

Impact of Rhizospheric and Phyllospheric Mycobiota on Plant Health of Tomato

Beenish Rasheed¹, Uzma Bashir¹, Karamat Ali Zohaib¹, Umara Mushtaq¹, Adnan Akhter¹, Waheed Anwar¹

¹Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan.

* Correspondence. Waheed Anwar; waheedanwar.dpp@pu.edu.pk

Citation | Rasheed. B, Bashir. U, Zohaib. K. A, Mushtaq. U, Akhter. A, Anwar. W, "Impact of Rhizospheric and Phyllospheric Mycobiota on Plant Health of Tomato", IJIST, Vol. 7 Issue. 2 pp 802-816, May 2025

DOI | <https://doi.org/10.33411/ijist/202572802816>

Received | April 16, 2025 **Revised** | May 05, 2025 **Accepted** | May 12, 2025 **Published** | May 14, 2025.

Tomato (*Solanum lycopersicum*) is a significant crop produced globally but suffers from numerous biotic and abiotic stresses when cultivated in fields. Among all the biological stresses, fungal diseases cause a sharp decline in yield and quality but may remain non-pathogenic and symptomless under certain fungal species throughout the plant's entire life cycle. This work aimed to isolate and purify the mycobiota from various parts of the tomato plant—stem, root, fruit, leaf, and rhizospheric soil—to determine the fungal communities present. Morphological and molecular identification established the presence of various fungal species, including *Aspergillus fumigatus*, *Acremonium* spp., *Pythium* spp., *Geotrichum candidum*, *Aspergillus parasiticus*, *Aspergillus carbonarius*, *Aspergillus terricola*, *Aspergillus flavus*, *Aspergillus oryzae*, and *Alternaria alternata*. The density and distribution of these fungi varied among different plant parts and soil, with *A. fumigatus* showing the highest frequency (80%) among all isolates. Fungal diversity analysis revealed notable differences in species richness and evenness across plant parts. The rhizospheric soil showed the highest fungal diversity (Shannon index = 2.31), followed by roots (2.05), while the leaf and fruit tissues exhibited lower diversity indices. The Simpson's index values also confirmed greater dominance and lower evenness in aboveground plant parts, indicating a more selective fungal colonization. A heat map was constructed to visually compare diversity metrics across plant parts. Moreover, the effect of microbiomes on tomato plant health, especially on chlorophyll content in the field, was also examined. The results indicate that tomato plant mycobiota play a positive role in plant health based on their interaction. Further studies need to be conducted to investigate the specific possible positive impact of individual fungal species and their interactive effect on plant health of tomato crops.

Keywords. Mycobiome, Fungal Identification, Chlorophyll Levels, Endophytes, Tomato.



Introduction.

Tomato (*Solanum lycopersicum*), a member of the Solanaceae family, is the second most widely cultivated vegetable crop after potato, largely due to its high nutritional value. It is grown globally because of its adaptability to diverse soil types and climatic conditions. However, tomato crops are susceptible to various biotic and abiotic stresses, as well as attacks from insect pests. Nearly more than 200 diseases have been reported on tomato plants globally which can be caused by various pathogens [1]. Interactions between organisms play a crucial role in shaping their distribution, community assembly, and functional roles within microbial ecosystems [2]. Plant-microbe interactions in agriculture increase plant productivity by facilitating nutrient uptake, activating factors that promote plant development, and tolerance to abiotic stressors [3]. The establishment of microbial communities in plants is shaped by a complex network of interactions occurring both above and below ground, involving the plants themselves, their surrounding environment, and associated microbes [4].

The plant mycobiome, consisting of fungi that colonize the host, engages in limited gene exchange with the plant and tends to develop the ability to produce bioactive secondary metabolites [5]. The microbiota of the rhizosphere and phyllosphere have been the subject of numerous published investigations. There are distinct and overlapping microbial pools in both plant compartments. Numerous microorganisms, including bacteria, viruses, fungi, algae, protists (nematodes, protozoa), and archaea, are found in the phyllosphere. According to [6], leaf surfaces with an oligotrophic environment that fosters interactions between microbes from the phyllosphere. As plant symbionts and soil decomposers, fungi are a common and incredibly diverse collection of organisms that are important to ecological and biogeochemical processes [7]. Plant health is attributed to the quantity of fungal variety, which may be measured using high throughput sequencing in conjunction with genetic markers [8].

The current work is to investigate the morphological and molecular characterization of microflora isolated from *Solanum lycopersicum*, their frequency of occurrence and pattern of distribution over various parts of the plant, determination of chlorophyll content and ascertain their effect on tomato plants.

Novelty Statement.

This study is the first to provide a comprehensive isolation and characterization of tomato-associated mycobiota from multiple plant tissues and rhizospheric soil under field conditions in the region. It uniquely highlights the predominance of *A. fumigatus* and suggests a potential beneficial role of endophytic and rhizospheric fungal communities in promoting plant health, particularly by enhancing chlorophyll content offering new insights into sustainable crop management through microbiome manipulation.

Material and Methods.

Investigation site.

The study was conducted in the field of the Faculty of Agricultural Sciences (FAS), University of the Punjab, Lahore, located at a latitude of 31°29'42"N, longitude of 74°17'49"E, and an elevation of 229 meters above sea level. It has a subtropical climate with monsoon rains (628.8 mm yearly), moderate winters (as low as 7°C), and hot summers (up to 39°C). The soil is alluvial and fertile, with a range of loamy to clayey, and the humidity is higher in the summer (around 74%).

Survey and Sample Collection.

Multiple surveys were carried out to collect the samples including the rhizospheric soil, roots, stem leaves, and fruit during different stages of Tomato plants. The study was conducted over six months starting in April 2022, using random sampling. Ten samples were collected from each part of the tomato plant, including root, stem, leaf, fruit, and

rhizospheric soil. Tools such as sterile forceps and scalpels were used for sample collection, while fungal identification was carried out using stereomicroscopy, culturing on PDA media, and PCR-based molecular techniques. Chlorophyll content was measured using a spectrophotometer. The samples were obtained from the field of the Faculty of Agricultural Sciences (FAS), University of the Punjab, Lahore, located at 31°29'42"N latitude, 74°17'49"E longitude, and an elevation of 229 meters above sea level. It has a subtropical climate with monsoon rains (628.8 mm yearly), moderate winters (as low as 7°C), and hot summers (up to 39°C). The soil is alluvial and fertile, with a range of loamy to clayey, and the humidity is higher in the summer (around 74%). Roots and surrounding soil at least 15-20 cm deep were targeted for investigation of the fungal community. Similarly, fresh emerging leaves as well as mature leaves above 5cm from the bottom were targeted. Young to mature red fruits were collected from plants. Multiple samples were collected, carried to the lab separately in polythene bags, and stored at 4 in the refrigerator till the isolation of fungi.

