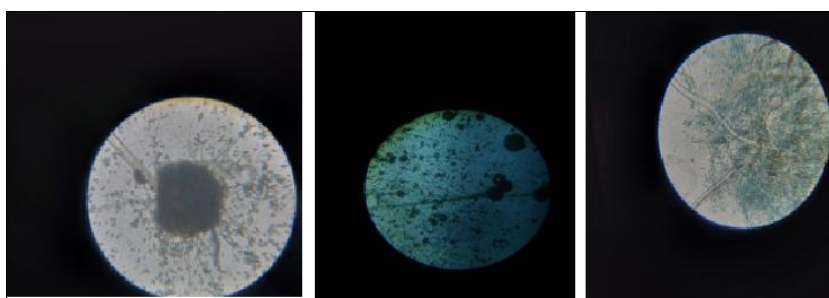
Photoplate 1: Morphological appearance of fungi isolated from *Elettaria cardamomum*.



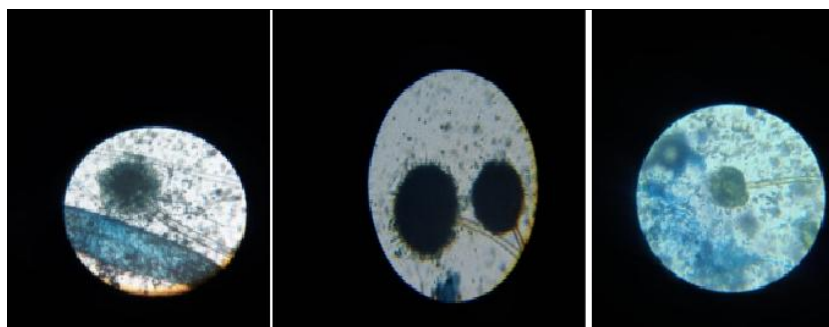
Photoplate 2: Morphological appearance of fungi isolated from *Cinnamomum verum*



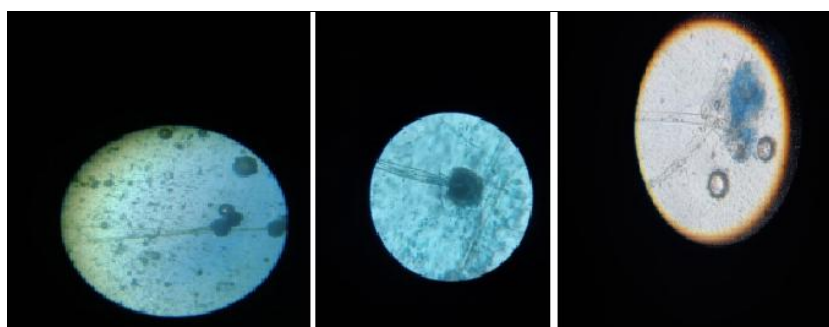
Photoplate 3: Morphological appearance of fungi isolated from *Cuminum cyminum*.



Photoplate 4: Staining images of fungi isolated from *Elettaria cardamomum*. (*Aspergillus* sp., *Rhizopus* sp. And *Penicillium* sp.)



Photoplate 5: Staining images of fungi isolated from *Cinnamomum verum* (*Aspergillus* sp., *Rhizopus* sp. And *Aspergillus* sp.)



Photoplate 6: Staining images of fungi isolated from *Cuminum cyminum* (*Aspergillus* sp., *Aspergillus* sp. And *Rhizopus* sp.)

Percentage Frequency of Fungal Species Associated with Selected spices

The percentage frequency of fungi isolated from the selected market spices is shown in the table. A total of 9 fungal isolates were obtained from the five spice samples. *Aspergillus* sp. was the most common fungus. It accounted for 6 isolates (66.66 %) of the total isolates. It was found in all 3 spices. Two isolates were obtained from cardamom, cinnamon, cumin.

Rhizopus sp. was the second most common fungus. A total of 2 isolates (22.22A%) were obtained. It was not found in cardamom.

It was present in cinnamon, cumin. *Penicillium* sp. was found only in cardamom. One isolate was obtained, which represented 6.67% of the total fungal isolates.

