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Fungal diversity associated with anthracnose in chili peppers of West Sumatra, Indonesia, using conventional and NGS approaches

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Abstract. *Warman R, Rahma H, Darnetty, Noveriza R. 2025. Fungal diversity associated with anthracnose in chili peppers of West Sumatra, Indonesia, using conventional and NGS approaches. Biodiversitas 26: 4257-4266.* Curly red chili plants are one of the leading horticultural commodities in West Sumatra, Indonesia. However, there has been a 90% decline in yield due to anthracnose disease. Anthracnose is caused by various fungi from the *Colletotrichum* genus and other fungi that interact within it. The objective of this study is to compare the diversity of fungi associated with anthracnose disease symptoms in West Sumatra using NGS (Next-Generation Sequencing) and conventional identification techniques. This study used a field survey method involving the collection of chili fruit samples with anthracnose symptoms and the isolation of associated fungi. The survey was conducted at various chili farming locations in South Pesisir District, West Sumatra, Indonesia, to obtain representative samples. Fungal identification was performed using conventional NGS methods. The results of identification using conventional techniques yielded 298 fungal isolates, which were divided into 8 groups based on macroscopic characteristics and 3 genera based on microscopic characteristics, namely *Colletotrichum* 78.18%, *Fusarium* 16.11%, and *Curvularia* 5.7% Meanwhile, the fungi obtained using NGS techniques comprised 10 genera: *Colletotrichum* 26.36%, *Peltula* 22.34%, *Moniliella* 18.73%, *Russula* 15.2%, *Linderina* 12.13%, *Fusarium* 1.13%, *Hydnum* 0.98%, *Rhexocercosporidium* 0.44%, *Crocinoboletus* 0.33%, and *Gymnopilus* 0.36%, with 198 species. The results of this study indicate that fungal diversity associated with anthracnose symptoms using NGS technology is higher than that identified using conventional methods. These findings regarding dominant pathogenic fungi and their high variability provide important guidance for designing appropriate control measures and optimizing fungicide application.

Keywords: Anthracnose, chili peppers, diversity, fungi, symbiosis

Abbreviation: NGS: Next-Generation Sequencing, PDA: Potato Dextrose Agar, WA: Water Agar

INTRODUCTION

Curly red chili peppers (*Capsicum annuum* L.) are a significant horticultural commodity in Indonesia, particularly in West Sumatra, where they hold strategic economic and social importance. Due to high consumer demand, chili peppers serve as a vital component of the national food supply chain. However, productivity in West Sumatra—especially in Pesisir Selatan District, one of the region's primary cultivation centers—remains low, averaging only 10 tons per hectare. This figure falls short of the optimal potential yield of 12.92 tons per hectare (Mareza et al. 2021). One of the major factors contributing to this yield gap is anthracnose infection. This disease not only diminishes the quantity of harvest but also compromises fruit quality, leading to significant losses for both farmers and the horticultural sector. The agro-climatic conditions in Pesisir Selatan District, marked by high humidity and average temperatures ranging from 27.1 to 28°C (Statistics of Pesisir Selatan District 2023), closely align with the optimal temperature for pathogen development, approximately 28°C (Uttamrao 2024). These conditions intensify the severity of anthracnose in chili crops. As a result, anthracnose has emerged as the most devastating disease affecting chili production, with the potential to reduce yields by up to 90% (Nurbailis et al. 2023).

Anthracnose in chili peppers is caused by fungi from the genus *Colletotrichum* Corda, 1831, which comprises a highly diverse species complex. Several species commonly associated with chili infection include *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Nurbailis et al. 2019), *Colletotrichum acutatum* J.H.Simmonds, *Colletotrichum aenigma* B.S.Weir & P.R.Johnst., *Colletotrichum scovillei* Damm, P.F.Cannon & Crous, *Colletotrichum truncatum* (Schwein.) Andrus & W.D.Moore, *Colletotrichum fructicola* Prihast., L.Cai & K.D.Hyde (Shi et al. 2021), and *Colletotrichum perseae* G.Sharma & S.Freeman (Sharma et al. 2022). This diversity indicates that anthracnose is a polyetiological disease, involving multiple pathogenic agents rather than a single causal species. *Colletotrichum* spp. may co-occur with other fungi, engaging in ecological interactions that range from pathogenic to endophytic or commensal (Newfeld et al. 2025). These associations can influence infection onset, disease severity, and the overall progression of disease development. During host invasion, pathogenic fungi actively reshape the plant microbiome, leading to shifts in microbial community composition within host tissues. Such shifts may

affect the efficacy of disease control and management strategies targeting anthracnose (Flores-Nunez and Stukenbrock 2024).

The interaction between *Colletotrichum* fungi and other fungal species is shaped by various factors, including environmental conditions, host plant genetics, and fungal community dynamics. In anthracnose-infected chili plants, symbiotic fungi play two distinct roles. First, Green et al. (2024) reported that greater diversity of pathogenic fungi correlates with increased disease severity. Conversely, Hane et al. (2020) highlighted that non-pathogenic symbiotic fungi found in symptomatic chili plants may function as biocontrol agents capable of suppressing disease progression under field conditions. Empirical evidence further supports the role of symbiotic fungi in enhancing plant resistance to pathogens. Garrastatxu et al. (2024) observed a positive association between fungal diversity and overall plant health, while Negi et al. (2024) demonstrated that the application of microbial consortia can reinforce host resistance. These beneficial microbes contribute to plant vitality not only through the production of phytohormones but also by supplying essential nutrients that support physiological processes (Singh et al. 2019).

