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RESEARCH ARTICLE

Mycoflora associated with black pepper in storage

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Abstract

Black pepper, known as the "King of Spices" and "Black gold," is a valuable spice native to India, commonly cultivated in tropical regions. However, it is prone to fungal growth and mycotoxin contamination, particularly during storage when moisture levels rise. Therefore a survey was conducted during July to December 2023, for identifying the mycoflora associated with black pepper in storage. Stored samples of the contaminated whole black pepper collected from pepper growers in 3 locations each from Thiruvananthapuram, Kozhikode, Idukki and Wayanad districts (Agro ecological units (AEU) 14, 15, 16 and 20) of Kerala, India. Twelve samples collected were examined to record the symptoms, then isolate the mycoflora associated with the samples and the prevalent contaminants present in it were characterized. The average moisture content of the samples was determined and found below 10 per cent for all the black pepper samples. Sixty one isolates of different fungi were isolated from the samples collected from different locations in Kerala. 14 isolates from Thiruvananthapuram, 16 from Kozhikode, 16 from Wayanad and 15 from Idukki were isolated from mouldy black pepper berries. Cultural and morphological studies of the 61 isolates were carried out and the isolates includes *Aspergillus* sp., *Penicillium* sp., *Syncephalastrum* sp., *Mucor* sp., *Colletotrichum* sp., *Helminthosporium* sp. Among the contaminants, one of the isolates of *Aspergillus* sp. observed from most of the samples collected was subjected to cultural, morphological and molecular characterization. Major contaminant associated with black pepper at storage was identified as *Aspergillus flavus*.

Keywords

Black pepper; Mycotoxin; Mycoflora storage; *Aspergillus* sp.; *Penicillium* sp.

Introduction

Black pepper (*Piper nigrum* Linn.), known as the "King of Spices" is a very valuable spice and medicinal crop of India. India is the most important producer of pepper accounting for about 50 per cent of the world production (1). Black pepper production in India is 64815.81 MT (2) of which 10-12% is exported. Kerala with an area of 82,761 ha under the crop produces 22000 MT (3) is a leading producer of the spice in India. It is cultivated across diverse agricultural environments in the state, spanning from sea level to high mountain ranges (4). In India, the anticipated loss of agricultural commodities after harvest falls between 20 and 50 per cent (5). The main cause of these losses is microbial contamination. Reports indicate that globally, 5-10 per cent of agricultural products are affected by mould contamination to the extent that they are unsuitable for consumption by human and animals (6).

The FAO in the United States found that about 25% of the world's food crops get contaminated by mycotoxins each year (7). The majority of mycotoxins are resistant to chemical and thermal treatments, making them difficult to eliminate during typical food processing procedures. Consequently, mycotoxins have emerged as a significant concern regarding the food safety standards necessary for the global trade of agricultural products intended for both human and animal consumption (8). Mycotoxins present a serious health risk, particularly in developing nations where poverty and malnutrition aggravate their effects by impairing the body's capacity to detoxify these harmful substances (9). Spices often get contaminated by mould and toxins because of how they're handled during processing (like harvesting, drying and storage) and because of the environment they're in. Hence, the quality deteriorates, and customer preference gradually decreases. A gradual reduction in essential oil and oleoresin content was observed in the black pepper. A particular concern is the presence of aflatoxins, which come from certain types of aflatoxigenic mould that can grow on spices (10). Because of the hot climate and improper storage, spices are highly prone to being contaminated with aflatoxins. These toxins are known to be harmful, causing mutations, birth defects and cancer. The fungi responsible for mycotoxin production areare fungi called *Aspergillus flavus* and *Aspergillus parasiticus*, which produce various types of aflatoxins such as aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) (11). In the EU, there are regulations that specify the permitted levels of aflatoxins in spices. The acceptable level for aflatoxin B1 (AFB1) is set at 5 μg/kg, and for the total amount of aflatoxins combined (AFB1 + AFB2 + AFG1 + AFG2), it's set at 10 μg/kg (12). Furthermore, the microbes found in spices are known to pose significant health risks, such as lung aspergillosis and mycoses. The tropical climate, characterized by high temperatures and humidity, combined with improper storage practices, negatively impacts the preservation of spices, cereal grains, oilseeds and similar products during storage (13).

Microbial spoilage is a significant issue in tropical and subtropical regions due to high humidity and temperature that promote mould growth. This leads to the quality deterioration of stored black pepper, resulting in a decline in its economic value in both domestic and global markets. Identifying the organisms responsible for deterioration is essential for its effective management. Therefore this study focused on identifying the mycoflora associated with black pepper during storage.

Materials and Methods

Sample Collection

A survey was conducted during the time period of July to December 2023, for collecting the stored samples of the whole black pepper from pepper growers in 3 locations each from Thiruvananthapuram (Agro ecological units (AEU) 14- Southern high hills), Kozhikode (AEU 15- Northern high hills), Idukki (AEU 16- Kumily high hills) and Wayanad districts (AEU 20- Wayanad central plateau) of Kerala, India for studying the mycoflora associated with the stored black pepper. Samples from 12 distinct sites were examined for visual symptoms and the contaminated ones were marked with specimen codes (Table 1). The type of storage and storage period of the black pepper samples were also recorded. The storage period of collected samples ranged from 1 to 2 years. The moisture content of all the collected samples were determined by moisture analyzer equipment (MX-50 Moisture Analyzer).

Isolation of Fungi

The collected berries were stored in sterile plastic bags during transportation to the laboratory. To isolate internally seed borne fungal contaminants from mouldy berries, the berries were surface sterilized with 1% sodium hypochlorite for approximately 30-60 seconds. Berries were then rinsed in three changes of sterile water. Subsequently, the berries were placed onto sterile Petri plates with potato dextrose agar (PDA) medium supplemented with Tagmycin (25 μg/mL) to prevent bacterial contamination. The plates were then incubated at 28 $\pm 2^{0}$C for 3-5 days. Fungal isolates were individually transferred to new PDA plates and subcultured twice by hyphal tip technique to ensure purity of cultures.