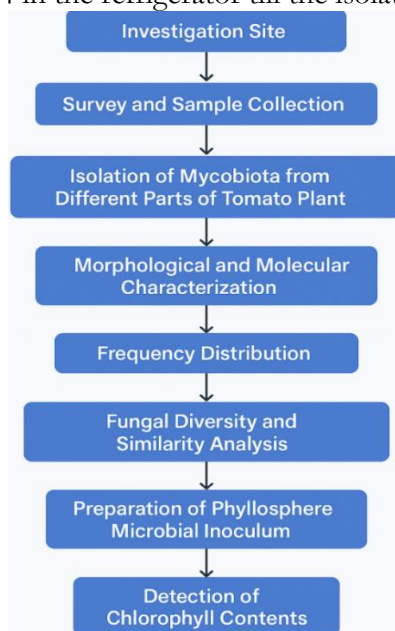


Figure 1. Flowchart of Methodology Diagram.

Isolation of Mycobiota.

For the isolation of mycobiota, Malt Extract Agar (MEA) media plates were prepared by using the composition (20g Agar, 20g Malt Extract, 1000g Distilled-water. Small pieces of tomato stem, root, leaf, and fruit (approximately 2 × 2 mm) were cut aseptically and inoculated onto MEA media plates as unsterilized samples. The same samples were surface disinfected using a 1% sodium hypochlorite [9] and incubated at 25±2 °C for 7 days for sterilized samples. For the isolation of fungi from rhizospheric soil, soil spread and serial dilution methods were used in the extraction of fungi from soil samples [10]. Purification of the isolated fungal species was carried out when mixed fungal colonies were observed. The cultures were incubated at 25 ± 2°C for 7 days. Pure Petri plates were stored at 4°C for morphological and molecular identification of fungi.

Morphological and Molecular Characterization.

Macroscopic characteristics like the color, texture, margins, pigmentation, concentric rings, and mycelium of the colonies were studied using a stereomicroscope. In microscopic identification, hyphal septation, texture, structure of conidia, shape, and size were studied under a compound microscope. Morphological identification was carried out by using a fungal dichotomous key Compendium of soil fungi [11]. For molecular characterization, total genomic DNA was extracted from all isolated fungal strains using a modified CTAB method as described by [12]. The integrity of the DNA was additionally verified using 0.8%

agarose gel electrophoresis. The ITS (Internal Transcribed Spacer) region of isolated fungal species was amplified using universal ITS primers [ITS1 forward (TCCGTAGGTGAACCTGCGG) and ITS4 reverse (TCCTCCGCTTATTGATATGC)] as outlined in previous studies [13]. The PCR reaction was conducted using following the protocol described by [14] The PCR products were sequenced, and sequence homology was analyzed using the NCBI online tool <https://www.ncbi.nlm.nih.gov/>.

Fungal Diversity and Similarity Analysis.

Fungal diversity was assessed based on the number of isolates obtained from different parts of the tomato plant (leaf, stem, root, fruit, and rhizospheric soil). Diversity indices including Species Richness (S), Shannon-Wiener Diversity Index (H'), Simpson's Index of Diversity ($1 - D$), and Evenness (E) were calculated using standard formulas. These indices were used to evaluate the composition and distribution of fungal species within each plant part.

The Sørensen Similarity Index (SSI) was applied to determine the degree of similarity between fungal communities of different plant parts. The index was calculated using the formula.

$$SSI = 2C / (A+B)$$

where C is the number of species common to both samples

A and B are the total number of species in each sample.

Average SSI values were also computed for each plant part against all others to assess overall similarity.

Heat Map Construction.

A heat map was generated using GraphPad Prism to visualize diversity indices across plant parts. The dataset included species richness (S), total isolates (N), Shannon index (H'), Simpson's index ($1-D$), evenness (E), and Sørensen similarity index (SSI). Data were entered in matrix format, with plant parts as rows and diversity metrics as columns. Color gradients represented relative values, aiding comparison among samples.

Preparation of Phyllosphere Microbial Inoculum.

The tomato leaves were placed into the falcon tubes with $MgCl_2$ buffer, and the sonication process was repeated to maximize microbial cell recovery from the phyllosphere [15]. Following this, the leaves were discarded, and the contents of both tubes were centrifuged at $7,197 \times g$ for 2 minutes to pellet the microbial cells. The supernatant, containing noncellular components like bacteriophages and other small organisms that don't pellet during centrifugation, was carefully removed, leaving 1 ml of the pelleted cells combined with 25 ml of supernatant from each tube, and the mixtures were pooled into a new 50-ml tube. The resulting supernatant (50 ml) was then mixed with 50 ml of 10 mM $MgCl_2$ and 0.001% Silwet L-77 (Bioworld, Dublin, OH, U.S.A.) in a spray bottle and this solution was subsequently sprayed onto tomato plants. Two treatments were applied to tomato plants in this study. The first group, labeled as the 'Sprayed Treatment,' was treated with a fungal inoculum spray, whereas the second group, termed the 'Control Treatment,' was left unsprayed. After 5 days of treatment, the chlorophyll contents were checked under a spectrophotometer.

Detection of Chlorophyll Contents.

