



## Fungal Flora Associated with *Capsicum Annum* and Their Impact on Plant Vigor

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**C**hilli is among the world's most popular vegetables, belonging to the family Solanaceae. Fungi are eukaryotic microorganisms that are found almost everywhere and are associated with plants and the rhizosphere. Some fungal flora associated with Capsicum annum affect the plant's health, whereas others do not cause any visible symptoms. In this research, fungal flora was isolated from various parts of Capsicum annum plants, including the stem, root, fruit, leaves, and soil. Furthermore, morphological characterization was performed to confirm the fungal species associated with *Capsicum annum*. *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus paraciticus*, *Colletotrichum sp*, and *Alternaria alternata* were isolated and characterized in this study. However, the density of the fungal flora varied across different parts of the chilli plants and in the rhizospheric soil. Furthermore, the impact of the phyllosphere microbial populations on plant health, specifically chlorophyll content, was assessed under sterilized conditions. It was observed that total chlorophyll content was higher in the presence of a microbial community as compared to its absence. The presence of these fungi collectively affected the chlorophyll contents and showed a direct proportional relationship with fungal flora.

**Keywords:** Fungal Characterization, Chlorophyll Contents, Frequency, Endophytic Fungi, Capsicum Health, Leaf Microbial Community (LMC).

### Introduction:

*Capsicum annuum* Linn, commonly known as chilli, belongs to the family Solanaceae, one of the most important vegetable crops. In India, chilli is grown for both domestic consumption and export, making it a significant cash crop. Both domestic and export-oriented chillies are grown in India. [1]. Hot red pepper, red chilli pepper, and pod pepper are some of its common names. However, its cultivation is frequently affected by fungal pathogens that impact yield and post-harvest quality, making the study of associated fungal flora essential for disease [2]. Chilli pepper yields are still low and hardly exceed 1.5 tons/ha compared to a potential yield of 15 tons/ha [3]. Five of the approximately 31 species in the genus Capsicum, *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens* are domesticated. Average output and cultivated area of dry and green peppers worldwide, and the majority of commercially grown pepper cultivars worldwide are members of the species *C. annuum*, notwithstanding their wide range of traits.

Historically, chilli was primarily used as a flavoring agent and medicinal crop, but now its applications have expanded to include fresh and processed vegetables, dried forms,

utilized as food colorant, grown as an ornamental plant, and the manufacture of extracts for the pharmaceutical and cosmetics industry [4]. Before its culinary use, chilli was likely used for medicinal purposes due to its nutrient richness, because of its abundance and variety of nutrients. Since ancient times, the general public has been able to gain knowledge about the utilization of these plants. Chilli is used not only in cooking but also as a raw material in various industries such as the pharmaceutical industry, pesticides, and others. However, chili production often drops drastically due to disease [5]. Climate change-induced alteration in composition and activities of plant microbiomes can affect host functions (warming and drought) on plant–microbiome interactions and on their ecological functions from genome to ecosystem scales. The plant microbiota consists of different microorganism bacteria, fungi, protists, nematodes, and viruses that inhabit different plant tissues that are accessible. The microbiome and the host plant develop intricate and dynamic relationships that inhabit the soil, rhizosphere, roots, and other plant parts.

The comprehension of ecological principles that influence the construction and function of microbiomes in response to climate change will enhance our comprehension of the characteristics of microbiomes, such as resilience and resistance, that enhance plant fitness in new climatic settings. Microbes vary in their metabolism, physiology, and susceptibility to moisture and temperature [1]. Due to its susceptibility to bacterial, fungal, and viral diseases, *Capsicum* production has declined significantly. The major fungal diseases are Die Back/ Anthracnose, Cercospora leaf spot, Phytophthora Leaf Blight and Fruit Rot, Fusarium Wilt, Powdery Mildew, and Damping-Off [6]. The present study was designed to assess the total fungal flora associated with different portions of Chilli plants under field conditions and their impact on plant health, particularly chlorophyll contents in plant leaves. This study aims to develop a future step towards sustainable management of chilli, that how different combinations of microbial communities affect plant health.