Table 1. Survey areas with specimen codes and GPS coordinates

Agro-Ecological Zone	Agro-Ecological Unit	District	Block	Panchayath	Specimen code	GPS Coordinates
High Hills	AEU 14 (Southern High Hills)	Thiruvananthapuram	Vamanapuram	Peringammala	14T1	8.4173°N, 77.0207°E
			Pothencode	Vithura	14T2	8.6753°N, 77.0852°E
			Pothencode	Aryanad	14T3	8.5785°N, 77.0852°E
	AEU 15 (Northern High Hills)	Kozhikode	Koduvally	Kodenchery	15K1	11.4323°N, 76.0073°E
			Perambra	Chakkittapara	15K2	11.5756°N, 75.8165°E
			Kunnummal	Narippatta	15K3	11°42′N, 75°42′E
	AEU 16 (Kumily High Hills)	Idukki	Azhutha	Kumali	16I1	9.6037° N, 77.1675° E
			Nedumkandam	Pampadumpara	16I2	9.7907800°N, 77.1578300°E
			Nedumkandam	Udumbanchola	16I3	9.8973° N, 77.1801° E
	AEU 20 (Wayanad Central Plateau)	Wayanad	Sulthan Bathery	Ambalavayal	20W1	11.6197°N, 76.2103° E
			Kalpatta	Muttil	20W2	11.6103°N, 76.0828° E
			Sulthan Bathery	Meenangadi	20W3	11.6596°N, 76.1726° E

Externally seed-borne fungi were isolated from mouldy berries without surface sterilization.

Cultural and morphological characteristics of the contaminants

A fresh culture of the associated organisms were grown in sterile petri dishes containing PDA media. A 5 mm mycelial disc from a seven day old culture of the contaminants were placed on the solidified sterile PDA medium and incubated at room temperature (28 ± 2°C). The growth pattern was monitored at 2, 5, 7 and 9 days after inoculation (DAI). Observations noted included cultural characteristics such as colony color, reverse plate color, culture margin, topography, zonation, substrate color and colony diameter (cm).

Morphological characters were observed using wet mount preparation and the slide culture technique outlined by Riddel (14). Glass rods, microscopic glass slides and coverslips were placed in a Petri plate with filter paper (9 cm) at the bottom. This slide culture unit was sterilized, then the filter paper was saturated with sterile water and glass slides were kept aseptically over the glass rods. 2% agar blocks were placed on the glass slide on which the contaminant was inoculated. Following inoculation, The coverslip was carefully placed over the agar piece after inoculation and incubated at room temperature (28 ± 2^{0}C). Observations were taken within 48-72 hours. A drop of lactophenol cotton blue (LPCB) stain was placed on a clean microscopic glass slide and the cover slip from the slide culture unit was placed on the drop of LPCB stain. The prepared slide was then examined under a compound light microscope (LEICA DM750) at 400X magnification. Detailed studies of the morphological characteristics, including the shape and size of vesicles, phialides, conidia, conidiophores, and seriation type were conducted using Leica LASEZ (version 3.4.0) imaging software.

Molecular characterization of the major contaminant

The molecular characterization of the major contaminant was done by using universal primers of ITS by the method of DNA barcoding. Fungal genomic DNA was isolated from 7-day-old mycelial mats grown in potato dextrose broth (PDB) medium. The DNA was extracted using a lysis buffer containing cetyl trimethyl ammonium bromide (CTAB) as described by Moller *et al.,* (15). The internal transcribed spacer (ITS) of the ribosomal RNA (rRNA) gene region in fungi was amplified using primers ITS-1F (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4R (5'-TCCTCCGCTTATTGATATGC-3').

Results

Sample Collection

Twelve samples of contaminated black pepper were collected with different period and type of storage. These samples were observed with crinkled, shriveled and wrinkled surfaces with white moldy growth, emitted a musty or earthy odor. Its moisture content was analyzed and values were in the range of 5.30- 7.22 per cent (Table 2).

Isolation of Fungi

A relatively high diversity of fungi was found among the analyzed black pepper samples. A total of 61 isolates were collected from twelve different locations in Kerala (Fig. 1a-1d). Among the 61 isolates, 23% isolates were from Trivandrum, 26% from Kozhikode, 26% from Idukki and 25% from Wayanad (Table 3). These isolates were purified using the hyphal tip technique. Morphological and cultural studies identified 11 of the 61 isolates as *Aspergillus flavus*, ten as *Aspergillus ochraceus*, seven as *Aspergillus niger,* seven as *Talaromyces pinophilus*, six as *Penicillium* sp., six as *Syncephalastrum* sp. and three as *Mucor* sp. (Table 3). Since the study primarily focused on major contaminant of black pepper samples, that is the isolate which is obtained from different location, *A. flavus* isolates was selected for further detailed molecular studies in order to confirm identity.