Leaves from both the 'Control' and 'Sprayed' treatments were collected to measure chlorophyll content. The leaves were ground in 15 mL of 80% acetone to create a fine paste or solution. The mixture was then centrifuged at 12,000 rpm for 5 minutes to allow the leaf debris to settle. The clear supernatant was carefully transferred to a cuvette, and absorbance was measured spectrophotometrically at wavelengths 665, 649, and 470 nm. Chlorophyll a, b, and carotenoids were evaluated using the following formula [16].

$$Chl\ a\ (mg/l) = 12.72 (A_{663}) - 2.59 (A_{645})$$

$$\text{Chl b (mg/l)} = 22 \cdot 88 (A_{645}) - 4 \cdot 67 (A_{663})$$

$$(\text{Total Chlorophyll content (mg/l)}) = \text{Chl a} + \text{Chl b}$$

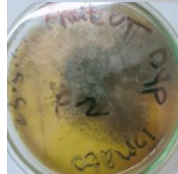
Result and discussion.

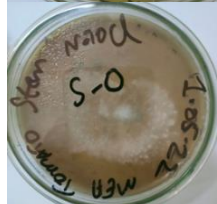
A total of 10 distinct fungal species associated with tomato plants were isolated on MEA media plates from various plant parts. Their morphological characterization was carried out by examining both macro and micro features, including the structure of their vegetative growth, reproductive structures, spores, and conidia. A comparison of the morphological and microscopic characteristics of each species from different genera is presented in Table 1.

Table 1. Isolated fungal flora from different parts of tomato plants.

Sr. No	Sample	Isolated fungal Species	Family
1	Soil	<i>Aspergillus fumigatus</i>	Ascomycota
		<i>Acremonium</i> sp.	Ascomycota
		<i>Pythium</i> sp.	Oomycota
		<i>Geotrichum candidum</i>	Ascomycota
		<i>Aspergillus parasiticus</i>	
		<i>Aspergillus oryzae</i>	
2	Root	<i>Aspergillus fumigatus</i>	Ascomycota
		<i>Aspergillus parasiticus</i>	
3	Fruit	<i>Aspergillus carbonarius</i>	Ascomycota
		<i>Aspergillus terricola</i>	
4	Stem	<i>Aspergillus flavus</i>	Ascomycota
		<i>Aspergillus oryzae</i>	
		<i>Aspergillus parasiticus</i>	
5	Leaf	<i>Alternaria alternate</i>	Dothideomycetes
		<i>Aspergillus fumigatus</i>	Ascomycota
		<i>Aspergillus carbonarius</i>	
		<i>Aspergillus flavus</i>	
		<i>Aspergillus oryzae</i>	

Table 2. Macroscopic and Microscopic Features of Fungi for Identification.

Fungal species	Macroscopic morphology	Microscopic morphology	Pictures
<i>A.fumigatus</i>	Powdery or velvety, Initially white, thereafter dark greenish to gray having a narrow white border, followed by the reversal to tan.	Conidiophores.Short smooth(<300µm) Phialides. uniseriate, typically only on upper upper two-thirds of the vesicle, parallel to the conidiophore axis.	
<i>A.flavus</i>	Velvety, yellow to green or brown, with a reverse that ranges from gold to reddish-brown.	Conidiophores. irregularly variable in length, coarse, pitted, and spiny. Phialides. biseriate and uniseriate, enveloping the whole vesicle and protruding in every direction.	
<i>A.oryzae</i>	Reverse uncolored or dull yellow; colonies floccose.	Stipe uncolored, rough surface, vesicle serration uniseriate, glucose spherical, conidia surface smooth.	
<i>A.parasiticus</i>	Reverse uncolored or in dull yellow to dull green shads; colonies floccose, not dense.	Stipe colorless, vesical uniseriate, spherical, conidia surface rough.	
<i>A.carbonarius</i>	Conidia black and uncrowded, reverse uncolored to slightly yellow, mycelium inconspicuous or as a white basal flat.	Conidial heads radiate, thick-walled, smooth to finely roughened, stipes uncolored or brownish near apices, vesicles spherical, biseriate, setulae over the entire vesicle surface, measuring.	

A. terricola	Colonies are fast-growing, dense, and velvety to floccose in texture. The reverse side is often pale yellow to light brown. Colonies are generally circular with well-defined margins. Droplets of clear or yellowish exudate may be present on the surface.	Vesicles are globose to subglobose, conidiophores are 300 to 600µ long by 6 to 8µ in diameter, and their walls seem granulate and uncolored. Conidial heads loosely radiate.	
Geotrichum candidum	White- to cream-coloured colonies are smooth and often membranous. White, dry, powdery to cottony colonies.	Chains of hyaline, smooth, one-celled, subglobose to cylindrical arthroconidia, measuring 6-12 × 3-6 µm, are formed when hyaline septate hyphae break apart.	
Alternaria alternata	Colonies filamentous, grey, dark brown, or black, velvety with club-shaped conidia in chains on septate hyphae.	Conidiophores are single or arranged in small clusters, straight or curved, occasionally geniculate, measuring 3–6 µm × 20–50 µm, with visible scars. Conidia are ellipsoidal, ovoid, or obclavate in shape.	
Acremonium sp.	Slow-growing colonies produce white, velvety colonies with thin, unbranched conidiophores and hyaline, one-celled conidia.	Mycelia awl-shaped, erect shielded from substratum or fasciculate aerial hyphae, conidia one-celled, hyaline or pigmented, in slimy heads or dry chains.	
Pythium sp.	Hyphae of Pythium species, similar to other members of the Pythiaceae family, are typically distinguished by the formation of coenocytic hyphae, which lack septations.	Oogonia typically house a single oospore, while antheridia feature an elongated, club-shaped structure.	

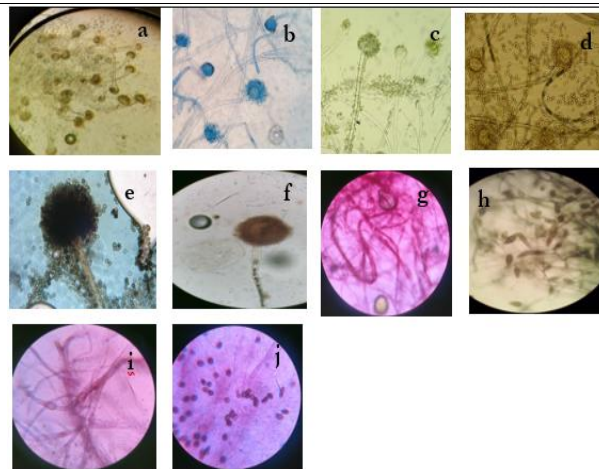


Figure 2. a) *A. fumigatus* b) *A. flavus* c) *A.oryzae* d) *A.paraciticus* e) *Acarbonarius* f) *A.terricola* g) *Geotrichum candidum* h) *Alternaria alternate* i) *Acremonium* sp. j) *Pythium* sp.