The above report was obtained from a series of fungal identification processes on chili peppers with anthracnose symptoms using conventional techniques. Conventional fungal identification techniques have long been used, including macroscopic and microscopic character observations, physiology, biochemistry, and pathogenicity tests to confirm the fungus's pathogenic or non-pathogenic nature. Despite its long-standing use, this conventional technique has the disadvantage of not being able to identify fungi at the species level. Furthermore, the identification process requires a considerable amount of time and cannot yield 100% results for fungi associated with anthracnose symptoms because some fungi do not grow on artificial media. Next-Generation Sequencing (NGS) identification techniques offer a more practical and sophisticated approach, enabling the detection of all fungi associated with anthracnose symptoms, down to the species level (Frey and Lilly 2015). NGS techniques will shorten identification times and accelerate decision-making for anthracnose control. Non-pathogenic fungi can be developed and utilized as biocontrol agents.

This paper aims to compare the diversity of fungi associated with chili peppers with anthracnose symptoms in West Sumatra using conventional identification techniques and NGS. This study is the first report comparing conventional and NGS diagnostic techniques to analyze fungal diversity in lowland chili peppers infected with anthracnose disease in West Sumatra and can be used as a guideline for the use of fungal biocontrol agents.

MATERIALS AND METHODS

Survey of locations for sampling chili peppers infected with anthracnose

The observation area was selected based on the primary chili production center in Pesisir Selatan District, West Sumatra, Indonesia, which is characterized by a humid agroclimate, average temperatures ranging from 27 to 28°C, and high rainfall—conditions conducive to the proliferation of anthracnose pathogens. To ensure data representativeness, symptomatic chili fruits were sampled from multiple farmers' fields using adequate biological replication, thereby capturing both intra- and inter-location variation proportionally. Sampling sites encompassed three sub-districts, each represented by three *nagari* (villages) with the largest chili cultivation areas and the highest recorded incidence of anthracnose. In total, three sampling points were established, as illustrated in Figure 1. Sampling was conducted using a purposive random method. Chili plants exhibiting anthracnose symptoms were selected, and 15 symptomatic fruits were collected from each sampling point. All samples were subsequently transported to the Central Laboratory of Universitas Andalas, Padang, for fungal isolation. The study was carried out from March to September 2024.

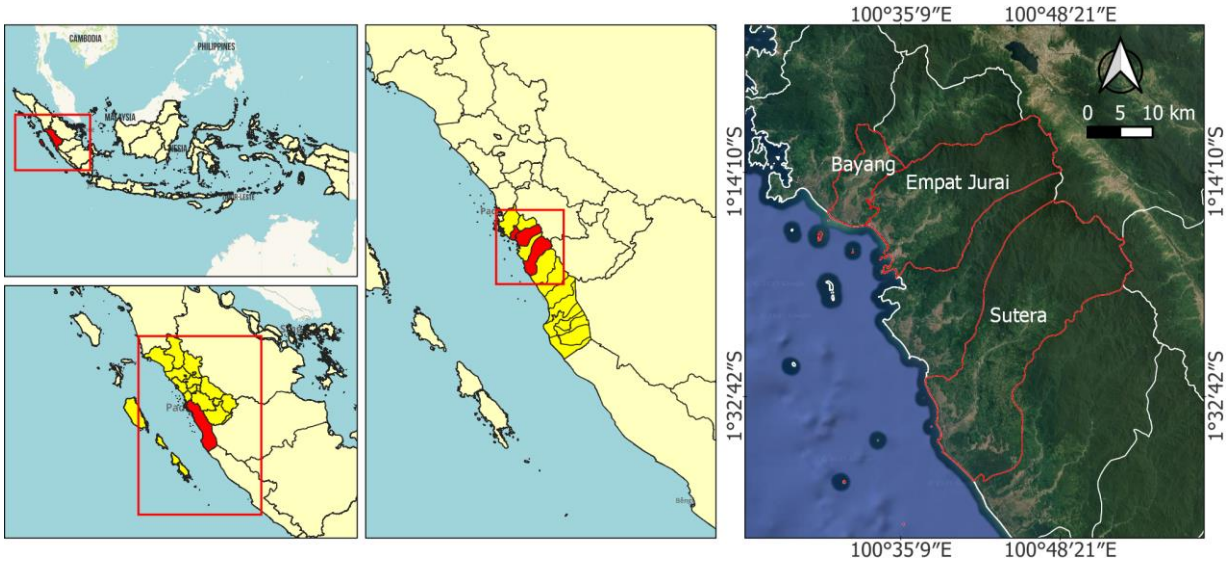

Figure 1. Location of chili plant sampling in Pesisir Selatan District, West Sumatra, Indonesia

Conventional identification techniques

Chili fruit samples from each location were isolated on Potato Dextrose Agar (PDA) media in 5 Petri dishes for each sample point. Chili fruits showing symptoms of anthracnose were cut into 1 × 1 cm pieces, with half of the pieces being diseased and half being healthy. The sample pieces were then surface-sterilized by soaking them in sterile distilled water for one minute, alcohol for one minute, and rinsing with sterile distilled water for one minute, then air-dried. The dried pieces were placed on PDA medium (10 mL) in petri dishes and incubated for 7 days at room temperature.

The fungi growing from the chili pepper pieces were then isolated into Petri dishes containing new PDA media and incubated for 5-7 days. Next, to obtain single spores, a fungal suspension was prepared by adding 5 mL of sterile distilled water to a test tube and incorporating pieces of the medium already colonized by the isolated fungi. The mixture was homogenized using a vortex, and the suspension was streaked onto Water Agar (WA) medium using a single inoculating needle. After a single spore or conidium germinates, the growing hyphae are transferred to PDA medium to obtain pure fungal isolates. The pure fungal colonies are observed for their macroscopic and microscopic characteristics. The macroscopic characteristics observed include colony color, shape, and texture. Meanwhile, the microscopic characteristics observed are the shape of the hyphae and conidia. The results of fungal characterization are compared with literature or fungal identification databases.

