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Seasonal Dynamics of Endophytic Fungal Diversity *Grevillea Robusta* (Silver Oak) Trees

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Abstract

This study delves into the impact of seasonal variations on the diversity of endophytic fungi inhabiting the foliage of *Grevillea robusta* trees along highways in Hawassa, Ethiopia. With 765 fungal isolates categorized into 73 distinct morphotaxa, 90.41% were identified across eight known genera, leaving 9.58% unidentified. Notably, Phoma and Pestalotiopsis emerged as the most diverse genera, presenting 13 and 11 morphotaxa respectively, while *Alternaria* and *Xylaria* showed lower diversity with 3 and 2 morphotaxa each. The research unveiled heightened fungal counts and diversity in trees situated in more polluted environments, leaves sampled during dry seasons, those exhibiting signs of disease, lower leaf sections, and midrib samples. These findings underscore the rich assortment of endophytic fungi associated with *Grevillea robusta* leaves within roadside plantations in Hawassa, emphasizing the need for further exploration of this fungal community's dynamics. Understanding the pivotal role of these endophytic fungi in tree health, ecosystem resilience, and potential applications in agriculture, forestry, and biotechnology remains imperative.

Key words: Endophytic Fungi, *Grevillea robusta*, Genera, Morphotaxa, Seasonal variation

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Introduction

Endophytic fungi stand as influential components in augmenting tree resilience against an array of environmental stressors, including drought, salinity, and extreme temperature fluctuations (Chitnis et al., 2020; Dhanyalakshmi et al., 2023). Their versatile contributions extend to bolstering disease resistance, facilitating essential nutrient uptake such as phosphorus and nitrogen, and catalyzing robust tree growth and development (Wang et al., 2023). Furthermore, these fungi exhibit a remarkable capacity to neutralize harmful compounds, fortifying adaptability while fostering biodiversity within vegetative communities (Iqbal et al., 2023). Unraveling the multifaceted roles of endophytic fungi in trees assumes paramount importance, serving as a linchpin in preserving forest vitality, reinforcing ecosystem resilience, and harboring potential applications across agricultural, forestry, and biotechnological domains.

Researchers exemplify endophytic fungi as a group that colonizes internal living tissues of plants without immediate detrimental effects, yet their impact might manifest under environmental stresses (Verma et al., 2017; Yan et al., 2019). Notably, numerous studies have underscored the pervasive presence of these fungi, estimating a vast array of over 1 million species residing within plants, exerting considerable influence on plant community dynamics and diversity (Field et al., 2018; Bajpai et al., 2019). Their significant role in shaping fungal biodiversity heightens their relevance in influencing plant community structures (Tomao et al., 2020).

This fungal cohort assumes a pivotal role as biocontrol agents against plant pathogens, actively impeding pathogenic invasions into host plants (Donald et al., 2021; Adeleke et al., 2022). However, their population dynamics exhibit sensitivity to climatic variations and host plant locales, demonstrating variability across plant species, regions, and fluctuating climatic conditions (Verma et al., 2017; Yan et al., 2019). Endophytic fungi have been isolated from diverse plant compartments, encompassing scale primordia, meristems, resin ducts, leaf segments with midribs, roots, stems, bark, leaf blades, petioles, and buds (Ganley et al., 2006). Their presence not only augments host plant defenses against pathogenic incursions but also mitigates damage inflicted by herbivores, thereby bolstering the overall plant existence (Herre et al., 2005).

In this context, this study endeavors to unravel the seasonal dynamics governing the assembly of endophytic fungi within *Grevillea robusta* trees cultivated in roadside plantations. Furthermore, it scrutinizes potential variations in endophytic fungal profiles between healthy and diseased-looking leaves, distinct leaf sections (including midribs), different crown locations (bottom, middle, and top), and across various sampling sites. The primary objective revolves around investigating the endophytic fungal communities associated with *G. robusta* leaves in roadside plantations and discerning the influences of diverse environmental factors on these interactions.

Materials and Methods

The research was undertaken within the bounds of Hawassa, situated in Southern Ethiopia, spanning from December 2020 to August 2021. This urban center serves as the capital of the Southern Nations, Nationalities, and Peoples' Regional State, positioned approximately 275 kilometers to the south of Addis Ababa, Ethiopia's capital. Hawassa is adorned with a diverse array of trees and shrubs cultivated within its environs. Geographically, the city's coordinates lie between 6º54' N latitude and 38º48'-38º33' E longitude (Mamuye et al., 2015).

Site selection and sample collection

The preliminary phase involved reconnaissance activities to delineate regions within Hawassa where *Grevillea robusta* trees were planted, conducted from December 2021 to August 2022, encompassing at least two distinct seasons—the dry and wet periods—over the course of a year. Subsequently, specific streets or roads housing *G. robusta* as roadside plantations were purposefully selected. Notably, three principal thoroughfares in the city were singled out: Hawassa-Yirgalem, Meneharia, and Haik Dar-Gabriel.

