International Journal of Biotechnology and Microbiology
www.biotechnologyjournals.com
Online ISSN: 2664-7680
Received: 15-07-2026, Accepted: 14-08-2026, Published: 30-09-2026
Volume 8, Issue 3, 2026, Page No. 102-106

Isolation and identification of fungi associated with red chilli and black pepper

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DOI: https://doi.org/10.66856/ijbm.2026.8.3.8064

Abstract
Spices are commonly stored for long periods and may become contaminated with fungi during processing, handling, and storage. The present study investigated the moisture content, pH, and fungal flora associated with market-collected red chilli (*Capsicum annuum*) and black pepper (*Piper nigrum*). The moisture content of red chilli was 4.55%, while black pepper showed a higher moisture content of 6.84%. The pH values were 6.15 for red chilli and 5.73 for black pepper. Fungi associated with the spice samples were isolated on Potato Dextrose Agar (PDA) medium and identified based on cultural and microscopic characteristics. Three fungal isolates were selected from each spice and coded as CA1–CA3 for red chilli and PN1–PN3 for black pepper. *Aspergillus* sp. was identified among the isolates from both spices, while *Rhizopus* sp. was also detected in black pepper. Among the recorded fungal isolates, *Aspergillus* sp. showed the highest frequency (83.33%), whereas *Rhizopus* sp. accounted for 16.66%. The results demonstrate that commercially available dried spices can carry fungal contaminants and that differences in moisture content and pH may influence their microbial quality during storage. The presence of predominantly *Aspergillus* species highlights the need for proper drying, storage, and handling practices to maintain the quality and safety of dried spices.

Keywords: Spices, fungi, black pepper, red chilli etc

Introduction

Spices are important food commodities that are widely used to improve the flavour, aroma, colour, and palatability of food. Red chilli (*Capsicum annuum*) and black pepper (*Piper nigrum*) are commonly consumed spices and are mainly marketed in dried form. During harvesting, drying, processing, transportation, and storage, spices may become contaminated with microorganisms, particularly fungi. Such contamination can reduce product quality and may also create food safety concerns when toxigenic fungi are present (Mandeel, 2005; Jeswal and Kumar, 2015) [1, 2, 5, 7].

Moisture content is an important factor affecting the storage stability of dried spices. Improper drying or storage under humid conditions may provide favourable conditions for fungal growth. Mandeel (2005) [1, 5] reported considerable variation in moisture content among dried spices and found that red chilli and black pepper were among the heavily fungal-contaminated spices examined. The study identified several fungal genera, including *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium*, and *Trichoderma*. These findings indicate that the physicochemical characteristics of spices can influence their susceptibility to fungal contamination.

Several fungal genera have been reported from dried chilli and other spice products. *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, and *Rhizopus* are among the commonly reported fungi associated with chilli products (Paterson *et al.*, 2019) [3, 10]. Among these, *Aspergillus* is frequently reported as a dominant fungal genus. Jeswal and Kumar (2015) [2, 7] reported that *Aspergillus flavus* and *A. niger* were the most dominant fungal species isolated from different Indian spices, including red chilli and black pepper. Similarly, *Aspergillus* species were reported as the predominant fungi in dried chilli samples collected from markets (Wikandari *et al.*, 2020) [4, 9].

The occurrence of *Aspergillus* in spices is important because some species are capable of producing mycotoxins. Aflatoxins and ochratoxin A are important mycotoxins associated with food and agricultural commodities. Jeswal and Kumar (2015) [2, 7] reported aflatoxin contamination in red chilli and ochratoxin A contamination in black pepper, along with toxigenic *Aspergillus* isolates. In their study, *A. flavus* isolated from red chilli showed aflatoxin-producing potential, while *A. ochraceus* from black pepper showed ochratoxin A-producing potential. These findings indicate that the presence of fungal contamination in spices may have implications for food safety.

Black pepper can also harbour a diverse fungal community. Studies on commercially available spices have reported *Aspergillus*, *Penicillium*, *Rhizopus*, and other storage fungi in black pepper (Mandeel, 2005; Nordin *et al.*, 2022). Nordin *et al.*, (2022) [1, 5] reported a high incidence of fungal contamination in whole black pepper and identified *A. flavus* and *A. niger* among the frequently isolated fungi. The occurrence and frequency of fungi may vary according to spice type, geographical origin, moisture conditions, processing, and storage practices.

Fungal contamination has also been reported in market-collected dried chilli. Wikandari *et al.*, (2020) [4, 9] found *Aspergillus* infection in a high proportion of dried chilli samples obtained from traditional and modern markets. Similarly, a study of chilli and chilli powder from South Indian markets reported the presence of *A. flavus* and *A. niger* and highlighted the potential importance of fungal contamination in commercially available chilli products

(Reddy *et al*., 2022) [6]. These studies demonstrate the need for regular monitoring of fungal contamination in dried spices.