Table 2. Period, type of storage and moisture content present in the

Sample code	Period of storage	Type of storage	Moisture content (in percent)
14T1	2 years	Sack	$(7.03 \pm 0.02)^{b}$
14T2	1.5 years	Air tight container	$(7.08 \pm 0.03)^{b}$
14T3	1 year	Polythene bag	$(6.12 \pm 0.02)^{g}$
15K1	2 years	Sack	$(7.22 \pm 0.02)^{a}$
15K2	1 year	Polythene bag	$(6.16 \pm 0.05)^{fg}$
15K3	1 year	Polythene bag	$(6.20 \pm 0.04)^{f}$
16I1	1.5 years	Sack	$(6.91 \pm 0.04)^{c}$
16I2	1 year	Newspaper	$(6.83 \pm 0.06)^{d}$
16I3	1 year	Sack	$(6.96 \pm 0.04)^{c}$
20W1	2 years	Polythene bag	$(6.63 \pm 0.05)^{e}$
20W2	1 year	Air tight container	$(5.82 \pm 0.06)^{i}$
20W3	1.5 years	Sack	$(5.98 \pm 0.05)^{h}$
Healthy	1 year	Air tight container	$(5.30 \pm 0.02)^{j}$

Cultural and morphological characteristics of the contaminants

The colony of *A. flavus* exhibited a yellow-green color with a white margin, had regular borders and a flat topography. Slight zonation and hyaline pigmentation were observed in the substrate or medium. Vesicle were subglobose/ globose having 18-20 micro meter in diameter. Non-pigmented, unbranched conidiophore with size of 400-500 µm x 3-5 µm were observed. Conidia was yellowish to olive globose shaped having 4-5µm in diameter. Phialides were ampulliform in shape. Based on the cultural and morphological studies, major contaminant was identified as *A. flavus.*

Molecular characterization of the major contaminant

The CTAB method was employed to extract fungal DNA from the predominant contaminant. PCR amplification of the DNA samples was performed with ITS primers, yielding a 500 bp amplicon (Fig. 2). The PCR product was then sequenced using ITS primers. The obtained sequences of the major contaminant were analyzed using the NCBI BLASTn program and compared with reported isolates in the NCBI gene bank. The sequence comparison showed 99% homology with the *A. flavus* strain (OM 240729.1), confirming the identity of the major contaminant as *A. flavus*. A phylogenetic tree was constructed, grouping the major contaminant and the *A. flavus* strain (OM 240729.1) into a single main cluster, indicating a shared evolutionary lineage (Fig. 3). After completing the molecular

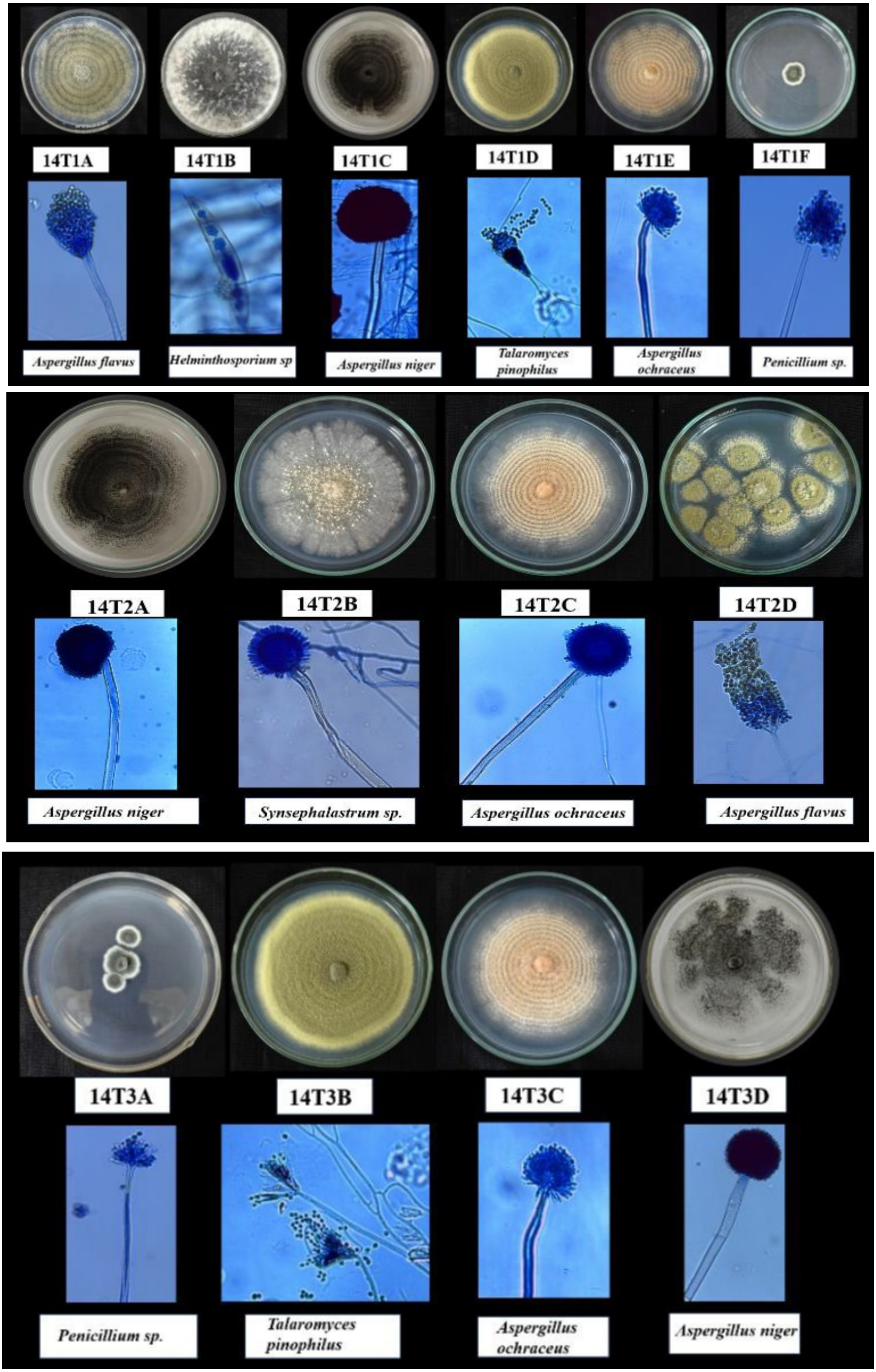

Fig. 1a. Different isolates obtained from the contaminated black pepper samples from Southern high hills and its microscopic images