### **Literature Review:**

*Capsicum annuum L.*, or chili pepper, is an important horticultural crop worldwide, prized for its food, medicinal, and economic value. Its productivity and general vigor, though, depend significantly on interactions with numerous microbial populations, such as fungi that colonize its rhizosphere, phyllosphere, and endosphere. Fungal flora of *C. annuum* can assume two functions, acting either as growth-suppressing pathogens or as functional organisms that enhance plant vigor by assuming numerous direct and indirect modes of action. Fungi infecting *C. annuum* can vary from plant diseases such as wilts, leaf spots, root rots, and damping-off diseases caused by *Fusarium spp.*, *Colletotrichum spp.*, *Alternaria spp.*, and *Rhizoctonia solani* [7] to plant growth-promoting endophytes and rhizospheric fungi such as *Trichoderma spp.* and *Penicillium spp.* that enhance plant growth through the process of nutrient solubilization, production of phytohormones, and systemic resistance induction [8].

Several studies have recorded the detrimental effects of pathogenic fungi on plant vitality. For instance, vascular wilting and severe yield reduction, particularly under high temperatures and humidity, are caused by *Fusarium oxysporum* f. sp. *capsici* [9]. *Colletotrichum truncatum* causes fatal defoliation, fruit rot, and early mortality in chili as its causal agent [10]. The extensive spread of these pathogens in various ecological regions is a perpetual threat to chili crop production. On the other hand, certain valuable fungal species are in the limelight for their contribution towards sustainable crop management. Endophytic fungi, *Trichoderma harzianum*, have been found to improve seed germination, root growth, and biomass production in *Capsicum annuum* by mechanisms such as mycoparasitism, competition, and inducing plant defense mechanisms [11]. In addition, the rhizospheric fungal population enhances plant strength through enhanced nutrient acquisition, particularly phosphorus solubilization by *Aspergillus niger* and *Penicillium sp* [12]. On a broader scale, the composition of fungal communities concerning chili plants is determined by several factors such as soil type,

climate, cultivation practices, and growth stages of the plant [13]. The description of fungal assemblages within the various compartments of a plant's roots, stems, leaves, and fruits are reflective of their ecological functions. Root-associated fungi, for example, are mostly implicated in nutrient transfer and disease management, while phyllosphere fungi may mediate plant stress processes [14].

New technologies in metagenomics and molecular biology have revolutionized the functional profiling and identification of fungal microbiomes of crops such as *Capsicum annuum*. These analyses have shown that microbial composition and diversity are positively correlated with plant health, with healthy plants typically having an increased proportion of disease-promoting fungi and a reduced prevalence of pathogen species [15]. Understanding the equilibrium between pathogenic and health-promoting fungal flora of *C. annuum* is of paramount importance for identifying integrated disease management practices as well as enhancing plant strength. Fungal bio-inoculants, crop rotation, as well as organic manure, are promising methods to shift the balance of the structure of fungi in the interest of plant health [16].

### Novelty Statement:

This research is the first to extensively isolate and describe fungal communities from various tissues and rhizospheric soil of *Capsicum annuum* in field conditions using a morphological method. Interestingly, it points to a direct positive association between the phyllosphere existence of fungi and higher chlorophyll content in sterilized conditions, which implies a possible plant growth-promoting activity of these fungi. The detection of several *Aspergillus* species and other pathogenic and non-pathogenic fungi from across the plant parts provides new information on the spatial distribution and functional importance of the fungal flora of chilli farming, providing novel points of view for crop management using microbiome.

### Objectives:

This research aims to explore the diversity, distribution, and functional significance of fungal flora on *Capsicum annuum* and assess their effects on plant vigor. Fungi on different parts of chilli plants, including roots, stems, leaves, and fruits, are capable of behaving as pathogens, endophytes, affecting plant growth and well-being in a variety of ways. The current study seeks to isolate, identify, and quantify both epiphytic and endophytic fungal species from various *C. annuum* tissues, determine their colonization patterns, and examine their possible impacts on plant vigor, such as content of chlorophyll and growth performance.

### Methodology:

#### Survey and Sample Collection:

A survey was conducted for the collection of chilli samples from the soil, stem leaves, and fruit of various chilli plants from the field of the Faculty of Agricultural Sciences (FAS), University of the Punjab, Lahore, during 2022. Roots and soil samples were collected at least 20-25 cm deep for investigation of the fungal community. Equivalently, mature leaves and young to mature chillies were collected. Different chilli samples were taken to the laboratory in separate polythene bags and then stored at 4°C in a refrigerator until the isolation of fungi.