The pH of a food product is another physicochemical factor that can influence microbial growth. Along with moisture content, pH can provide useful information about the conditions present in a food product and its possible susceptibility to microbial contamination. Determination of moisture content and pH, together with isolation and identification of associated fungi, can therefore provide a basic assessment of the microbiological quality of dried spices.

Although fungal contamination of spices has been investigated in different geographical regions, the fungal profile of market-available spices may vary with location, storage conditions, handling practices, and product quality. Local studies are therefore important for documenting the fungal flora associated with commercially available spice samples. Cultural and microscopic identification provides a useful initial approach for detecting and characterizing fungal contaminants.

Therefore, the present study was undertaken to determine the moisture content and pH of selected red chilli (*Capsicum annuum*) and black pepper (*Piper nigrum*) samples, isolate the associated fungi using Potato Dextrose Agar (PDA) medium, and identify the fungal isolates based on their cultural and microscopic characteristics. The frequency of the isolated fungal genera was also determined to identify the predominant fungi associated with the selected spice samples.

Materials and methods

Collection of spices

Frequently utilized spices such as cinnamon, black cardamom, red chile, cumin, and black pepper 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) [1, 5].

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. Them 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) [1, 5].

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. black pepper showed 6.84% moisture while Red chili showed the lower moisture content (4.55%). The pH also varied among the spice samples. Red chili showed pH value 6.15 while black pepper had a pH of 5.73. 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	Red chili	4.55	6.15
2	Black Papper	6.84	5.73

Isolation of mycoflora associated with the collected spices

The selected spices were examined for the presence of fungal flora. The samples included red chilli (*Capsicum annuum*) and black pepper (*Piper nigrum*). 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 red chilli were coded as CA1, CA2, and CA3 while the black pepper isolates were coded as PN1, PN2, and PN3. The coding system used for the fungal isolates is given in Table 2.

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.

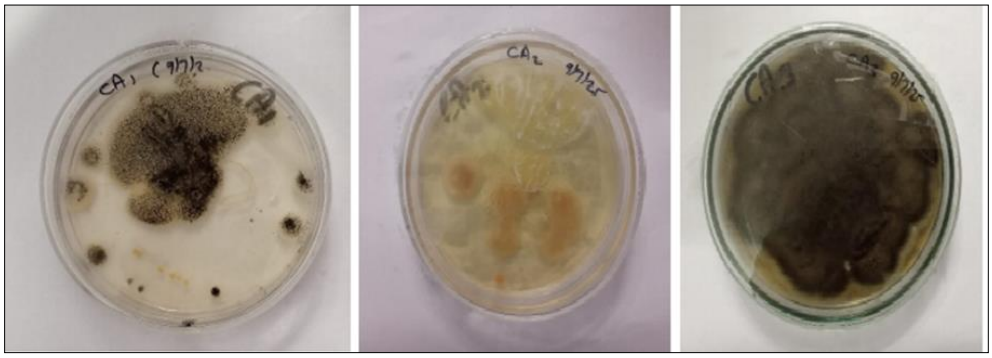
Cultural and Microscopic Identification of Isolated Fungi

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 fungal isolates from red chilli (*Capsicum annuum*) showed characteristics of *Aspergillus* sp. Similarly, the fungi isolated from black pepper (*Piper nigrum*) were identified as *Aspergillus* sp., *Rhizopus* sp., and *Aspergillus sp.*

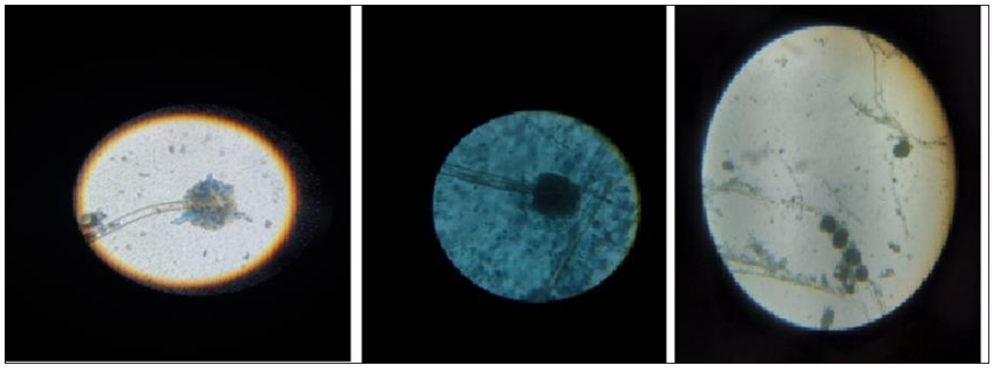
Among the tested spices, Aspergillus and Rhizopus were the most commonly observed fungal genera. *Penicillium* sp. was found only in cardamom. The cultural and microscopic features of the isolated fungi are shown in the respective photoplates. These results confirm the presence of different fungal contaminants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