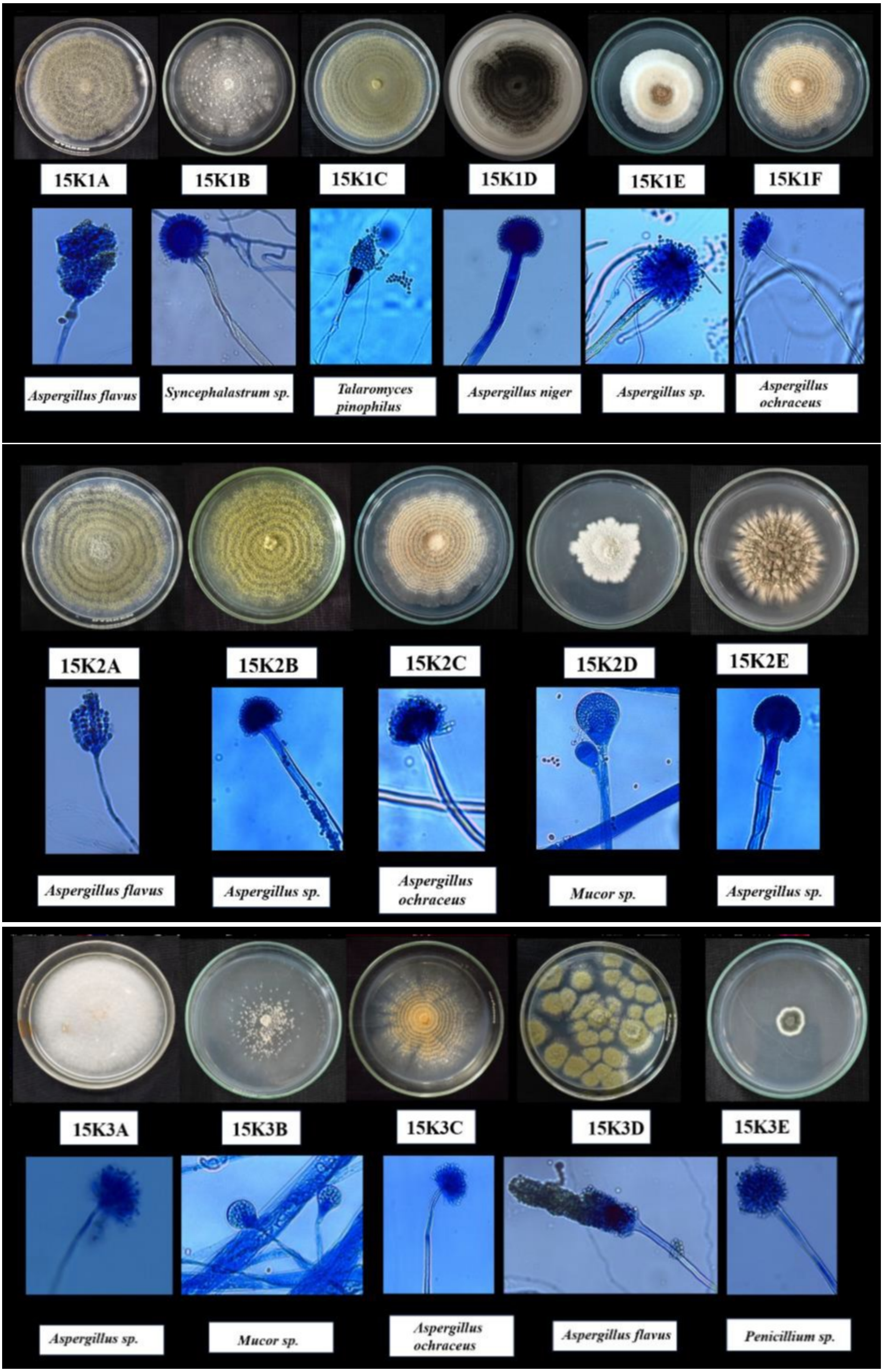

Fig. 1b. Different isolates obtained from the contaminated black pepper samples from Northern high hills and its microscopic images