The identified fungi were subjected to a pathogenicity test to determine whether the fungi obtained were pathogenic or non-pathogenic. Pathogenic fungi will cause blackish and necrotic lesions, while non-pathogenic fungi do not cause morphological changes in the tested chili fruit. The pathogenicity test was conducted on 3 chili varieties of Akar for each isolate with the criteria of healthy and physiologically ripe fruit. The first stage, the chili fruit was surface sterilized by immersing it in sterile distilled water for one minute, then immersed in 70% alcohol for one minute and rinsed again with sterile distilled water for one minute, then air-dried. The dried chili fruit was injured with a sterile needle. The purified fungal isolate with a formula of 15 hsi was cut using a 7 mm cork borer and attached to the wound point, covered with sterile moist cotton. The chili fruit was placed in a mica plastic box lined with moist filter paper with a plastic straw on top as a support, the box was closed and incubated until symptoms of anthracnose disease appeared.

Fungal DNA extraction, PCR amplification, and fungal identification with Next-Generation Sequencing (NGS)

Fungal DNA was extracted from chili fruit tissues showing anthracnose symptoms using the Quick-DNA MagBead Plus Kit (Zymo Research, D4082) according to the manufacturer's protocol. Initial quantification and purity of the extracted genomic DNA were evaluated using a NanoDrop 2000 spectrophotometer (Thermo Scientific). PCR amplification was performed targeting the internal transcribed spacer (ITS) region of fungal rDNA using universal primers ITS1 and ITS4 and the KOD Multi & Epi PCR kit (KME-101), under standard amplification conditions. Successful amplification and PCR product size were confirmed by agarose gel electrophoresis (1.5%) with ethidium bromide staining. Accurate determination of PCR product quantity was performed using the Qubit dsDNA HS Assay kit (Thermo Scientific). Purified amplicons were then sent to a third-party sequencing facility for metagenomic sequencing. Sequencing was performed using the Oxford Nanopore MinION platform, using a FLO-MIN106 flow cell and the SQK-LSK109 library preparation kit. Basecalling was performed using Guppy software, and raw read quality was evaluated using NanoPlot and filtered with NanoFilt. A fungal index was constructed using the NCBI RefSeq database (https://ftp.ncbi.nlm.nih.gov/refseq/TargetedLoci/). Downstream analysis and visualization were performed using Pavian (https://github.com/fbreitwieser/pavian), Krona Tools (https://github.com/marbl/Krona), and RStudio with R version 4.3.3 (https://www.R-projec).

Raw data were trimmed with adapters and removed sequences shorter than 200 bp or with a Phred score < 20. Paired-end sequences were merged, and chimeric sequences were filtered using UCHIME. OTUs were clustered at 97% similarity and taxonomically mapped against the UNITE fungal ITS database (version 9.0) with an identity threshold of ≥ 97%. Rare taxa (singletons and those with a relative abundance < 0.01% across samples) were excluded to minimize artifacts in downstream analyses.

RESULTS AND DISCUSSION

Diversity of fungi associated with anthracnose disease symptoms in chili plants

The isolation and purification of chili peppers with anthracnose symptoms yielded 298 fungal isolates. Based on macroscopic characteristics, including colony shape and color, the fungi were grouped into 8 groups. Meanwhile, based on microscopic characteristics, including conidia shape, the fungi were grouped into 3 genera, which are dominated by the Colletotrichum 233 isolates (78,19%), *Fusarium* Link, 1809 48 isolates (16.11%), and *Curvularia* 17 isolates (5.7%). The results of conventional identification techniques are shown in Table 1 and Figure 2.

The pathogenicity test of eight fungal isolates revealed that four groups (1, 3, 6, and 7) were pathogenic, while groups (2, 4, 5, and 8) were non-pathogenic. Pathogenic fungi induced symptoms characterized by black or brown spots that progressively developed into larger, circular lesions with concentric rings. These lesions were typically circular or concave and contained moist acervuli exhibiting pink to orange coloration. In contrast, non-pathogenic fungi did not induce any anthracnose symptoms (Figure 3). The results of the pathogenicity test are presented in Figure 2.

Fungal identification techniques using Next-Generation Sequencing (NGS)

The results of fungal DNA isolation using ITS1 and ITS4 primer pairs from chili fruits showing anthracnose symptoms can be seen in Figure 4, where the DNA band size is 700 bp.

Table 1. Characteristics of fungi associated with anthracnose symptoms using conventional identification techniques

Group	Characteristics of fungi	Identification results	Identification reference
1 (1 isolate)	The colonies were round, with a slightly convex center, uneven margins, and a white coloration. Conidia were elliptical in shape and borne at the tips of conidiophores.	*Colletotrichum* spp.	Liu et al. (2022)
2 (11 isolates)	Colonies exhibited an uneven shape and were flat in elevation, with a relatively smooth surface, even margins, and a white coloration. Microconidia were elliptical and aseptate (non-septate).	*Fusarium* spp.	Moura et al. (2020)
3 (12 isolates)	The colonies were round and flat-topped, with smooth, even margins and a white coloration featuring a darker center. Conidia were cylindrical in shape and aseptate (non-septate).	*Colletotrichum* spp.	Armand et al. (2023)
4 (1 isolate)	The colonies were round, flat-topped with a slight central protrusion, and exhibited a white coloration. Conidia were spherical in shape, accompanied by the formation of dark, globose appressoria at the tips of transparent hyphae. Ascomata structures were also observed.	*Colletotrichum* spp.	Hodiyah et al. (2024)
5 (48 isolates)	The colonies were irregular in shape, flat-topped with a slight central prominence, and displayed uneven edges with a white coloration. Both microconidia and macroconidia were elongated and slightly curved, septate, and tapered at one end.	*Fusarium* spp.	Khan et al. (2021)
6 (72 isolates)	The colonies were round with a flat-topped surface and smooth margins. Conidia were curved and crescent-shaped, tapering at both ends, and aseptate (non-septate).	*Colletotrichum* spp.	Martins et al. (2024)
7 (136 isolates)	The colonies were round with flat elevations and smooth margins, spreading symmetrically across the culture plate. They exhibited a coloration ranging from white to cream. Conidia were elongated-oval in shape and aseptate (non-septate).	*Colletotrichum* spp.	Huang et al. (2024)
8 (17 isolates)	The fungal colonies exhibited a round shape with a flat surface and a slight central elevation. Margins were smooth, and the colony coloration was predominantly black. Conidia were slightly oval to moderately curved in shape, typically possessing four septa.	*Curvularia* spp.	Kiss et al. (2019)