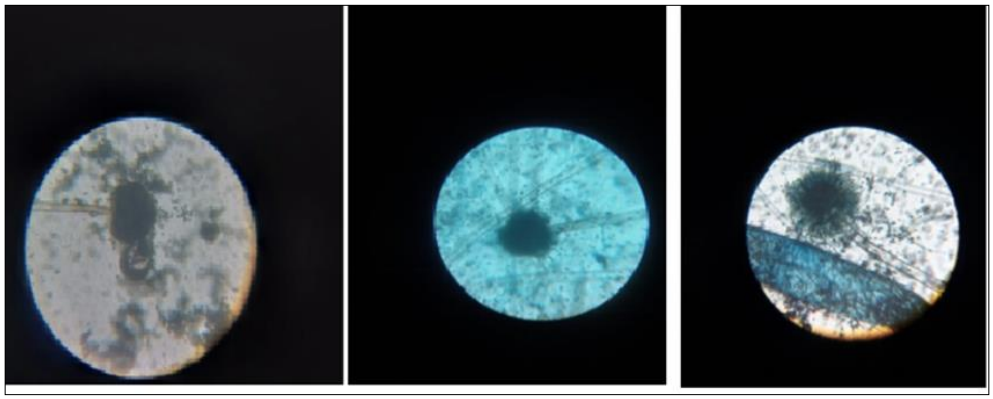
Photoplate 1: Morphological appearance of fungi isolated from *Capsicum annuum*.



Photoplate 2: Morphological appearance of fungi isolated from *Piper nigrum.*



Photoplate 3: Staining images of fungi isolated from *Capsicum annuum* (*Aspergillus sp*.)



Photoplate 4: Staining images of fungi isolated from *Piper nigrum (Aspergillus sp., Rhizopus sp. And Aspergillus 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 6 fungal isolates were obtained from the five spice samples. *Aspergillus* sp. was the most common fungus. It accounted for 5 isolates (83.33%) of the total isolates. It was found in both spices.

Rhizopus sp. was the second most common fungus. A total of 1 isolates (16.66%) were obtained. It was not found in cardamom or red chilli. It was present in cinnamon, cumin, and black pepper.

The results show that *Aspergillus* sp. was the predominant fungal genus in the selected spices. *Rhizopus* sp. was found less frequently. 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	% frequency of fungi		Total
		Red Chilli: *Capsicum annuum*	Black papper: *Piper nigrum*	
1	Aspergillus sp.	3	2	83.33
2	Rhizopus sp.	0	1	16.66

Discussion

The present study investigated the physicochemical characteristics and fungal contamination of market-collected red chilli (*Capsicum annuum*) and black pepper (*Piper nigrum*). The results showed differences in moisture content and pH between the two spice samples. Red chilli had a lower moisture content (4.55%) than black pepper (6.84%), while the pH of red chilli (6.15) was higher than that of black pepper (5.73). These differences may be related to the processing, drying, storage, and handling conditions of the spices. Moisture is particularly important for the microbiological stability of dried food products because increased moisture availability can support the growth of storage fungi (Mandeel, 2005) [1, 5].

The moisture content recorded in the present study was relatively low in both spice samples. Similar variation in moisture content among dried spices has been reported by Mandeel (2005) [1, 5], who also observed fungal contamination in commercially available spices. The higher moisture content recorded in black pepper compared with red chilli may provide relatively more favourable conditions for fungal survival and growth. However, fungal development is influenced by several factors, including water activity, temperature, storage duration, packaging, and environmental humidity. Therefore, moisture content alone cannot be considered responsible for fungal occurrence.

The pH values of the samples were 6.15 in red chilli and 5.73 in black pepper. Both samples were mildly acidic to near neutral. Food pH can influence the growth and survival of microorganisms, although many storage fungi are capable of growing over a relatively broad pH range. The differences observed between the two spices may be associated with their natural chemical composition and processing conditions. Similar variations in physicochemical properties among spice products have been reported in previous studies (Mandeel, 2005; Jeswal and Kumar, 2015) [1, 2, 5, 7].

Fungal growth was detected in both red chilli and black pepper samples during cultivation on Potato Dextrose Agar medium. This observation confirms that dried spices available in the market can harbour viable fungal propagules. Spices pass through several stages, including harvesting, drying, processing, transportation, marketing, and storage. Contamination may occur at any of these stages. Mandeel (2005) [1, 5] reported fungal contamination in several imported spices and identified *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium*, and other fungal genera. Such findings support the observation that dried spices can act as carriers of storage fungi.