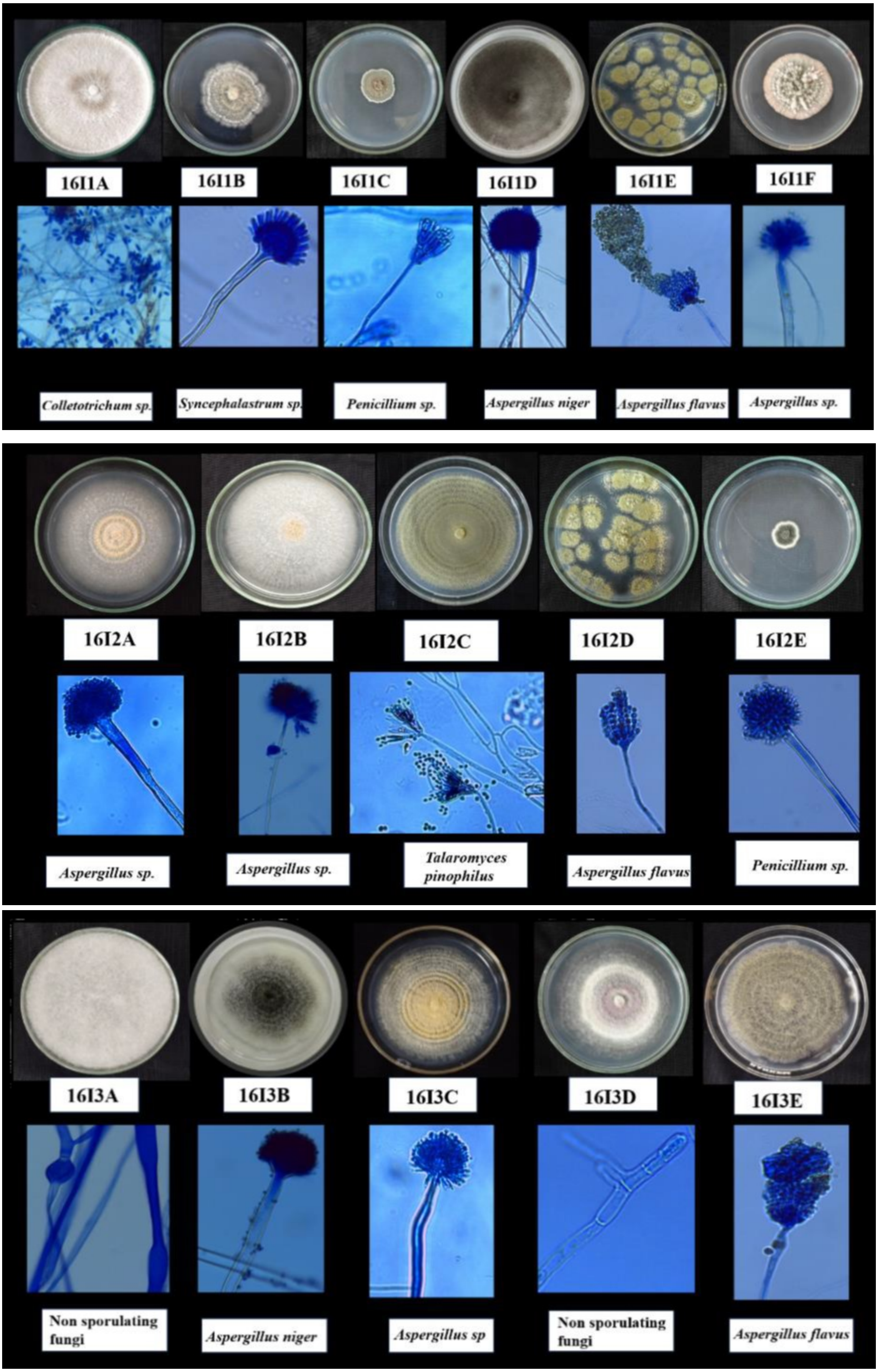

Fig. 1c. Different isolates obtained from the contaminated black pepper samples from Kumily high hills and its microscopic images

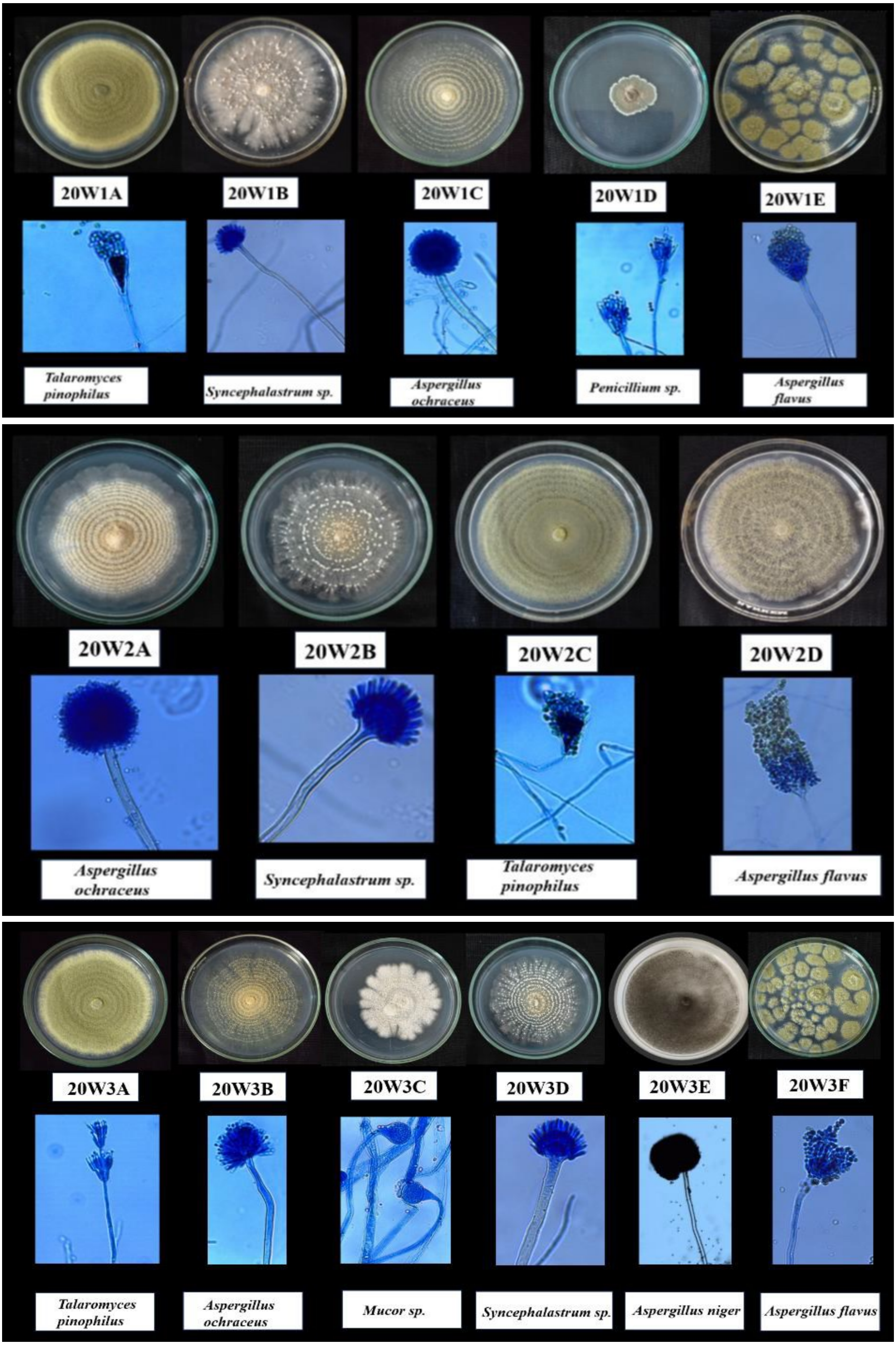

Fig. 1d. Different isolates obtained from the contaminated black pepper samples from Wayanad central plateau and its microscopic images