#### Isolation of Fungal Flora:

Malt Extract Agar (MEA) plates were prepared by using the following composition: 20 g agar, 20 g malt extract, and 1000 mL distilled water for the isolation of chilli fungal flora [17]. For the unsterilized method, small fragments of chilli stem, root, leaf, and fruit (about 2 × 2 mm) were cut aseptically and inoculated onto the media Plates. For surface sterilized methods, samples were sterilized with 0.1 % sodium hypochlorite solution [18] and incubated for 7 days at 25±2 °C. For the isolation of fungal flora from soil samples, two methods were used: one was soil spread, and the second was serial dilution methods [19]. Purification of isolated fungal species was performed when multiple fungi appeared; the cultures were then

incubated for 7 days at  $25\pm2^{\circ}\text{C}$  then pure cultures were stored at  $4^{\circ}\text{C}$  for macroscopic and microscopic characterization of fungal flora.

### Morphological Characterization:

Microscopic and macroscopic characters were observed by using a stereomicroscope and a compound microscope. Morphological identification of isolated fungal flora was carried out by using a fungal dichotomous key [20].

### Microbial Community and Total Chlorophyll:

*Capsicum annum* healthy leaves were cut into different pieces and placed in a centrifuge tube, and completely immersed in  $\text{MgCl}_2$ . The centrifuge tube was sonicated in a sonicator for 15 min at  $25^{\circ}\text{C}$ , then vortexed for 10s. To increase the recovery of microbial cells from the phyllosphere, a second sonication step was performed to dislodge tightly adhered microbes that may not be removed by the initial washing. From both tubes, leaflets were then discarded and again centrifuged for 3 min at  $6000 \times g$  to pellet cells, and 25ml supernatant was removed from both tubes and combined in a new centrifuge tube for microbial community. Supernatant was added to a spray bottle with  $\text{MgCl}_2$  buffer and 0.1% Tween 80. [21]. Two *Grewia asiatica* plants were selected. One *Grewia asiatica* plants were sprayed with  $\text{MgCl}_2$  buffer for control, and one was sprayed with leaf inoculum under sterilized conditions. After this, check the chlorophyll content of the leaf.

To check the chlorophyll content, the *Grewia asiatica* leaves were collected individually from two plants. In a pestle mortar, fresh chilli leaves of 1 g were macerated, 5 mL of 80% acetone solution was mixed with 3mL ethanol, and fill this mixture in 10 mL tubes, then stirred for 2 min. This mixture was left for 35 min, then centrifuged for 8 min at 1000 rpm. At the wavelengths of 663 nm and 645 nm, absorbance readings were performed [22].

The obtained values were put in the given formula.

$$\text{Chlorophyll a (mg/g)} = 12.7(\text{OD A663}) - 2.69(\text{OD A645})$$

$$\text{Chlorophyll b (mg/g)} = 22.9(\text{OD A645}) - 4.68(\text{OD A663})$$

$$\text{Chlorophyll total (mg/g)} = 8.02(\text{OD A663}) + 20.2(\text{OD A645})$$

### Analysis of Fungal Community Composition and Diversity Metrics:

Fungal diversity metrics were measured on the basis of the number of isolated fungal species recovered from various segments of the chilli plant. Diversity indices such as Species Richness (S), Shannon-Wiener Diversity Index ( $H'$ ), Simpson's Index of Diversity ( $1 - D$ ), and Evenness (E) were computed by using standard equations. The similarity indices were employed for examining the variety and range of fungal flora species in every plant part, which was used to measure the similarity between fungal communities of various plant parts and calculated with the following formula.

$$\text{SSI} = 2C / (A+B) \quad (1)$$

### Results:

The fungal flora isolated from *Capsicum annum* were: *Aspergillus fumigatus*, *A.parasiticus*, *A.flavus*, *Alternaria alternata*, *Aspergillus terreus*, *Aspergillus niger*, and *Colletotrichum* spp. Members of the Ascomycota family, such as *Aspergillus* spp., are found in large numbers on or around the plant *Capsicum annum* parts as compared to other families of fungi. Ascomycota, sometimes also called sac fungi or ascomycetes, is the biggest phylum of fungi, with over 64,000 species. Ascomycota fungi play a critical role in carbon and nitrogen cycling, soil stability, plant biomass degradation, and endophytic interactions in arid environments. Ascus, or ascospores, is a reproductive structure found in almost all ascomycetes. Most of them are terrestrial, parasitic, unicellular, and multicellular fungi. Mycelium consists of septate and branched hyphae, with a cell wall composed of chitin. A total of 7 isolates belonging to 3 genera: *Aspergillus*, *Alternaria*, and *Colletotrichum*.