The isolated fungal flora from *Solanum lycopersicum* were; *Aspergillus fumigatus*, *Acremonium* sp., *Pythium* sp., *Geotrichum candidum*, *A.parasiticus*, *A.carbonarius*, *A.terricola*, *A.flavus*, *A.oryzae*, *Alternaria alternate*. Members of the Ascomycota phylum, particularly species of *Aspergillus*, were found in greater abundance on or around various parts of the tomato plant compared to fungi from other families.

The detection of Ascomycota in both below-ground (soil and root) and above-ground (stem, leaf, and fruit) parts of the tomato plant highlights their potential role in the tomato microbiome. These results highlight the ecological importance of Ascomycota fungi in tomato agroecosystems and their possible impact on plant health and productivity.

Molecular Identification of Fungal Isolates.

The fungal isolates were identified based on sequencing of the PCR-amplified products, followed by nucleotide homology analysis using the NCBI BLASTn tool. All sequences showed 100% query coverage and 100% identity with reference sequences available in the GenBank database, indicating a high level of confidence in the species identification. The BLASTn results revealed that the isolates belonged to a diverse group of fungal genera, including *Aspergillus*, *Alternaria*, *Geotrichum*, *Acremonium*, and *Pythium*. Among the identified species, *Aspergillus fumigatus* exhibited the highest Max Score (1306), followed by *Pythium* sp. (1386), and *Aspergillus oryzae* (1138). A complete list of BLASTn results, including Max Score, Total Score, Query Coverage, E-value, Percentage Identity, and GenBank Accession Numbers, is presented in Table 3.

Table 3. BLASTn results of PCR-sequenced fungal isolates showing sequence similarity with reference sequences in the GenBank database.

Description	Max. Score	Total Score	Query Value	E Value	Per. Ident	Accession
<i>Aspergillus flavus</i>	1096	1096	100%	0.0	100.00%	KX852295.1
<i>Aspergillus fumigatus</i>	1306	1306	100%	0.0	100.00%	OR939714.1
<i>Aspergillus ooze</i>	1138	1138	100%	0.0	100.00%	NR_135395.1
<i>Aspergillus parasiticus</i>	977	977	100%	0.0	100.00%	NR_151784.1
<i>Aspergillus carbonarius</i>	1118	1118	100%	0.0	100.00%	NR_111094.1
<i>Aspergillus terricola</i>	1005	1005	100%	0.0	100.00%	NR_151785.1
<i>Alternaria alternate</i>	1125	1125	100%	0.0	100.00%	JN122073.1
<i>Pythium</i> sp.	1386	1386	100%	0.0	100.00%	NC_027966.1
<i>Geotrichum candidum</i>	701	701	100%	0.0	100.00%	KU176111.1

Acremonium sp.

898

898

100%

0.0

100.00%

KT878347.1

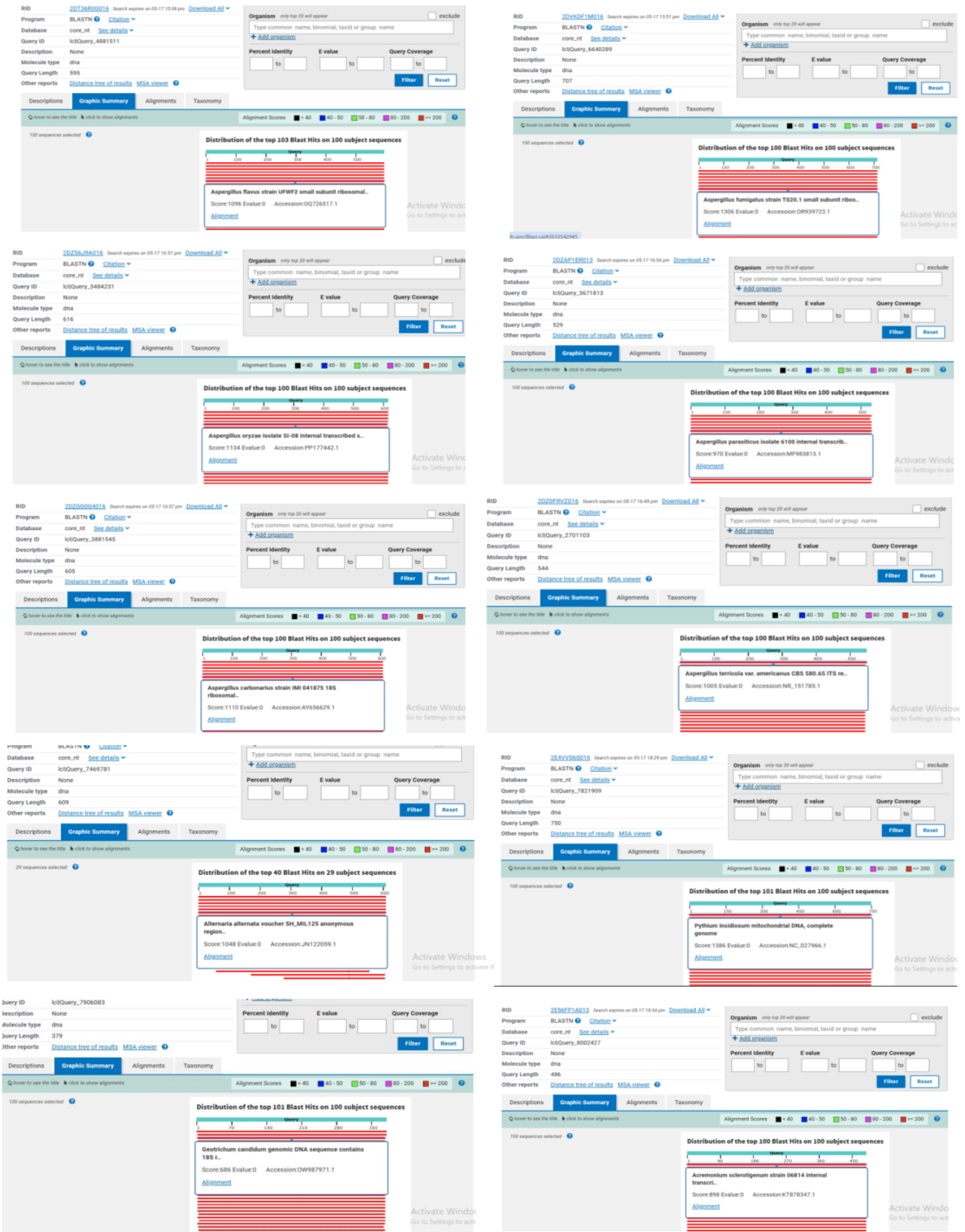


Figure 3. NCBI blast homology analysis of all 10 isolates.