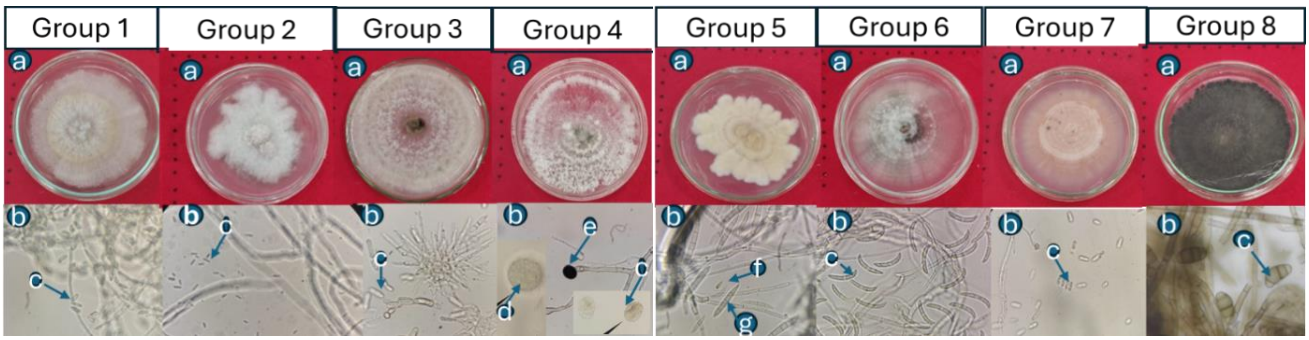

Figure 2. Characteristics of fungi isolated using conventional techniques. a. Macroscopic appearance of fungi in a 15-hour-old Petri dish, b. Microscopic appearance of fungi under a microscope at 100× magnification, c. Conidia, d. Conidiomata, e. Apresoria, f. Microconidia, g. Macroconidia

Distribution of fungi associated with chili peppers showing symptoms of anthracnose

The results of the NGS analysis showed the presence of 198 species with a total population of 39,074 CFU, comprising more than 10 OTUs (Operational Taxonomic Units) associated with anthracnose symptoms in chili.

The distribution of fungal taxa can be seen in Figure 5. At the domain level, all sequences belong to the eukaryotes, with 71,400 CFUs belonging to the fungal kingdom, which consists of four phyla: Ascomycota (23,700 CFUs), Basidiomycota (14,700 CFUs), Mucoromycota (84 CFUs), and Zoopagomycota (5,600 CFUs); Rhizopogonaceae 84 CFU, and 27,316 CFU from other phyla. The Ascomycota phylum consists of 5 families, namely Glomerellaceae (10,300 CFU), Peltulaceae (8,730 CFU), Nectriaceae (447 CFU), Helotiales (171 CFU), and 4,052 CFU from other families. Each family consists of 1 genus, namely *Colletotrichum*, which includes 3 species: *C. truncatum* 3,810 CFU, *Colletotrichum jasminigenum* Wikee, K.D.Hyde, L.Cai & McKenzie 1,380 CFU, and *Colletotrichum aciculare* Jayaward., Tangthir. & K.D.Hyde 276 CFU, plus 4,834 CFU from other genera. The genus *Peltula* has one species, *Peltula polyphylla* Q.X.Yang & X.L.Wei, with 8,730 CFU, the genus *Fusarium* has 447 CFU and 5 CFU from other genera, and there is one species of *Fusarium solani* (Mart.) Sacc. complex with 178 CFU and 264 CFU from other species.

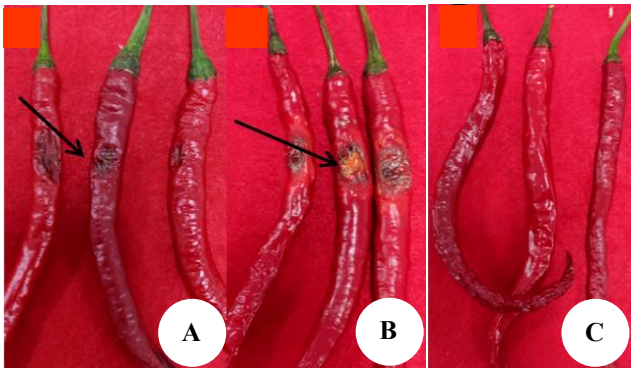

Figure 3. Anthracnose symptoms resulting from pathogenicity testing. A. Chili peppers with anthracnose symptoms from inoculation with fungus group 4, B. Chili peppers with anthracnose symptoms from inoculation with fungus group 5, C. Peppers without symptoms from inoculation with group 8

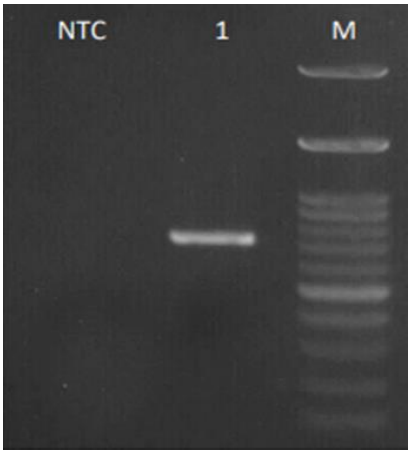

Figure 4. Visualization of fungal DNA bands with ITS1-ITS4 primers measuring 700 bp. NTC = negative control