## Percentage Frequency of Isolated Fungal Flora from Various Parts of Chilli:

Fungal flora distribution was examined in 5 samples collected from different parts of the chilli plant, that is, leaves, stems, fruit, roots, and rhizospheric soil. Fungal flora identified in chilli plant parts included *Aspergillus fumigatus*, *A.parasiticus*, *A.flavus*, *Colletotrichum* sp, *A.niger*, and *Alternaria alternata*, with different occurrence frequencies. The percentage frequency of *A. flavus* and *A.fumigatus* was highest at 80% followed by *A.parasiticus* and *A.niger* (60%) and *A. terreus* and *Colletotrichum* sp (40%), and *Alternaria alternata* (20%), as shown in Table 1.

**Table 1.** Distribution of isolated fungal species from different parts of chilli and their relative frequency of occurrence.

Sr. no.	Isolated fungal flora			No. of isolated species			Total isolates	f	$\chi^2$
		Leaf	Stem	Root	Fruit	Soil			
1	<i>Aspergillus fumigatus</i>	1	0	1	1	1	4	80%	1
2	<i>Aspergillus terreus</i>	0	0	0	1	1	2	40%	3
3	<i>Colletotrichum</i> sp	0	0	0	1	0	1	40%	4
4	<i>Aspergillus niger</i>	0	1	0	1	1	3	60%	2
5	<i>Aspergillus parasiticus</i>	0	1	1	0	1	3	60%	2
6	<i>Aspergillus flavus</i>	1	1	1	1	0	4	80%	1
7	<i>Alternaria alternata</i>	1	0	0	0	0	1	20%	4
	Total						18		
		p = 0.34					df = 9		

## Morphological Characterization of Isolated Fungal Flora:

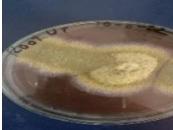
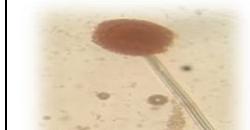
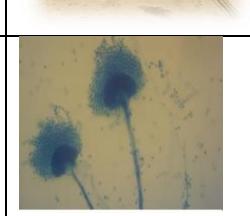
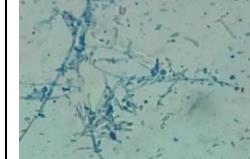
Morphological characterization of isolated fungal flora involves the visible features such as colony color, texture, shape, and growth pattern, as well as microscopic features like spore type, hyphal structure, and reproductive structures were observed as shown in Table 2.

## Analysis of Fungal Species Diversity Across Different Chilli Parts:

A total of seven fungal flora was isolated, with varying presence across the leaf, stem, root, fruit, and soil. Among these, *Aspergillus flavus* and *A. fumigatus* were the most widespread, each colonizing four different plant parts, indicating their adaptability and ecological dominance. The fruit and soil samples exhibited the highest species richness (S = 5 and S = 4, respectively) and total number of isolates (N = 5 for both), reflecting high fungal load and diversity in these environments. In contrast, the leaf, stem, and root samples showed lower species richness (S = 3), with fewer isolates and more uneven species distributions. Diversity indices further illustrated this variation. The fruit had the highest Shannon diversity index ( $H' = 1.609$ ) and evenness ( $E = 1.0$ ), indicating not only the presence of multiple fungal species but also a balanced distribution among them. Soil also showed high diversity ( $H' = 1.386$ ;  $E = 0.998$ ), followed by the stem ( $H' = 1.099$ ), root ( $H' = 0.918$ ), and leaf ( $H' = 0.636$ ). Simpson's index (1-D) echoed similar trends, with the fruit and soil again ranking highest.

Pairwise comparison using the Sørensen similarity index (SSI) revealed moderate similarity between plant parts, with the highest average similarity observed between fruit and soil ( $SSI \approx 0.70$ ), suggesting potential overlap in fungal colonization sources or environmental interactions. Lower similarity values were observed between the leaf and other parts ( $SSI \approx 0.45$ ), indicating a more unique fungal community on the leaf surface. Overall, these results highlight both shared and distinct fungal communities across chilli plant parts, with soil and fruit serving as key reservoirs of fungal diversity Table 3.