Occurrence Frequency of Fungal Isolates on different parts of Tomato Plants.

Fungal distribution was analyzed in 10 samples collected from tomato leaves, stems, fruit, roots, and rhizospheric soil. The highest frequency of fungal occurrence was recorded in the rhizospheric soil (60%), followed by both fruit and stem samples (30% each). The root samples exhibited the lowest fungal presence at 20%, as illustrated in Figure 4.

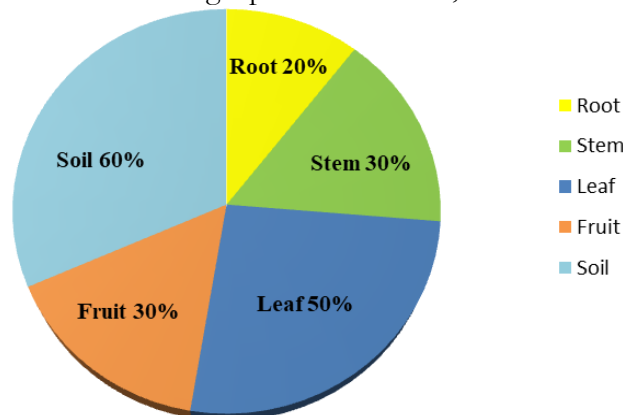


Figure 4. Percentage of fungi found on tomato plant (*Solanum lycopersicum*) parts.

Fungi identified in Tomato plant parts included *Aspergillus fumigatus*, *Acremonium* sp., *Pythium* sp., *Geotrichum candidum*, *A.parasiticus*, *A.carbonarius*, *A.terricola*, *A.flavus*, *A.oryzae*, *Alternaria alternate*, with varying occurrence frequencies. *A.fumigatus* had the highest occurrence at 80%, followed by *A.oryzae* and *A.parasiticus* (60%) *A. flavus* and *A. carbonarius* (40%), and *A. terricola*, *Acremonium* sp., *Pythium* sp., *Geotrichum candidum* and *Alternaria alternate* (20%) as shown in Table 4.

Sr. no.	Fungi name	No. of Individual species isolates					Total isolates	f	χ^2
		Leaf	Stem	Root	Fruit	Rhizospheric soil			
1	Aspergillus fumigatus	1	0	1	1	1	4	80%	1
2	Acremonium sp.	0	0	0	0	1	1	20%	4
3	Pythium sp.	0	0	0	0	1	1	20%	4
4	Geotrichum candidum	0	0	0	0	1	1	20%	4
5	Aspergillus parasiticus	0	1	1	0	1	3	60%	2.01
6	Aspergillus carbonarius	1	0	0	1	0	2	40%	3.0
7	Aspergillus terricola	0	0	0	1	0	1	20%	4
8	Aspergillus flavus	1	1	0	0	0	2	40%	3.0
9	Aspergillus oryzae	1	1	0	0	1	3	60%	2.01
10	Alternaria alternate	1	0	0	0	0	1	20%	4
Total							19		
p = 0.34							df = 9		

Table 4. Different fungi are found on Tomato plant (*Solanum lycopersicum*) parts.

f = occurrence frequency in percentage χ^2 = Chi-square value p = probability df= degree

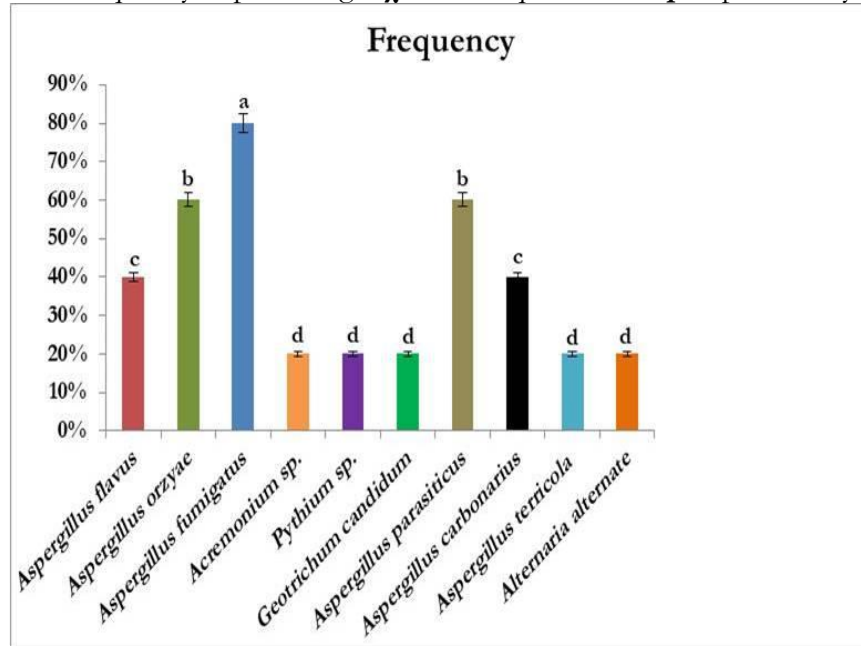


Figure 5. Percentage of individual fungi found on tomato plant (*Solanum lycopersicum*) parts. Vertical bars show standard errors of means. Values with different letters at their top show significant differences ($P \leq 0.05$) as determined by Tukey's Test.

Plant Health Metrics and Fungal Frequency Comparison.