From each of these designated sites, a delib-

erate selection process identified four standing trees exhibiting both healthy and diseased-looking foliage. Three leaves, chosen randomly from either the bottom, middle, or top branches of each tree at a rate of one leaf per branch, were meticulously plucked. These leaf samples were promptly secured in properly labeled, perforated polyethylene bags to ensure appropriate ventilation and transported to the Forest Pathology Laboratory at Wondo Genet College of Forestry and Natural Resources.

Isolation of leaf associated with endophytic fungi

The process of isolating leaf-associated endophytic fungi from *Grevillea robusta* leaves was meticulously conducted on individual leaf samples. Leaf segments of 5×5mm^2 dimensions were meticulously excised using a pair of scissors. From each leaf, eight distinct leaf pieces were obtained—four from each of the two halves of the leaf blade and an additional four pieces spanning from the bottom of the midrib to its tip—carefully placed in separate and appropriately labeled Petri dishes. Each Petri dish contained leaf pieces sourced from a single leaf.

Before the actual surface sterilization procedure employing hydrogen peroxide (H_2O_2), the prepared leaf pieces were sprayed just once with 70% ethanol as a surfactant agent using a sprayer (I Litter et al., 2010). Subsequently, surface sterilization was carried out by immersing the leaf pieces in a 33% hydrogen peroxide (H_2O_2) solution for one minute, followed by three to five consecutive rinses using sterilized water, as per the method described by Guo et al. (2001). A separate set of leaf pieces, intended for use as controls, underwent washing solely with sterile water, without the surface sterilization step involving H_2O_2.

The surface-sterilized leaf pieces were aseptically transferred to sterile and dried filter paper to remove excess liquid. Following this, the surface-sterilized leaf segments were inoculated onto sterile malt extract agar (MEA) plates, at a rate of four-leaf pieces per Petri dish. Leaf pieces derived from both the leaf blade and midrib were separately labeled and inoculated onto distinct plates. These plates were then incubated at a temperature range of 25-30 ºC for a duration of 5-10 days, facilitating the growth of endophytic fungal colonies on the medium. Regular checks for fungal growth were conducted within this incubation period.

Upon observing fungal growth at the culmination of approximately one week of incubation, individual fungal species were carefully and aseptically isolated by excising a small piece of mycelium plug using a sterile scalpel blade. These isolated fungi were then transferred to new sterile MEA plates. Instances where multiple fungi were found in a single sub-cultured plate led to further sub-culturing of each individual fungus onto new sterile MEA plates. These pure cultures were temporarily stored at a refrigerated temperature of 4-8 ºC until morphological examinations were undertaken.

Identification of Leaf-Associated Endophytic Fungi Based on Morphological Characteristics

The process of identifying leaf-associated endophytic fungi involved a meticulous assessment of both cultural and morphological attributes of the mycelia and reproductive structures, including conidia or other spore types. Several criteria were employed to characterize these fungal entities, encompassing distinct cultural and morphological characteristics.

Physical features

The evaluation of the upper and lower surfaces of cultures was conducted, with a focus on colors such as dark, brown, gray, yellow, or other identifiable colors (Caselli et al., 2017). A meticulous examination of colony margin shape and mycelial growth patterns was carried out, encompassing features such as fluffiness. Additionally, assessments of aerial hyphae, submerged hyphae, and the formation of hair-like tufts from hyphae were performed (Currah et al., 1997).

Morphotaxa Categorization

The isolated fungi were systematically categorized into morphotaxa, aiming to identify them at least to the genus level based on culture characteristics and spore morphology (Ofgea et al., 2015).

Unidentified taxa, while displaying distinct culture and conidial characteristics, couldn't be conclusively assigned to any known genera.

Microscopic Examination

Lactophenol and blue cotton staining solutions were used for the purposes of microscopic examination and evaluation of fungal spores without hyaline pigment, focusing on features such as shape, color, isolation and the presence or absence of specialized appendages on the spores (Thomas et al., 1991; Rathnayaka et al., 2023).

Conidial Morphological Features

Conifer characteristics, including spore shape and color, separation rate, and the presence or absence of specialized appendages on spores, were carefully examined. This comprehensive assessment of cultural and spore morphological characteristics allowed for the categorization and characterization of the fungal isolates into distinct morphotaxa, aiding in the identification of known genera and highlighting unidentified taxa based on their unique attributes (Naranjo-Ortiz et al., 2019).