**Table 2.** Macroscopic and microscopic characteristics of fungal species isolated from chilli.

Isolated fungus	Macroscopic morphology	Picture	Microscopic morphology	Picture
<i>A. fumigatus</i>	Velvety appearance and first white, then turning dark gray with a narrow white border.		Conidiophores are Short, smooth, and phialides are uniseriate and only on the upper two-thirds of the vesicle and parallel to the axis of the conidiophore.	
<i>A. flavus</i>	Velvety in appearance and yellow to green in color.		Conidiophores are variable in length, rough, spiny, and Phialides are uniseriate and biseriate and also cover the entire vesicle.	
<i>A. parasiticus</i>	Reverse uncolored dull yellow to dull green shades; colonies floccose, not dense.		Stipe colorless, vesicular, uniseriate, spherical, conidia surface rough	
<i>Alternaria alternata</i>	Colonies filamentous, grey, dark brown, or black		Conidiophores are single to small groups and sometimes geniculate, with scars. <u>Conidia</u> ovoid and obclavate,	
<i>A. niger</i>	Initially white, quickly becoming black with conidial production.		Hyphae septate hyline Conidial head radiat. Metulae and phialides cover the entire vesicle. Conidia are brown to black, very rough, globose, and measure 4 $\mu\text{m}$ in diameter.	
<i>A. terreus</i>	Conidia are pale brownish orange to yellow centrally. Pale orange to greyish orange mycelium, white, usually inconspicuous reverse in greyish yellow shades.		Conidial heads in compact columns, stipes smooth-walled walled uncolored, spherical or pyriform, biseriate, tightly backed phialides, vesicular, wide	
<i>Colletotrichum sp</i>	Colonies are typically grayish-white with a cottony		Conidia were cylindrical, conidiophores straight, and contained branched hyphae.	

**Table 3.** Diversity Indices and Sorenson Similarity of Isolated Fungal flora from various parts of chilli.

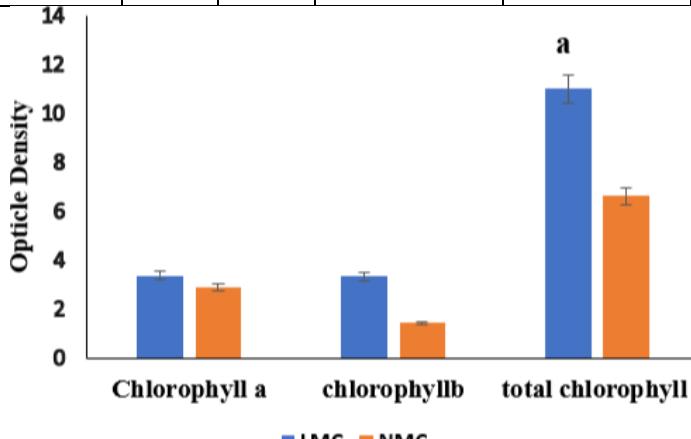
Plant Part	S (Species Richness)	N (Total Isolates)	H' (Shannon Index)	Simpson (1-D)	Evenness (E)	Avg. Sørensen Similarity (SSI)
Leaf	3	3	1.099	0.667	1.0	0.446
Stem	3	3	1.099	0.667	1.0	0.518
Root	3	3	1.099	0.667	1.0	0.601
Fruit	5	5	1.609	0.8	1.0	0.542
Soil	4	4	1.386	0.75	1.0	0.524

**Effect of Leaf Microbial Community on Chlorophyll Content:**

Optical Density (OD) value for LMC at 663nm was 0.31, and at 645nm was 0.21. Chlorophyll a was 3.37, and chlorophyll b was 3.35 total chlorophyll content was 11. On the other hand, the OD value for the NMC at 663nm was 0.252, and at 645nm was 0.114. Chlorophyll a was 2.89, and chlorophyll b was 1.43 total chlorophyll content was 6.62. As shown in Table 4, the total chlorophyll was higher in the LMC treatment (11) as compared to the NMCs treatment (6.62), demonstrating a significant increase of approximately 66.2%. These results suggest that LMC treatment enhances chlorophyll biosynthesis or retention, potentially contributing to improved photosynthetic efficiency in treated samples. Therefore, plants with leaf communities had high chlorophyll content as compared to those plants that were not sprayed with the leaf microbial community, and had a positive impact on the plant growth.

**Table 4.** Optical density values and chlorophyll content in LMC and no microbial community (NMC) treatments of chilli.