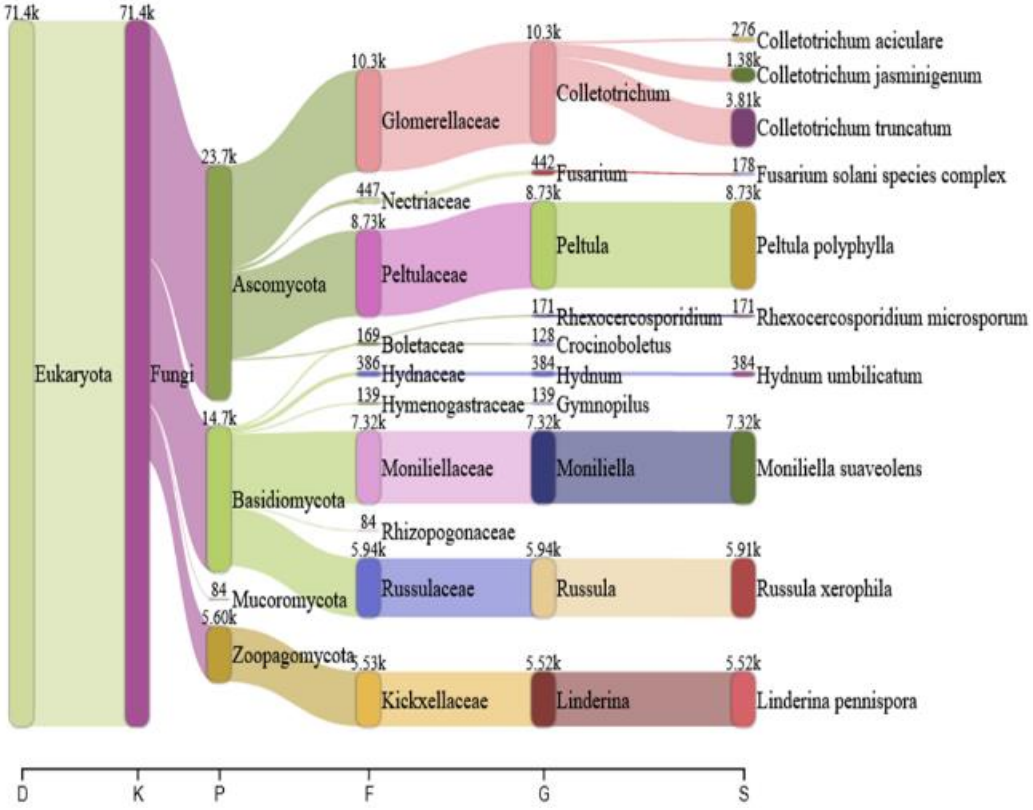

Figure 5. Taxonomic distribution of fungal diversity associated with anthracnose symptoms in chili peppers. The relative abundance of fungi that are symbiotic with anthracnose disease

The phylum Basidiomycota is divided into six families: Moniliellaceae, genus *Moniliella* Stolk & Dakin, with the species *Moniliella suaveolens* (Lindner) Arx at 7,240 cfu; Russulaceae, genus *Russula* Pers., with the species *Russula xerophila* (M.E.Sm. & Trappe) Trappe & T.F.Elliott at 5,910 cfu and 30 cfu of other species; the family Hydnaceae, genus *Hydnum* L., with the species *Hydnum umbilicatum* Peck at 384 CFU and 2 CFU from other genera, the family Hymenogastraceae, genus *Gymnopilus* P.Karst., 1879, at 139 CFU. The family Boletaceae, genus *Crocinoboletus* N.K.Zeng, Zhu L.Yang & G.Wu, 2014, at 128 CFU, the family Rhizopogonaceae at 84 CFU, and 703 CFU. Phylum Zoopagomycota with the family Kickxellaceae at 5,530 CFU and 70 CFU from other families with the genus *Linderina* Raper & Fennell, 1952 and the species *Linderina pennispora* Raper & Fennell at 5,520 CFU. The distribution of fungi associated with anthracnose symptoms on chili peppers can be seen in Figure 5.

The relative abundance of fungi associated with anthracnose symptoms at the genus level is dominated by *Colletotrichum*, *Peltula*, *Moniliella, Rusula*, and *Linderina*. Other genera detected include *Fusarium*, *Hydnum, Rhexocercosporidium*, *Crocinoboletus*, and *Gymnopilus*. At the species level, the dominance is characterized by *P. polyphylla, M. suaveolens, R. xerophila, Linderina pennispora* Raper & Fennell, *Colletotrichum*, *C. truncatum*, and *C. jasminigenum*, with a smaller presence of species such as *Hydnum umbilicatum* Peck, *Crocinoboletus pinetorum* N.K.Zeng, L.L.Wu, Zhi Q.Liang & S.Jiang, and *Rhexocercosporidium microsporum*. The abundance of fungal taxa associated with anthracnose symptoms can be seen in Figure 6, and the visualization of their crowns can be seen in Figure 7.