The results show that *Aspergillus* sp. was the predominant fungal genus in the selected spices. *Rhizopus* sp. was found less frequently, while *Penicillium* sp. showed the lowest occurrence. The high frequency of *Aspergillus* suggests that this genus was a major part of the fungal flora associated with the market-collected spices. Its presence in all the tested spices also indicates that *Aspergillus* can commonly survive or grow on dried spice products during handling and storage.

Table 2: % Frequency of the isolated fungi.

S no.	Name of the fungi	Number of fungi			% Frequency
		Cardamom: <i>Elettaria cardamomum</i>	Cinnamon: <i>Cinnamomum verum</i>	Cumin: <i>Cuminum cyminum</i>	
1	<i>Aspergillus</i> sp.	2	2	2	66.66%
2	<i>Rhizopus</i> sp.	0	1	1	22.22%
3	<i>Penicillium</i> sp.	1	0	0	11.11%

Discussion

The present study evaluated the moisture content, pH, and fungal flora associated with commercially available cinnamon (*Cinnamomum verum*), cardamom (*Elettaria cardamomum*), and cumin (*Cuminum cyminum*) collected from a local market in Jaipur, Rajasthan. Differences were observed in both moisture content and pH among the three spice samples. Cumin showed the highest moisture content (9.11%), followed by cinnamon (6.36%) and cardamom (5.15%). The pH ranged from 4.23 in cumin to 7.45 in cardamom. These differences may be related to the nature of the spices, processing methods, drying conditions, packaging, and storage practices.

Moisture content is an important factor affecting the stability of dried spices. Higher moisture availability can support the survival and growth of storage fungi, particularly when environmental humidity and storage

conditions are favourable. In the present study, cumin had the highest moisture content (9.11%) and also showed fungal contamination by both *Aspergillus* and *Rhizopus*. Mandeel (2005) ^[4] reported considerable variation in moisture content among commercially available spices and demonstrated the occurrence of several fungal genera in these products. The results support the importance of moisture control during drying and storage of spices.

The moisture content recorded in cardamom was the lowest (5.15%) among the three spices. Nevertheless, fungal growth was detected in the sample, with *Aspergillus*, *Rhizopus*, and *Penicillium* identified. This indicates that moisture content alone does not determine fungal contamination. Other factors such as water activity, storage duration, temperature, packaging, handling, and initial contamination can influence fungal survival. Similar observations have been reported in studies of commercially

available spices, where fungal occurrence varied considerably among products despite differences in physicochemical characteristics (Mandeel, 2005; Perrone *et al.*, 2018)^[4, 6].

The pH values also differed considerably among the spices. Cardamom showed the highest pH (7.45), whereas cumin had the lowest pH (4.23). Cinnamon showed an intermediate pH of 6.14. The occurrence of fungi across this pH range indicates that the isolated genera were able to survive under different physicochemical conditions. Fungi generally show considerable adaptability to environmental conditions, and pH should therefore be considered together with moisture and other storage factors when evaluating fungal contamination.

A total of nine fungal isolates were obtained from the three spice samples. The isolates represented three fungal genera: *Aspergillus*, *Rhizopus*, and *Penicillium*. *Aspergillus* was the predominant genus, with six isolates representing 66.67% of the total isolates. It was detected in all three spices. The predominance of *Aspergillus* observed in the present study agrees with earlier reports showing that this genus is frequently associated with dried spices. Mandeel (2005)^[4] reported *Aspergillus* among the major fungal genera isolated from imported spices. Similarly, Perrone *et al.*, (2018)^[6] reported frequent occurrence of *Aspergillus* species in commercially available spices and highlighted their ability to survive and grow under storage conditions.

The occurrence of *Aspergillus* in all three tested spices suggests that this genus may be well adapted to the conditions associated with dried spice products. The ability of some *Aspergillus* species to grow at relatively low water availability may contribute to their frequent recovery from dried food commodities. However, the present study identified the isolates only at the genus level based on cultural and microscopic characteristics. Therefore, it is not possible to determine whether the isolates belong to toxigenic species without further molecular identification.