Sr.no	Treatments	OD (645)	OD (663)	Chlorophyll a	Chlorophyll b	Total chlorophyll
1	LMC	0.21	0.31	3.37	3.35	11
2	NMC	0.114	0.252	2.89	1.43	6.62



**Figure 1.** Comparison of chlorophyll a, chlorophyll b, and total chlorophyll content in chilli under leaf microbial community (LMC) and no microbial community (NMC) treatments. Error bars represent standard deviation. Different letters above bars indicate statistically significant differences at  $p \leq 0.05$  as determined by Tukey's Test.

**Discussion:**

In this study, the finding is that fungal flora associated with chilli mostly belongs to the family Ascomycota. In the present study, seven different fungal flora were isolated, which were associated with the chilli plant. According to reports, a large number of the isolated fungi are pathogenic to the seeds of many crops, resulting in foliar diseases, fruit root, root rot, damping off, seed rot, and wilt. *Aspergillus digitatum* isolated *Aspergillus niger* and *Aspergillus*

flavus from chilli fruits [23]. In the recycling of nitrogen and carbon, the saprophytic fungus *Aspergillus fumigatus* performs a vital role in the environment [24]. Its natural biological niche is in the soil, where it lives and grows on organic debris. This species of fungus is among the most widely distributed on Earth because of its flying conidia, even though it is not the most prevalent type [25]. Numerous bioactive compounds have been reported to be produced by *A. fumigatus*. Several secondary metabolites derived from endophytic *A. fumigatus* have been identified. Microorganisms are composed of biosynthetic gene clusters that regulate secondary metabolite synthesis. Significant attempts have been undertaken recently to increase the chemical diversity of microbial cultures. In this study, the other isolated fungi were *Aspergillus parasiticus*.

Aflatoxin-producing *A. parasiticus* is a plant pathogen that causes liver cancer. Aflatoxin B1, B2, G1, G2, and mycotoxins are strong carcinogens produced by this fungus. Aflatoxin B1 is thought to be the most powerful naturally occurring carcinogen of them all. The fungus is typically found in soil, where it contributes to the deterioration of plant matter. It may also opportunistically colonize both people and animals [26]. Another isolated fungus was *Aspergillus Flavus*. The genus *Aspergillus* includes more than 100 species, the majority of which are known to grow well on conventional synthetic or semi-synthetic medium, and around 50 of which have been demonstrated to generate hazardous compounds. *Aspergillus flavus* is one of these species. *Aspergillus flavus*, which dates back to 1806, is a well-known and distinct species that belongs to the *Aspergillus* sect. *Flavi*. It has close kinship with *A. parasiticus*, *A. oryzae*, and *A. sojae*, the latter two of which are crucial to Asia's production of fermented foods. *Alternaria alternata* was also included in isolated fungal flora. *Alternaria alternata* is the fungus that causes numerous plant diseases, including rots, blights, and leaf spots. It is an opportunistic pathogen that affects more than 380 plant species as hosts. This study showed that *Aspergillus terreus* and *Aspergillus niger* were associated with chilli plants. In many recent studies, the pathogenic effect of the fungal pathogens has been reported.

This fungus also belongs to the genus *Aspergillus* and *Colletotrichum* spp. It is concluded that the fungal flora associated with chilli affects the plant health. Molecular characterization confirms the presence and frequency level in the soil. The fungal community of chilli plants is crucial for determining plant physiology, nutrient acquisition, and biotic and abiotic stress resistance. Chilli crops are regularly colonized by Ascomycota phylum fungi with both beneficial endophytes and pathogens. Endophytic fungi such as *Aspergillus terreus* and *Trichoderma harzianum* were demonstrated in various studies to increase capsaicin content, plant growth, and disease resistance [27]. Nonetheless, pathogens like *Aspergillus flavus* also infect the chilli crop under opportunistic conditions to cause stunted growth, wilting, or rotting of the fruits [28]. Thus, the functional importance of these species is strongly context-dependent, and ecological and molecular research are necessary to distinguish between beneficial and deleterious strains in the mycobiome of chilli.

### Conclusion:

This work gives a detailed description of fungal floras inhabiting various tissues and rhizospheric soil of *Capsicum annuum* under natural conditions. Successful isolation and identification of various species of fungi, such as multiple *Aspergillus* spp., *Colletotrichum* sp., and *Alternaria alternata*, indicate the diversity and occurrence of mycobiota in chilli plants. Notably, the existence of these fungal communities, especially within the phyllosphere, was found to have a positive effect on chlorophyll content, which suggests their possible role in enhanced plant health. These results highlight the importance of plant-associated fungal flora and introduce possibilities for investigating useful microbial interactions in sustainable chilli production and crop management practices.

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