Isolation Rate (IR)

The Isolation Rate (IR) serves as a crucial metric, calculated by determining the quotient of the number of isolates obtained from leaf segments and the total number of leaf segments incubated. This quotient, expressed as a percentage, allows for the measurement of fungal species richness within a leaf (Lv et al., 2010), formulated as:

$$\text{IR (\%)} = \frac{\text{Number of obtained isolates from leaf segments}}{\text{Total number of incubated leaf segmented}} \times 100$$

Relative Frequency (RF)

The Relative Frequency (RF) was computed as the total number of isolates from a single taxon divided by the total number of isolate taxa obtained from all tissues. This metric was instrumental in determining the most frequently isolated taxon among the entire array of isolated taxa in the study (Lv et al., 2010), calculated as:

$$\text{RF (\%)} = \frac{\text{Number of isolates of a fungal taxon}}{\text{Total number of isolates of all fungal taxon}} \times 100$$

Shannon Diversity Index (H)

The Shannon Diversity Index (H) was employed to characterize species diversity within the community across selected sites. This index, calculated for factors assumed to influence the diversity of endophytic fungi, was determined using the following formula:

$$H = -\sum_{i=1}^{R} p_i \ln p_i$$

Data analysis

The data obtained from the study underwent rigorous statistical analysis utilizing SPSS version 27. Several key parameters were calculated to assess and quantify the fungal species richness and diversity within the leaf segments.

Results

Endophytic Fungal Isolates from *G. robusta* Leaves

The investigation yielded a total of 765 endophytic fungal isolates, retrieved from 576 leaf fragments of *Grevillea robusta* trees, with 288 fragments from the midrib and an equal count from leaf blade fragments. Of these isolates, 700 were provisionally identified, classifying into eight genera: *Altarnaria*, *Aspergillus*, *Botryosphaeria*, *Fusarium*, *Penicillium*, *Pestalotiopsis*, *Phoma*, and *Xylaria*. The remaining 65 isolates represented seven distinct morphological groups that remain unidentified.

Within the identified genera, *Pestalotiopsis* (195), *Botryosphaeria* (181), *Phoma* (101), and *Fusarium* (90) constituted the most prevalent isolates, comprising 25.49%, 23.66%, 13.20%, and 11.76% of the total isolates, respectively. Conversely, *Xylaria* had the fewest isolates, accounting for 0.65% (5 isolates) of the total.

The distribution of these endophytic fungal isolates across the three sampling sites exhibited slight variability. The Hawassa-Yirgalem main road site housed the highest number of isolates (299, 39.08% of the total), while the Haik Dar-Gebriel church road site harbored the lowest number (222, 29.01% of the total) as depicted in Figure 1.

Botryosphaeria predominantly populated the

Hawassa-Yirgalem main road site with 90 isolates, while the Meneharia-South Star hotel road and Haik Dar-Gebriel church road sites were dominated by *Pestalotiopsis*, accounting for 70 and 59 isolates, respectively.

Seasonal Variation and Leaf Health Impact on Endophytic Fungal Isolates in *G. robusta*

Collection of leaf samples from *Grevillea robusta* trees occurred across two distinct periods: the dry and wet seasons. The dry season exhibited the highest count of endophytic fungal isolates, comprising 58.82% (450), while the wet season presented the lowest count at 41.17% (315) across all sampling sites (Figure 2).

During the dry season, the Hawassa-Yirgalem main road yielded the most endophytic fungal isolates (180, 40%), contrasting with the least count from Haik Dar-Gebriel road (119, 26.44%). In the wet season, Meneharia-South road had the highest count (119, 37.77%), whereas Meneharia-South Star hotel road displayed the lowest count (93, 29.52%). Statistical analysis did not indicate significant differences at $p>0.05$. Notably, *Fusarium* spp. and *Xylaria* spp. were absent from specific sites during the wet season, further accentuating the seasonal variations (Figure 2).

Within each sampling site, a varied number of endophytic fungal isolates were retrieved from symptomatic (diseased-looking) versus asymptomatic (healthy-looking) *G. robusta* leaves. Symptomatic leaves harbored a higher count of fungal isolates (415, 54.24%) compared to asymptomatic ones (350, 45.75%) across all sites (Table 1).

The distribution of specific fungal genera varied among sites and leaf health conditions. *Botryosphaeria* was prevalent (57, 7.45%) in diseased-looking leaves at the Hawassa-Yirgalem main road site, while *Alternaria* was the least identified (6, 0.78%). In asymptomatic leaves at the same site, *Botryosphaeria* dominated (33, 4.31%), followed by *Pestalotiopsis* (31, 4.05%) and *Xylaria* (Table 1).

At Meneharia-South Star, *Pestalotiopsis* led (36, 4.71%) in asymptomatic leaves, while *Fusarium* followed closely (25, 3.27%). Conversely, *Alternaria* and *Xylaria* were least identified in diseased-looking leaves at this site (Table 1).