The impact of fungal presence on plant health was assessed by measuring plant health metrics, including chlorophyll content. The results revealed a significant correlation between fungal presence and the plant health metrics (chlorophyll content) of the tomato plant. The presence of beneficial endophytic or mycorrhizal fungi was associated with improved nutrient uptake and increased chlorophyll concentration, suggesting a positive impact on plant vigor.

Fungal Species Diversity and Similarities between Different Tomato Plant Parts.

A total of 10 fungal species were isolated from different parts of the tomato plant (leaf, stem, root, fruit, and rhizospheric soil). Diversity was assessed using species richness (S), Shannon Index (H'), Simpson's Index ($1 - D$), and Evenness (E). The rhizospheric soil showed the highest richness ($S = 6$) and diversity ($H' = 1.609$), followed by the leaf ($S = 5$; $H' = 1.493$). The root had the lowest richness ($S = 2$; $H' = 0.693$), indicating lower fungal colonization as shown in Table 4. Evenness was high across all parts ($E \geq 0.929$), suggesting balanced species distribution. The Sørensen Similarity Index revealed that the leaf shared more fungal species with other parts (average SSI = 0.412), while the fruit showed the lowest similarity (SSI = 0.238), indicating a more distinct fungal community.

Table 5. Diversity indices and average Sørensen Similarity Index for fungal species on different tomato plant parts.

Plant Part	S (Species Richness)	N (Total Isolates)	H' (Shannon Index)	Simpson ($1 - D$)	Evenness (E)	Avg. Sørensen Similarity (SSI)
Leaf	5	5	1.609	0.800	1.000	0.412
Stem	3	3	1.099	0.667	1.000	0.349
Root	2	2	0.693	0.500	1.000	0.300
Fruit	3	3	1.099	0.667	1.000	0.238
Soil	6	6	1.609	0.833	1.000	0.330

Heat Map Analysis of Fungal Diversity.

A heat map was constructed to compare fungal diversity across plant parts using six indices: species richness (S), total isolates (N), Shannon index (H'), Simpson's index (1-D), evenness (E), and Sørensen similarity index (SSI). Soil and Leaf showed the highest diversity (S = 6 and 5; H' = 1.609), indicating rich and balanced fungal communities. Root had the lowest diversity (S = 2; H' = 0.693; 1-D = 0.500), suggesting limited colonization. All parts exhibited maximum evenness (E = 1.000), while similarity was highest in Leaf (SSI = 0.412) and lowest in Fruit (SSI = 0.238), indicating distinct fungal compositions. Overall, the heat map highlights the soil and leaf as key reservoirs of fungal diversity, while root and fruit tissues host more specialized communities.

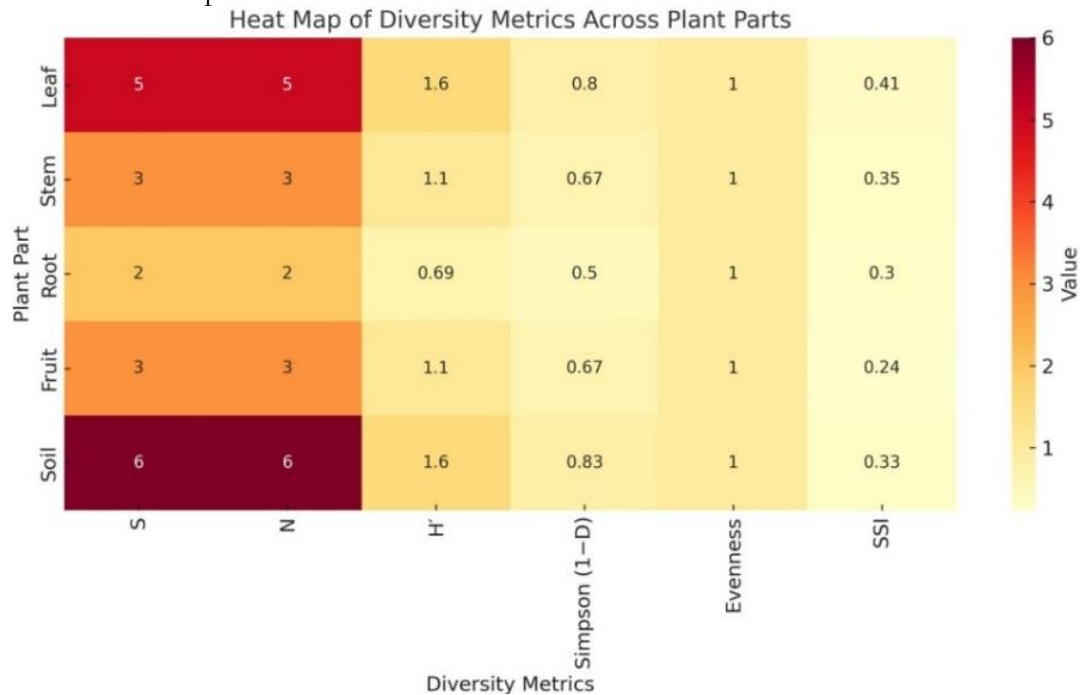


Figure 6. Heat map showing fungal diversity metrics across plant parts. Darker shades indicate higher values for each index (S, N, H', 1-D, E, SSI).

Effect of Phyllosphere Microbial Inoculum on Photosynthetic Pigments.

The data presented in Figure 7 indicate that chlorophyll (a, b) and total chlorophyll content were elevated in the treated plants that received phyllosphere microbial inoculum. Microbial inoculants appear to positively influence the growth and development of tomato plants, leading to increased levels of chlorophyll and other photosynthetic pigments. However, chlorophyll a, b, and total chlorophyll in the control treatment were decreased where phyllosphere microbial inoculum was not sprayed when compared with the sprayed treatment.

Table 6. Measurement of photosynthetic pigments of tomato plants.