Table 3. Isolates obtained from the samples and its characteristics

Sample code	Isolate name	Identified characteristics
14T1	*Aspergillus flavus*	Yellow-green colony color having globose shaped yellowish to olive colour conidia
	Helminthosporium sp.	White and denser mycelium at centre and dark brown at periphery, hyphae was septate, pale brown to brown with branched, erect conidiophores bearing 27.5- 45.5× 6.5- 10 µm conidia
	A. niger	Abundant aerial mycelium with brown to black globose shaped conidial heads, 3.5 - 4.5 µm conidia
	Talaromyces pinophilus	Floccose to funiculose deep green colony with pale yellow margin, conidiophores borne from aerial hypha bearing subglobose conidia, metulae 8- 12 × 2.5- 3.5µm and phialides 9- 11× 2- 3µm present
	A. ochraceus	Golden yellow coloured colony with little aerial mycelium, Globose yellow to orange conidia
	Penicillium sp.	Flattened, velvety grayish green colonies with white towards the periphery, erect conidiophores bearing grayish green conidia having 3- 3.5× 3.5- 4 µm
14T2	*A. niger*	Abundant aerial mycelium with brown to black globose shaped conidial heads
	Syncephalastrum sp.	Fluffy, cottony white mycelium bearing sporangiophore with apical vesicles
	A. ochraceus	Golden yellow coloured colony with little aerial mycelium, Globose yellow to orange conidia
	A. flavus	Yellow-green colony color having globose shaped yellowish to olive colour conidia
14T3	*Penicillium* sp.	Flattened, velvety grayish green colonies with white towards the periphery, erect conidiophores bearing grayish green conidia having 3- 3.5× 3.5- 4 µm
	T. pinophilus	Floccose to funiculose deep green colony with pale yellow margin, conidiophores borne from aerial hypha bearing subglobose conidia, metulae 8- 12 × 2.5- 3.5µm and phialides 9- 11× 2- 3µm present
	A. ochraceus	Golden yellow coloured colony with little aerial mycelium, Globose yellow to orange conidia
	A. niger	Abundant aerial mycelium with brown to black globose shaped conidial heads
15K1	*A. flavus*	Yellow-green colony color having globose shaped yellowish to olive colour conidia
	Syncephalastrum sp.	Fluffy, cottony white mycelium bearing sporangiophore with apical vesicles
	T. pinophilus	Floccose to funiculose deep green colony with pale yellow margin, conidiophores borne from aerial hypha bearing subglobose conidia, metulae 8- 12 × 2.5- 3.5µm and phialides 9- 11× 2- 3µm present
	A. niger	Abundant aerial mycelium with brown to black globose shaped conidial heads
	A. ochraceus	Golden yellow coloured colony with little aerial mycelium, Globose yellow to orange conidia
15K2	*A. flavus*	Yellow-green colony color having globose shaped yellowish to olive colour conidia
	Aspergillus sp.	Cedar green to dark dull green colonies with long condiophores, globose to radiate conidial head bearing globose to subglobose conidia, globose vesicle and sterigmata present
	A. ochraceus	Golden yellow coloured colony with little aerial mycelium, Globose yellow to orange conidia
	Mucor sp.	White coloured colony showed submerged mycelia, globose to subglobose sporangia bearing sporangiospores and subglobose to pyriform columella present
	Aspergillus sp.	Yellow green tinge colonies with long conidiophores, small conidial head bearing globose to subglobose, echinulate conidia
15K3	*Aspergillus* sp.	Floccose white colony with long conidiophore, biseriate sterigmata, radiate conidial head bearing globose, echinulate conidia
	Mucor sp.	White coloured colony showed submerged mycelia, globose to subglobose sporangia bearing sporangiospores and subglobose to pyriform columella present
	A. ochraceus	Golden yellow coloured colony with little aerial mycelium, Globose yellow to orange conidia
	A. flavus	Yellow-green colony color having globose shaped yellowish to olive colour conidia
	Penicillium sp.	Flattened, velvety grayish green colonies with white towards the periphery, erect conidiophores bearing grayish green conidia having 3- 3.5× 3.5- 4 µm
16I1	*Colletotrichum* sp.	White to grey floccose aerial septate mycelium, cylindrical conidiophores bearing 11.5- 19.5 × 3-5 µm
	Syncephalastrum sp.	Fluffy, cottony white mycelium bearing sporangiophore with apical vesicles
	Penicillium sp.	Flattened, velvety grayish green colonies with white towards the periphery, erect conidiophores bearing grayish green conidia having 3- 3.5× 3.5- 4 µm
	A. niger	Abundant aerial mycelium with brown to black globose shaped conidial heads
	A. flavus	yellow-green colony color having globose shaped yellowish to olive colour conidia
	Aspergillus sp.	Yellow green tinge colonies with long conidiophores, small conidial head bearing globose to subglobose, echinulate conidia
16I2	*Aspergillus* sp.	Zonate, dull yellow orange colony with long conidiophores, globose conidial heads and adhering conidial chains with globose to subglobose conidia, globose vesicle and biseriate sterigmata present
	Aspergillus sp.	Floccose white colony with long conidiophore, biseriate sterigmata, radiate conidial head bearing globose, echinulate conidia
	T. pinophilus	Floccose to funiculose deep green colony with pale yellow margin, conidiophores borne from aerial hypha bearing subglobose conidia, metulae 8- 12 × 2.5- 3.5µm and phialides 9- 11× 2- 3µm present
	A. flavus	Yellow-green colony color having globose shaped yellowish to olive colour conidia
	Penicillium sp.	Flattened, velvety grayish green colonies with white towards the periphery, erect conidiophores bearing grayish green conidia having 3- 3.5× 3.5- 4 µm