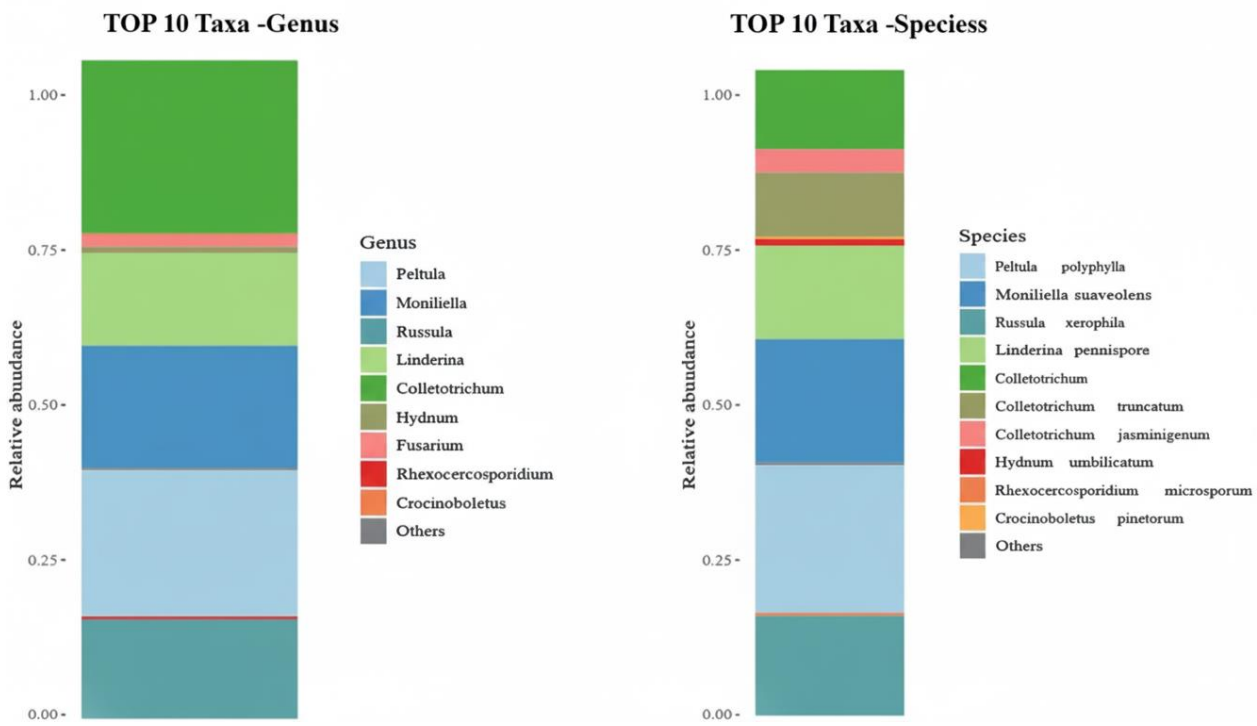

Figure 6. Relative abundance of fungi associated with anthracnose symptoms in chili peppers using NGS analysis

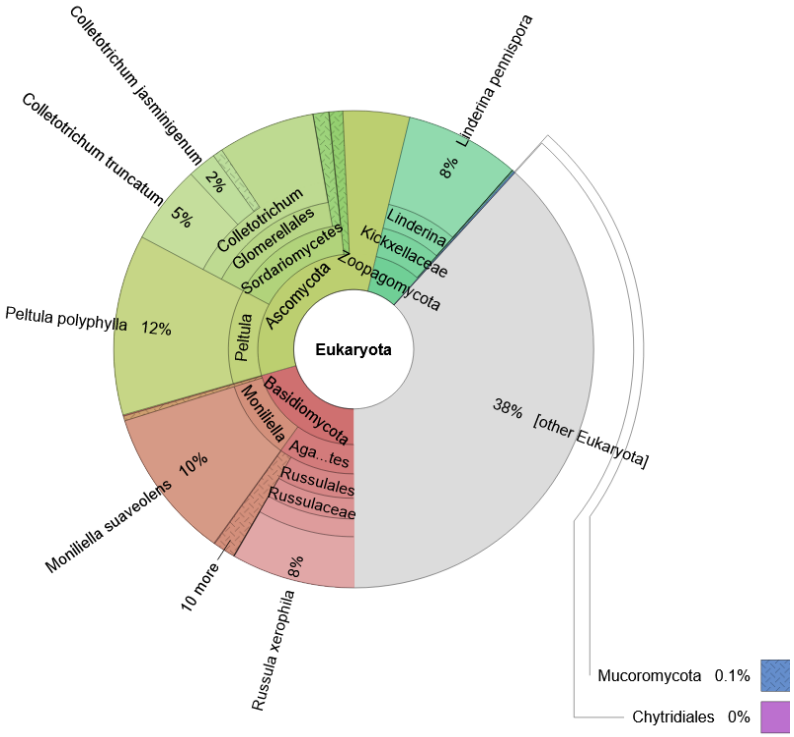

Figure 7. Visualization of the abundance of fungi associated with anthracnose symptoms in chili peppers

Figure 8 presents the diversity analysis of fruit samples with and without anthracnose symptoms, revealing that anthracnose-affected fruit exhibits higher species richness and microbial diversity compared to healthy fruit. The number of observed Operational Taxonomic Units (OTUs) in diseased fruit reached a median of approximately 360, while healthy fruit showed only around 300 OTUs, indicating greater directly detectable richness. This trend is further supported by the Chao1 (750) and ACE (700) indices, which were markedly higher than those in healthy fruit (Chao1: 500; ACE: 500), suggesting the presence of more unobserved or rare taxa. In terms of diversity, the Shannon index was slightly higher in diseased fruit (2.2) than in healthy fruit (2.1), reflecting a more even distribution of species, while the Simpson index (0.86 vs. 0.82) and Inverse Simpson index (7.0 vs. ~5.0) indicate lower species dominance and greater community balance in symptomatic samples. Additionally, the Fisher's Alpha value was higher in anthracnose-affected fruit (55) compared to healthy fruit (42.5), reinforcing the conclusion that diseased fruit harbors greater species richness even when accounting for log-normal distribution patterns.

The heatmap below (Figure 9) illustrates the relative abundance of fungal species associated with anthracnose symptoms. The species *C. truncatum, C. jasminigenum, C. aciculare, Colletotrichum eriobotryae* Damm & C.J.Huang, *C. scovillei, Fusarium tonkinense* (Bugnic.) O'Donnell, Geiser & T.Aoki, *F. solani,* and *Fusarium duplospermum* are species with high distribution and abundant diversity on chili peppers exhibiting anthracnose symptoms.