Sr. no.	Treatments	OD (645)	OD (663)	Chl a	Chl b	Total Chl
1	Control Treatment	0.3	0.31	3.12	1.62	4.74
2	Sprayed Treatment	0.29	0.34	4.1	3.39	7.49

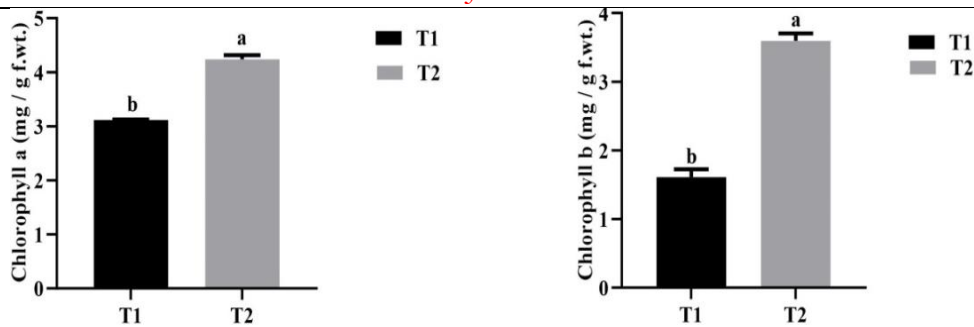


Figure 7. Effect of phyllosphere microbial inoculum on photosynthetic pigments. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant differences ($P \leq 0.05$) as determined by Tukey's Test.

Discussion.

Our findings indicate that the tomato mycobiome is predominantly composed of fungi from the Ascomycota phylum, reinforcing their significance in a variety of ecosystems, including soil, subsoil, plants, deep-sea sediments, aquatic environments, and even the atmosphere [17]. Microbial consortia play a crucial role in maintaining plant health [18]. However, there remains a limited understanding of microbiomes associated with wild plant species and their crop wild relatives. In most cases, bulk soil serves as a reservoir for plant-associated microbial communities, from which a specific subset is recruited into the rhizosphere, largely influenced by root exudates and rhizodeposits [19]. The tomato plant mycobiome plays a significant role in plant health and physiological processes, such as chlorophyll content. Fungi that are beneficial to the plant, like some endophytes, promote tomato growth and photosynthetic performance. *Aspergillus terreus* has been reported to enhance fresh and dry weight in tomato plants through improved nutrient acquisition and enhanced photosynthetic potential [20]. In the same vein, co-inoculation of *Aspergillus violaceofuscus* and *Bacillus licheniformis* under drought stress increased total chlorophyll content significantly, showing improved tolerance to stress [21]. The above results imply that beneficial microbiomes have a positive influence on tomato physiology by enhancing chlorophyll production and growth. *Aspergillus* species are opportunistic pathogens, that have adverse effects on plant health and chlorophyll content. For example, *Aspergillus flavus* has been cited as an endophytic leaf colonizer in tomatoes, causing decreased plant vigor and photosynthetic performance [22].

In this study, *Aspergillus fumigatus* was successfully isolated from the surfaces of soil, roots, leaves, and fruit, indicating its strong ecological adaptability and ability to thrive across various plant-associated environments. Previous studies have documented the widespread occurrence of *Aspergillus* species, including *A. niger* and *A. flavus*, on tomato roots, stems, leaves, and fruits [23]. Furthermore, *A. niger* often contaminates tomato products, resulting in post-harvest rotting and financial loss [21]. According to [24], *A. flavus* also showed colonization rates of 10.25% in several tomato regions, indicating that it may be a pathogen. The most common fungal species in soil samples were discovered to be *Acremonium* and *Pythium*, which is not surprising considering their well-established biological niches as soilborne fungi. According to [25], their finding raises the possibility of interactions with the tomato rhizosphere, which may affect nutrient intake and root health. The findings show how the mycobiome plays a complex role in regulating tomato health and productivity.

Conclusion.

As potential pathogens and growth boosters, the tomato mycobiome in general and the *Aspergillus* species in particular have two roles to perform. Knowing their distribution

patterns and functional activities is essential for both sustainable tomato production and effective disease management.

Acknowledgement.

The authors would like to thank all contributing institutions.

Author's Contribution.

Beenish Rasheed (BR) Umara Mushtaq (UM) & Karamat Ali Zohaib (KAZ) proposed the methodology and carried out the experiments, whereas Adnan Akhter (AA) and Uzma Bashir (UB) designed the study. Beenish Rasheed (BR) & Karamat Ali Zohaib (KAZ) gathered and examined data. Waheed Anwar (WA) made contributions to the draft of the work and insightful commentary and crucial edits for the finished version. Their combined efforts produced a thorough and significant study result.

Conflict of interest. There is no conflict of interest in this paper.

Reference.

- [1] S. M. K. and A. K. Ragupathi KP, PR Renganayaki, S Sundareswaran, "Seed mycobiome of tomato cultivar PKM 1," *J. Entomol. Zool. Stud.*, vol. 8, no. 6, pp. 1843–1846, 2020, doi. <https://doi.org/10.22271/j.ento.2020.v8.i6y.8094>.
- [2] R. Poudel, A. Jumpponen, M. Kennelly, C. Rivard, L. Gomez-Montano, and K. A. Garrett, "Integration of system phenotypes in microbiome networks to identify candidate synthetic communities. a study of the grafted tomato rhizobiome," *bioRxiv*, p. 874966, Dec. 2019, doi. [10.1101/2019/874966](https://doi.org/10.1101/2019/874966).
- [3] S. J. M. Mousami Poudel, Yiming Meng, Rodrigo Mendes, Lilian Abreu, C. Guillermo Bueno, Svetlana Folimonova, Karen A Garrett, "The Role of Plant-Associated Bacteria, Fungi, and Viruses in Drought Stress Mitigation," *Front. Microbiol.*, vol. 12, 2021, doi. <https://doi.org/10.3389/fmicb.2021.743512>.
- [4] M. K. Natalia Naumova, Olga Baturina, Taisia Nechaeva, "Root and Rhizosphere Microbiome of Tomato Plants Grown in the Open Field in the South of West Siberia under Mineral Fertilization," *Horticulture*, vol. 8, no. 11, p. 1051, 2022, doi. <https://doi.org/10.3390/horticulturae8111051>.
- [5] "Microbiome-Host Interactions," *Microbiome-Host Interact.*, Feb. 2021, doi. [10.1201/9781003037521/MICROBIOME-HOST-INTERACTIONS-DHANASEKARAN-DHIRAJ-PAUL-AMARESAN-SANKARANARAYANAN-YOGESH-SHOUCHE](https://doi.org/10.1201/9781003037521/MICROBIOME-HOST-INTERACTIONS-DHANASEKARAN-DHIRAJ-PAUL-AMARESAN-SANKARANARAYANAN-YOGESH-SHOUCHE).
- [6] D. R. P. Pragma Adhikari, Yeonyee Oh, "Current Status of Early Blight Resistance in Tomato. An Update," *Int. J. Mol. Sci.*, vol. 18, no. 10, p. 2019, 2017, doi. <https://doi.org/10.3390/ijms18102019>.
- [7] M. A. Vinothini Kamalanathan, Nakkeeran Sevugapperumal, Saranya Nallusamy, Suhail Ashraf, Kumanan Kailasam, "Metagenomic Approach Deciphers the Role of Community Composition of Mycobiome Structured by *Bacillus velezensis* VB7 and *Trichoderma koningiopsis* TK in Tomato Rhizosphere to Suppress Root-Knot Nematode Infecting Tomato," *Microorganisms*, vol. 11, no. 10, p. 2467, 2023, doi. <https://doi.org/10.3390/microorganisms11102467>.
- [8] E. T. Vladimír Půža, "Interactions between Entomopathogenic Fungi and Entomopathogenic Nematodes," *Microorganisms*, vol. 11, no. 1, p. 163, 2023, doi. <https://doi.org/10.3390/microorganisms11010163>.
- [9] Q. S. Dong, Chunjuan, Wang, Lingling, Li, Qian, "Epiphytic and Endophytic Fungal Communities of Tomato Plants," *Hortic. Plant J.*, vol. 7, no. 1, pp. 38–48, 2021, doi. <https://doi.org/10.1016/j.hpj.2020.09.002>.
- [10] S. C. J. Nakuleshwar Dut Jasuja, Richa Saxena, Subhash Chandra, "Isolation and identification of microorganism from polyhouse agriculture soil of Rajasthan," *Afr. J. Microbiol. Res.*, vol. 7, no. 41, pp. 4886–4891, 2013, doi. <https://doi.org/10.5898/ajmr.7.41.4886>.