	Non sporulating fungi	-
	A. niger	Abundant aerial mycelium with brown to black globose shaped conidial heads
16I3	*Aspergillus* sp.	Zonate, dull yellow orange colony with long conidiophores, globose conidial heads and adhering conidial chains with globose to subglobose conidia, globose vesicle and biseriate sterigmata
	Non sporulating fungi	-
	Aspergillus flavus	Yellow-green colony color having globose shaped yellowish to olive colour conidia
20W1	*T. pinophilus*	Floccose to funiculose deep green colony with pale yellow margin, conidiophores borne from aerial hypha bearing subglobose conidia, metulae 8- 12 × 2.5- 3.5μm and phialides
	Syncephalastrum sp.	Fluffy, cottony white mycelium bearing sporangiophore with apical vesicles
	A. ochraceus	Golden yellow coloured colony with little aerial mycelium, Globose yellow to orange conidia
	Penicillium sp.	Flattened, velvety grayish green colonies with white towards the periphery, erect conidiophores bearing grayish green conidia having 3- 3.5× 3.5- 4 μm
	A. flavus	Yellow-green colony color having globose shaped yellowish to olive colour conidia
20W2	*A. ochraceus*	Golden yellow coloured colony with little aerial mycelium, Globose yellow to orange conidia
	Syncephalastrum sp.	Fluffy, cottony white mycelium bearing sporangiophore with apical vesicles
	T. pinophilus	Floccose to funiculose deep green colony with pale yellow margin, conidiophores borne from aerial hypha bearing subglobose conidia, metulae 8- 12 × 2.5- 3.5μm and phialides 9- 11× 2- 3μm present
	A. flavus	Yellow-green colony color having globose shaped yellowish to olive colour conidia
20W3	*T. pinophilus*	Floccose to funiculose deep green colony with pale yellow margin, conidiophores borne from aerial hypha bearing subglobose conidia, metulae 8- 12 × 2.5- 3.5μm and phialides 9- 11× 2- 3μm present
	A. ochraceus	Golden yellow coloured colony with little aerial mycelium, Globose yellow to orange conidia
	Mucor sp.	White coloured colony showed submerged mycelia, globose to subglobose sporangia bearing sporangiospores and subglobose to pyriform columella present
	Syncephalastrum sp.	Fluffy, cottony white mycelium bearing sporangiophore with apical vesicles
	A. niger	Abundant aerial mycelium with brown to black globose shaped conidial heads
	A. flavus	Yellow-green colony color having globose shaped yellowish to olive colour conidia

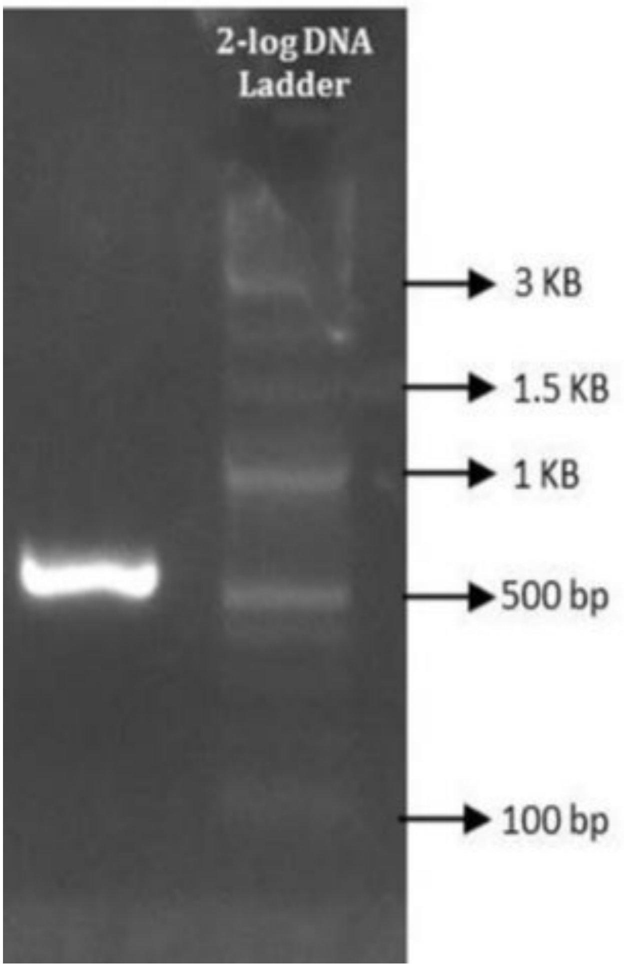

Fig. 2. Agarose gel electrophoresis image of PCR amplification of major contaminant of stored black pepper sample using ITS 1 and ITS 4