Discussion

Conventional methods for identifying anthracnose-causing fungi typically capture only a limited portion of the disease-associated microbial community, as they tend to prioritize easily cultured species that produce conspicuous symptoms, thereby emphasizing epidemiologically dominant pathogenic groups. Pathogenicity testing confirmed *Colletotrichum* as the primary causal agent, characterized by concentric blackish-brown lesions, consistent with the findings of Wang et al. (2024a,b) and Chowdhury et al. (2023). In addition to *Colletotrichum*, *Fusarium* and *Curvularia* were also identified in association with anthracnose symptoms, contributing to increased disease severity and highlighting the complexity of secondary pathogen interactions within the infected host.

Next-Generation Sequencing (NGS) approaches have revealed significantly greater fungal community diversity, with distinct differences in alpha diversity, and have enabled the identification of previously unculturable microorganisms. This technique provides both taxonomic and genomic insights, even for organisms that were previously unknown. Through NGS, ten dominant genera were successfully identified, including *Colletotrichum*, *Fusarium*, *Moniliella*, *Peltula*, *Linderina*, *Russula*, *Hydnum*, *Rhexocercosporidium*, *Gymnopilus*, and *Crocinoboletus*. The primary advantage of NGS over conventional methods lies in its capacity to detect unculturable taxa, including latent species and minor populations, thereby offering a more comprehensive view of the microbial community (Kankam et al. 2022). A particularly noteworthy finding was the detection of *M. suaveolens*, an opportunistic fungus previously reported only as a pathogen in fish and humans (Pawar et al. 2022; Ibrahem et al. 2024). Its potential role as a phytopathogen in plants, however, remains largely unexplored. The elevated alpha diversity observed in anthracnose-affected fruit may reflect shifts in microbiota composition triggered by plant stress or pathogen invasion. Increased diversity is often associated with colonization by opportunistic microbes or disruption of microbial homeostasis. In contrast, the lower diversity values observed in healthy fruit suggest a more stable and regulated microbial community, which may contribute to maintaining plant health.

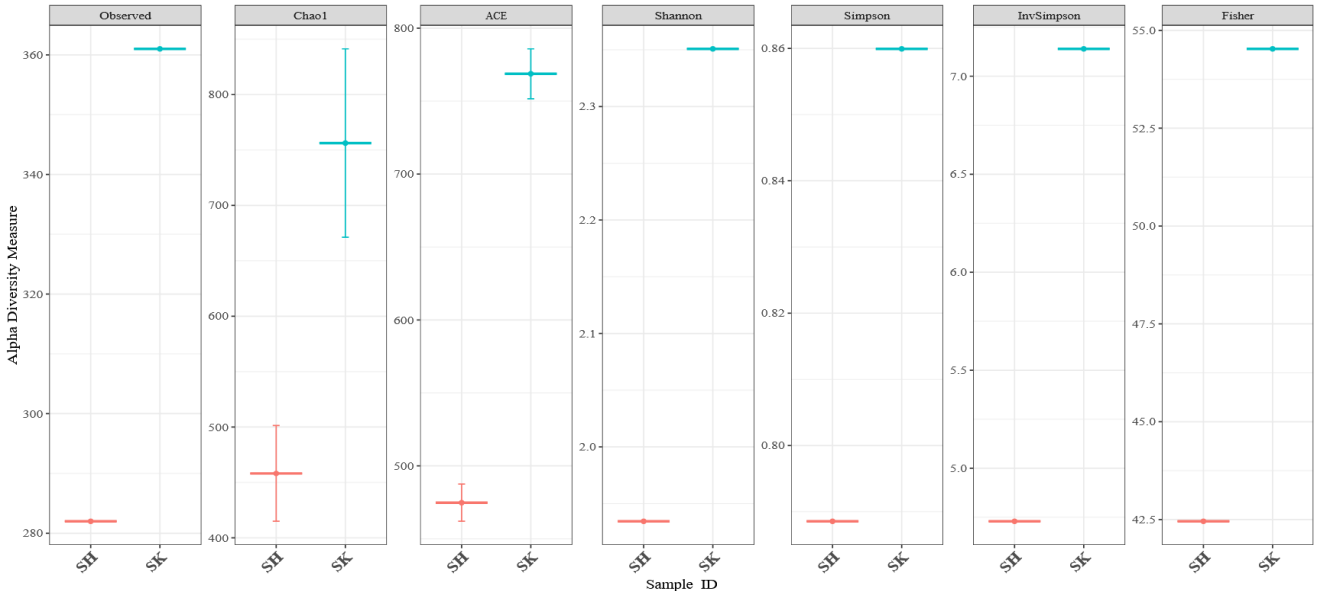

Figure 8. Distribution of alpha diversity indices (Observed OTUs, Chao1, ACE, Shannon, Simpson, Inverse Simpson, and Fisher's Alpha) in healthy fruit samples (SH) and anthracnose-infected fruit samples (SK)