<https://doi.org/10.5897/AJMR2012.2413>.

- [11] T. H. A. K. H. Domsch, W. Gams, "Compendium of Soil Fungi, 2nd ed," *Eching, Ger. IHW-Verlag*, 2007.
- [12] J. S. S. Yanmei Zhang, Zewei Song, "Evaluation of DNA Extraction Methods for Microbial Community Profiling in Deadwood Decomposition," *Microbiologyopen*, p. e70007, 2024, doi. <https://doi.org/10.1002/mbo3.70007>.
- [13] G. S.-S. Claudia A. Jasalavich, Victor M. Morales, Lawrence E. Pelcher, "Comparison of nuclear ribosomal DNA sequences from *Alternaria* species pathogenic to crucifers," *Mycol. Res.*, vol. 99, no. 5, pp. 604–614, 1995, doi. [https://doi.org/10.1016/S0953-7562\(09\)80720-X](https://doi.org/10.1016/S0953-7562(09)80720-X).
- [14] P. S. V. Kumar, R. Gupta, "Distribution and frequency of *Aspergillus* species on tomato plants," *Mycobiology*, vol. 50, no. 2, pp. 123–130, 2022.
- [15] S. M. Abdoul Razack Sare, Gilles Stouvenakers, Mathilde Eck, Amber Lampens, Sofie Goormachtig, M. Haïssam Jijakli, "Standardization of Plant Microbiome Studies. Which Proportion of the Microbiota is Really Harvested?," *Microorganisms*, vol. 8, no. 3, p. 342, 2020, doi. <https://doi.org/10.3390/microorganisms8030342>.
- [16] N. D. Portela et al, "Effect of DNA extraction method in gut fungal community assessment," *J. Fungal Res*, vol. 12, no. 2, pp. 45–56, 2025.
- [17] L. P. Yixuan Lu, Xiao Wang, Lucineidy C. S. de S. Almeida, "Environmental Factors Affecting Diversity, Structure, and Temporal Variation of Airborne Fungal Communities in a Research and Teaching Building of Tianjin University, China," *J. Fungi*, vol. 8, no. 5, p. 431, 2022, doi. <https://doi.org/10.3390/jof8050431>.
- [18] C. Xiong *et al.*, "Rare taxa maintain the stability of crop mycobiomes and ecosystem functions," *Environ. Microbiol.*, vol. 23, no. 4, pp. 1907–1924, Apr. 2021, doi. 10.1111/1462-2920.15262.
- [19] G. A. T. & C. W. S. M. A. Cregger, A. M. Veatch, Z. K. Yang, M. J. Crouch, R. Vilgalys, "The *Populus* holobiont. dissecting the effects of plant niches and genotype on the microbiome," *Microbiome*, vol. 6, no. 31, 2018, doi. <https://doi.org/10.1186/s40168-018-0413-8>.
- [20] N. A. S. El-Sayed, A. Youssef, "Growth enhancement of tomato plants by *Aspergillus terreus* endophytes," *Plants*, vol. 13, no. 16, p. 2218, 2023.
- [21] Z. A. M. Ahmad, S. Khan, "Occurrence of *Aspergillus* species in post-harvest tomato fruits," *Int. J. Food Microbiol.*, vol. 298, pp. 1–6, 2019.
- [22] Z. L. M. Jamil, M. Tariq, "Endophytic colonization by *Aspergillus flavus* and its impact on tomato plant health," *Phytopathol. Mediterr.*, vol. 59, no. 3, pp. 429–437, 2020.
- [23] S. Kumar, R. Singh, P. L. Kashyap, and A. K. Srivastava, "Rapid detection and quantification of *Alternaria solani* in tomato," *Sci. Hortic. (Amsterdam)*, vol. 151, pp. 184–189, 2013, doi. <https://doi.org/10.1016/j.scienta.2012.12.026>.
- [24] S. K. R. Sharma, V. Patel, "Colonization frequency of *Aspergillus flavus* on tomato plants," *J. Plant Pathol.*, vol. 103, no. 2, pp. 1–8, 2021.
- [25] Q. L. H. Zhang, Z. Wang, "Soilborne fungi associated with tomato rhizosphere," *Appl. Soil Ecol.*, vol. 149, p. 103474, 2020.



Copyright © by authors and 50Sea. This work is licensed under Creative Commons Attribution 4.0 International License.