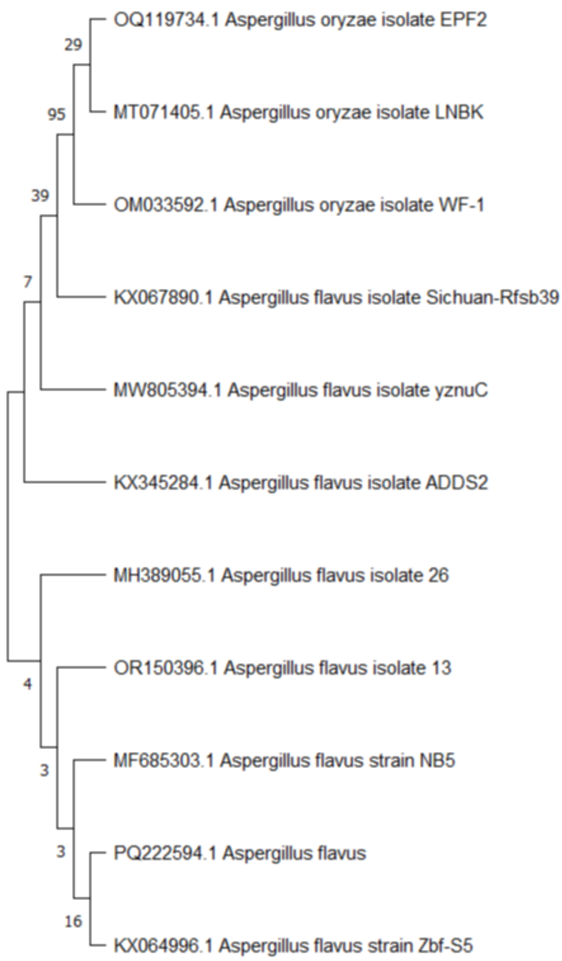

Fig. 3. Phylogenetic tree of *Aspergillus flavus* isolate

characterization, the isolate was deposited in the Genbank and the accession number was obtained as PQ222594.

Discussion

Spices are the major sources of foreign currency for India, which includes Black pepper, white pepper, cinnamon, clove, nutmeg, mace etc. Among these, black pepper makes up a significant portion. Mycotoxin contamination in spices impacts the international trade of these commodities. Even though India accounts for 50% of world production, it is true that there was very little information from India on mould infestation of spices by toxigenic fungi and the production of mycotoxins. Therefore, the current investigation on the mycoflora of stored black pepper was undertaken.

The results of the current study indicated that black pepper underwent deterioration and spoilage by various fungal contaminants during storage. Storage conditions also influenced the amount of mould growth in the samples. Sacks, polythene bags and airtight containers were generally used for storage. Samples stored in sacks showed a higher number of contaminants compared to those stored in other types of containers. Maintaining proper storage conditions to prevent mould growth helps to reduce quality deterioration. There was a correlation between the moisture content of the samples and the number of isolates. Higher moisture content favored increased mould growth.

Fungi such as *Aspergillus niger, A. flavus, A. ochraceus, Penicillium* sp.*, Syncephalastrum* sp.*, Mucor* sp.*, Talaromyces pinophilus*, *Colletotrichum* sp. were found commonly associated with black pepper samples. The morphological and cultural characteristics of major contaminant were examined by growing them on PDA medium and preparing wet mounts and microscopic slide cultures. Afzal *et al.* (16) studied about the morphology of *Aspergillus* and observed that conidial head of *A. flavus* usually have a radiate structure and measure between 250 and 350 µm in diameter. The cultural and morphological studies revealed that the conidiophore was colorless, coarsely roughened and is less than one millimetre in length, with a width of 8 to 12 µm and a wall thickness of 1 to 2 µm. The vesicle is subglobose to globose, with a diameter of 25 to 30 µm. The sterigmata are arranged in two layers. The metulae measure 5 to 8 µm in length and 3 to 4.5 µm in width. The phialides are ampulliform, measuring 6 to 10 µm in length and 3 to 4 µm in width. The conidia are either globose or subglobose and have a diameter of 3 to 4.5 µm in their study. Based on the cultural and morphological studies the major contaminant was identified as *Aspergillus flavus*. *Aspergillus flavus* was reported in black pepper (17), ginger (18) and cinnamon (19). Frimpong *et al.* (20) surveyed capsicum pepper for the occurrence of fungal flora, *A. flavus, A. niger*, *A. fumigatus*, *Penicillium citrinum*, *Fusarium solani* and *F. equiseti*. Mir *et al.* (21) reported that *A. flavus, A. niger*, *Rhizopus stolonifera*, *Penicillium arenicola* and *P. oxalicum* were the most common fungi associated with spoilage of turmeric, cloves, black pepper, cumin and cinnamon.

Aspergillus flavus was identified as a major contaminant since it was isolated from different locations with different agro ecological conditions from black pepper samples. The identity of the fungus was confirmed by molecular studies and the accession number is PQ222594. This fungus is capable of producing aflatoxins in stored products, with contamination being particularly favored by high moisture content, as moist conditions promote fungal growth. Optimal aflatoxin production occurs at 85% relative humidity, while 95% relative humidity significantly increases production (22). *A. flavus* can survive across a broad temperature range, with 28°C to 37°C being optimal for growth (23). Although aflatoxin production occurs over a wide temperature range, 25°C to 35° C is ideal (24). Generally, high temperatures lead to greater AFB production than AFG, while at low temperatures, both AFB and AFG are produced equally (25). Therefore, relative humidity, storage temperature and proper drying of agricultural products should be maintained to prevent mould infestation.

This study reveals that berries were readily invaded by storage fungi during storage, leading to loss of quality and degradation, which reduced their value for export, food and processing, thereby creating a significant economic loss for our country. Additionally, these fungi contributed to the airspora of spice storage facilities, potentially causing health hazards.

Conclusion

The primary focus of this study was to evaluate fungal contamination in black pepper, often regarded as the king of spices. Results indicated the fungal presence in all black pepper samples examined. The prevalent fungi species identified included *A. niger, A. flavus, A. ochraceus, Penicillium* sp.*, Syncephalastrum* sp.*, Mucor* sp.*, Talaromyces pinophilus*. Consequently, the study inferred that these spices harbor fungi, which could potentially produce mycotoxins, thereby compromising the quality of the spices. The research emphasizes the necessity to explore and adopt physical methods for managing fungal infections in spices to safeguard and improve their quality. The significant levels of fungal contamination and the strong mycotoxin-producing ability of the mycotoxigenic species found in this study necessitate effective food safety management practices for the production, processing and storage of black pepper berries before they are released to the local market.

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Authors' contributions

SST- Planned the experiment

JNB-Carried out the experiment and wrote the manuscript with the support from CBN, SGS

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest.

Ethical issues: None

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