Rhizopus was the second most frequent genus in the present study, accounting for 2 isolates (22.22%). It was detected in cinnamon and cumin but was not observed in cardamom. The occurrence of *Rhizopus* in dried spices has also been reported previously. Mandeel (2005)^[4] identified *Rhizopus* among the fungal genera associated with imported spices. Its presence in the present study indicates that storage fungi other than *Aspergillus* can also occur in commercially available spice products.

Penicillium sp. was detected only in cardamom and accounted for 1 isolate (11.11%). The lower frequency of *Penicillium* compared with *Aspergillus* and *Rhizopus* may be related to differences in the ability of these fungi to survive under the specific conditions of the sampled spice products. Previous studies have also reported *Penicillium* among the fungal contaminants of dried spices (Mandeel, 2005; Elshafie *et al.*, 2002)^[1, 4]. The detection of *Penicillium* in only one spice in the present study may also reflect the limited number of samples examined.

The fungal profile observed in the present investigation is comparable with previous studies of spices. Elshafie *et al.*, (2002)^[1] reported several fungal genera, including *Aspergillus*, *Penicillium*, and *Rhizopus*, from commercially available spices. Jeswal and Kumar (2015)^[3] also reported *Aspergillus* species as dominant fungal contaminants in Indian spices. These similarities suggest that *Aspergillus*,

Rhizopus, and *Penicillium* are commonly associated with dried spice products across different geographical regions.

The occurrence of *Aspergillus* is of particular interest because some species within this genus can produce mycotoxins. Jeswal and Kumar (2015)^[3] reported aflatoxins, ochratoxin A, and citrinin in some Indian spice samples and identified potentially toxigenic fungal species. Perrone *et al.*, (2018)^[6] also emphasized the mycotoxigenic potential of some *Aspergillus* species isolated from spices. The present study did not determine mycotoxin production, and the detection of *Aspergillus* sp. should not be interpreted as confirmation of mycotoxin contamination. Species-level identification and direct mycotoxin analysis would be required to establish such a relationship.

The presence of fungal contaminants in all three spice samples highlights the importance of proper post-harvest management. Spices may be exposed to fungal contamination during drying, processing, transportation, marketing, and storage. Maintaining appropriate moisture levels, using clean handling practices, and protecting products from excess humidity can help reduce fungal growth. Regular microbiological assessment of market-available spices can also provide useful information about their quality.

The present study has some limitations. Only three spice samples and a limited number of fungal isolates were examined. Identification was based on cultural and microscopic characteristics and was not confirmed using molecular techniques. In addition, mycotoxin production was not investigated. Future studies should include a larger number of samples from different markets and seasons, molecular identification of fungal isolates, and direct analysis of important mycotoxins.

In conclusion, the present findings demonstrate that cinnamon, cardamom, and cumin available in the local market can harbour different fungal genera. *Aspergillus* was the predominant fungus, followed by *Rhizopus* and *Penicillium*. The differences in moisture and pH among the spices indicate that physicochemical characteristics may contribute to differences in fungal occurrence. The findings emphasize the importance of proper drying, storage, and hygienic handling of dried spices to maintain their microbiological quality.

Conclusion

The present study confirmed the presence of fungal contaminants in commercially available cinnamon (*Cinnamomum verum*), cardamom (*Elettaria cardamomum*), and cumin (*Cuminum cyminum*) collected from a local market in Jaipur, Rajasthan. Moisture content ranged from 5.15% to 9.11%, while pH ranged from 4.23 to 7.45. A total of nine fungal isolates were obtained and identified as *Aspergillus* sp., *Rhizopus* sp., and *Penicillium* sp. *Aspergillus* sp. was the predominant fungus, accounting for 66.67% of the total isolates, followed by *Rhizopus* sp. (22.22%) and *Penicillium* sp. (11.11%). The occurrence of these fungi indicates that dried spices can carry fungal contaminants during processing, handling, and storage. Proper drying, hygienic handling, moisture control, and suitable storage conditions are important for maintaining the microbiological quality of spices. Further studies using molecular identification and mycotoxin analysis are recommended to determine the species-level identity and food-safety significance of the isolated fungi.

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