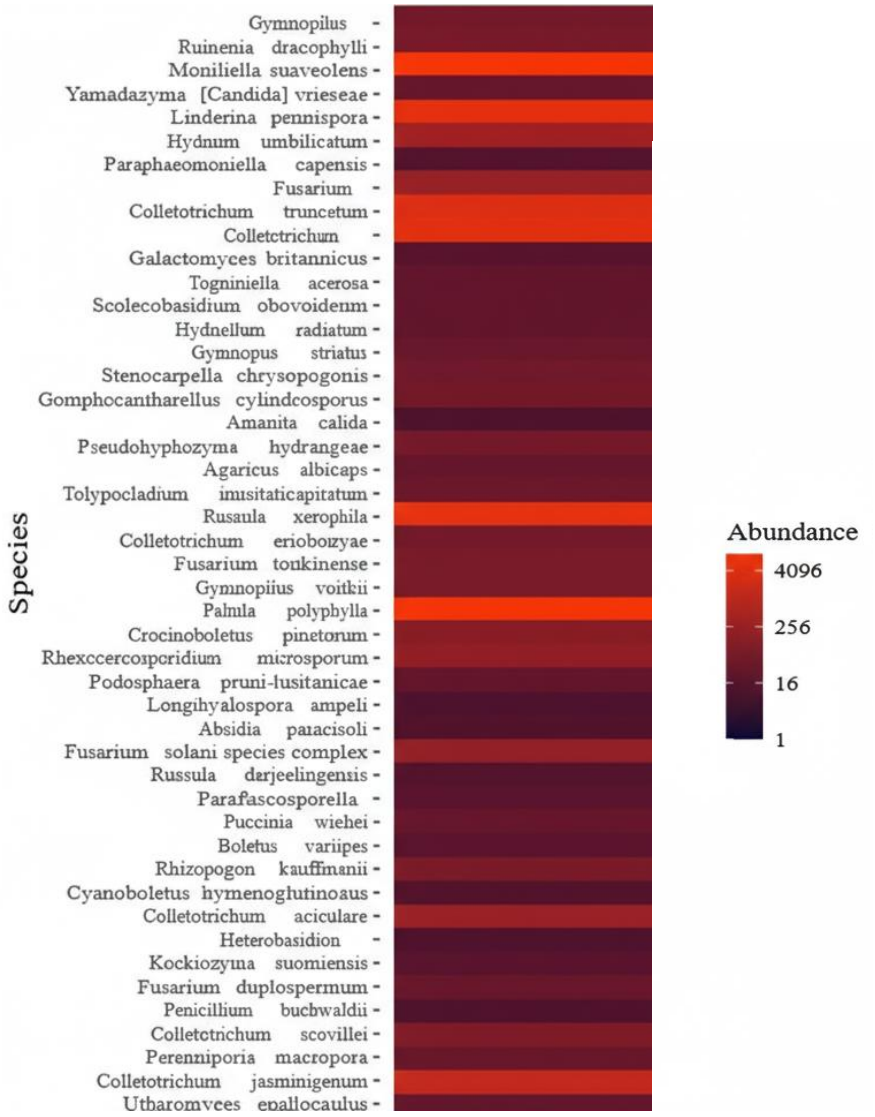

Figure 9. Heatmap of relative abundance of fungal species associated with anthracnose symptoms on chili peppers

The NGS technique revealed the presence of non-pathogenic fungi with important ecological functions, particularly mycorrhizal species that serve as potential biocontrol agents but cannot be cultivated using PDA media. This finding aligns with the report by Firdu and Dida (2024), which states that mycorrhizae can only be cultivated in living plant tissue. For example, *R. xerophila* facilitates phosphorus exchange with *Quercus* L. species (Zhou et al. 2022), while *H. umbilicatum* enhances nutrient uptake—especially phosphorus—and helps maintain microbial ecosystem balance and plant nutrient absorption (Manz et al. 2021). *Crocinoboletus pinetorum*, an ectomycorrhizal fungus, demonstrates strong adaptability to nutrient-poor environments and increases plant stress tolerance through symbiotic interactions (Xue et al. 2024). Additionally, *L. pennispora* produces indole-3-acetic acid (IAA), lindoline A and B, and chitinase, all of which have potential as biocontrol agents (Rassbach et al. 2023; Reynolds et al. 2023). These findings confirm that the chili ecosystem affected by anthracnose represents a complex microbial consortium, where pathogens, saprophytes, and symbionts interact both synergistically and competitively to modulate disease dynamics.

The dominance of *Colletotrichum* as the primary pathogen responsible for anthracnose disease in chili is consistent with reports from various Asian countries, including Indonesia, Malaysia, Sri Lanka, Thailand, and Taiwan. According to de Silva et al. (2019), seven *Colletotrichum* species were identified in chili fruits exhibiting anthracnose symptoms: *Colletotrichum endophyticum* Manamgoda, Udayanga, L.Cai & K.D.Hyde, *C. fructicola*, *Colletotrichum karsti* You L.Yang, Zuo Y.Liu, K.D.Hyde & L.Cai, *Colletotrichum plurivorum* Damm, Alizadeh & Toy.Sato, *C. scovillei*, *Colletotrichum siamense* Prihast., L.Cai & K.D.Hyde, and *Colletotrichum tropicale* E.I.Rojas, S.A.Rehner & Samuels. Additionally, three novel species were described: *Colletotrichum javanense* D.D.de Silva, Crous & P.W.J.Taylor, *Colletotrichum makassarense* D.D.de Silva, Crous & P.W.J.Taylor, and *Colletotrichum tainanense* D.D.de Silva, Crous & P.W.J.Taylor. Similarly, Katoch et

al. (2017) reported seven *Colletotrichum* species isolated from anthracnose-infected chili fruits in India, namely *C. truncatum* (syn. *Colletotrichum capsici*), *Colletotrichum coccodes* (Wallr.) S.Hughes, *Colletotrichum karsti* You L.Yang, Zuo Y.Liu, K.D.Hyde & L.Cai, *Colletotrichum kahawae* J.M.Waller & Bridge, *Colletotrichum nymphaeae* (Pass.) Aa, *C. fructicola*, and *C. gloeosporioides*. However, the broad spectrum of non-pathogenic fungal species revealed in this study is rarely detected using conventional morphology-based approaches. As noted by Wang et al. (2024a,b), pathogenic fungal infections can influence the diversity, network complexity, and stability of endophytic fungal communities within plant tissues.

The detection of rare species exclusively through NGS confirms that the ecosystem of anthracnose-infected chili fruits also serves as a reservoir of hidden microbial biodiversity, which has remained largely inaccessible using conventional methods. Metagenomic analysis enables the identification of both pathogenic and non-pathogenic taxa, some of which may shift their ecological roles depending on environmental conditions, thereby offering valuable insights into plant disease epidemiology (Piombo et al. 2021). The consistency of these findings with global metagenomic studies further underscores the power of NGS in advancing our understanding of plant disease dynamics by uncovering cryptic taxa that may contribute to both disease progression and suppression.

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