The fungal isolates in the present study were identified using cultural and microscopic characteristics. The isolates obtained from red chilli were identified as *Aspergillus* sp., while the isolates from black pepper included *Aspergillus* sp. and *Rhizopus* sp. The occurrence of *Aspergillus* in both spices is consistent with previous studies that have identified this genus as an important component of the fungal flora associated with dried spices. Jeswal and Kumar (2015) [2, 7] reported *A. flavus* and *A. niger* as dominant fungal species in Indian spices, including chilli and black pepper.

The high frequency of *Aspergillus* sp. observed in the present study is an important finding. *Aspergillus* accounted for 83.33% of the recorded isolates, whereas *Rhizopus* sp. accounted for 16.66%. The predominance of *Aspergillus* is consistent with earlier reports of fungal contamination in dried spices. Mandeel (2005) [1, 5] reported *Aspergillus* among the frequently isolated fungi from imported spices. Similarly, Jeswal and Kumar (2015) [2, 7] found *Aspergillus* species to be dominant among fungi isolated from Indian spices. The repeated occurrence of *Aspergillus* in different spice products may be related to its ability to survive under relatively dry storage conditions.

The detection of *Rhizopus* sp. in black pepper also agrees with previous observations of storage fungi in dried spices. Mandeel (2005) [1, 5] reported *Rhizopus* among the fungal genera associated with commercially available spices. Although *Rhizopus* showed a lower frequency than *Aspergillus* in the present study, its occurrence indicates that more than one fungal genus can be associated with dried spice products.

The presence of *Aspergillus* in food commodities is of particular importance because some species within this genus can produce mycotoxins under suitable environmental conditions. Aflatoxins are mainly associated with species such as *A. flavus* and *A. parasiticus*, while some other *Aspergillus* species can produce ochratoxin A. Jeswal and Kumar (2015) [2, 7] reported aflatoxins and ochratoxin A in Indian spice samples and detected toxigenic fungal isolates. Similarly, Wikandari *et al.*, (2020) [4, 9] reported fungal contamination and mycotoxin-related concerns in dried chilli. The present study did not determine mycotoxin production, so the detection of *Aspergillus* in our samples should not be interpreted as evidence of mycotoxin contamination. Further studies using molecular identification and direct mycotoxin analysis would be required to establish this relationship.

The fungal contamination observed in the present study may also be related to post-harvest handling and storage conditions. Dried spices are often transported and stored for

extended periods before consumption. Poor ventilation, exposure to atmospheric humidity, inappropriate packaging, and repeated handling may increase the possibility of fungal contamination. Paterson *et al.,* (2019) [3, 10] highlighted the importance of environmental and processing conditions in fungal contamination and mycotoxin development in *Capsicum* products. Therefore, proper drying, moisture control, hygienic handling, and suitable storage conditions are important for maintaining the microbiological quality of dried spices.

The results of the present study also indicate the value of simple microbiological screening of commercially available spices. Cultural observation followed by microscopic examination can provide preliminary information about fungal contamination. However, morphological identification has limitations because closely related fungal species may show similar cultural and microscopic characteristics. Molecular methods such as ITS sequencing can provide more reliable species-level identification. This would be particularly useful for *Aspergillus* isolates because species-level identification is important when assessing potential food-safety risks.

In the present study, *Aspergillus* was the predominant fungal genus, while *Rhizopus* occurred at a lower frequency. The differences in moisture content and pH between red chilli and black pepper also demonstrate that the physicochemical characteristics of spices vary among products. The findings support the need for appropriate drying, storage, and handling practices to minimize fungal contamination. Further studies involving molecular identification, larger sample sizes, seasonal sampling, and direct analysis of mycotoxins would provide a more comprehensive assessment of the fungal and food-safety status of commercially available spices.

Conclusion

The present study confirmed the presence of fungal contaminants in commercially available red chilli (*Capsicum annuum*) and black pepper (*Piper nigrum*). Red chilli showed a moisture content of 4.55% and pH of 6.15, while black pepper showed 6.84% moisture and pH of 5.73. Fungal growth was observed on PDA medium, confirming that dried spices can carry viable fungal flora. Based on cultural and microscopic characteristics, *Aspergillus* sp. was detected in both spices, while *Rhizopus* sp. was also identified from black pepper. *Aspergillus* sp. was the predominant fungus, accounting for 83.33% of the recorded isolates, whereas *Rhizopus* sp. accounted for 16.66%. These findings highlight the importance of proper drying, hygienic handling, and suitable storage conditions for maintaining the microbiological quality of dried spices. Further studies using molecular identification and mycotoxin analysis are recommended to confirm the fungal species and determine their potential food-safety significance.

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