Comparatively, *Pestalotiopsis* (25, 3.27%) dominated in asymptomatic leaves at Meneharia-South Star, while *Alternaria* was least identified (2, 0.26%) in diseased-looking leaves. *Botryosphaeria* was prominent in diseased-looking leaves at Haik Dar-Gebriel, with *Xylaria* being the least discovered (Table 1).

Notably, diseased leaves consistently housed higher fungal colony counts than healthy leaves across all sites, although statistical significance wasn't observed at $p>0.05$.

During both dry and wet seasons, more endophytic fungal isolates were recovered from midrib leaves compared to leaf blade samples. Midrib samples exhibited higher isolation frequency than leaf blade samples throughout both seasons (Table 2).

Table 1. Endophytic fungal isolates recovered from leaves of G.robusta trees from diseased and healthy-looking leaves from the three sampling sites

Genus	Sampling sites						
	Hawassa -Yirgalem		M eneharia -South Star		Haik Dar-Gebriel		
	Diseased	Health	Diseased	Health	Diseased	Health	Total
Altarnaria	6	4	2	2	9	5	28
Aspergillus	8	8	10	7	3	6	42
Botryosphaeria	57	33	19	24	32	16	181
Fusarium	14	9	25	14	13	16	91
Penicillium	14	11	7	10	9	6	57
Pestalotiopsis	35	31	34	36	30	29	195
Phoma	30	16	14	15	12	14	101
Xylaria	-	1	2	-	2	-	5
Unidentified	8	14	14	9	6	14	65
Total	172	127	127	117	116	106	765

Table 2. Number and frequency of endophytic fungal isolates recovered from different leaf parts during dry and wet seasons at the three sampling sites

Season	Sampling sites and leaf parts	No isolates	RF (%)
Dry	Hawassa-Yirgalem, midrib	101	22.44
	Hawassa-Yirgalem, leaf blade	77	17.11
	Meneharia-South Star, midrib	89	19.78
	Meneharia-South Star, leaf blade	65	14.44
	Haik Dar-Gebriel, midrib	63	14.00
	Haik Dar-Gebriel, leaf blade	55	12.22
Wet	Hawassa-Yirgalem, midrib	77	24.44
	Hawassa-Yirgalem, leaf blade	44	13.97
	Meneharia-South Star, midrib	47	14.92
	Meneharia-South Star, leaf blade	43	13.65
	Haik Dar-Gebriel, midrib	64	20.32
	Haik Dar-Gebriel, leaf blade	40	12.70

Discussion

Variations in fungal abundance and species composition were evident among the three sampling sites. This variability might be attributed to socio-economic activities and environmental factors unique to each site. Notably, the Hawassa-Yirgalem main road site, characterized by the presence of a textile industry and higher traffic density, exhibited higher occurrence of *Botryosphaeria* (90) and *Phoma* (49) isolates compared to the Meneharia-South (43 and 30) and Haik Dar-Gebriel (48 and 26) sites (Figure 1).

Both *Botryosphaeria* and *Phoma*, recognized as *Ascomycetous endophytes* with pathogenic potential, displayed higher counts in the highly impacted Hawassa-Yirgalem main road site. The increased isolation of these genera might be linked to environmental pollutants adversely affecting the microbial communities in *G. robusta* leaves, potentially compromising the protective role of these endophytic microorganisms. However, not all encountered endophytic genera adhered to this pattern, though the data lacked statistical significance at $p>0.05$.

Previous studies have highlighted the presence of similar fungal species as endophytes in various tree seeds across different countries, suggesting a widespread occurrence (Pautasso et al., 2015; Vaz et al., 2018; Harrison et al., 2020). Climate and habitat disturbance have been identified as influential factors affecting microfungi diversity, aligning with the current findings (Genevieve et al., 2019).

Seasonal variations exhibited higher fungal isolates during the dry season compared to the wet season across all sampling sites. The dry season's environmental stresses—higher temperature, lower soil moisture, relative humidity, and air pollutants—might have compromised the endophytic communities, rendering the plant leaves more susceptible to secondary infections (Jones et al., 2012; Delgado-Ospina et al., 2021).

The disparity in endophytic fungal isolates among sites was more pronounced during the dry season than the wet season, attributed to water stress in the latter season. This aligns with studies showcasing variations in endophytic flora concerning host and geographical factors (Froehlich et al., 2000; Martins et al., 2016).

Studies conducted in other regions have highlighted differences in endophytic fungal isolates based on dry and wet seasons, with varying counts recorded across seasons. However, these findings might not entirely align with our study outcomes, suggesting varied ecological factors at play (Osono et al., 2008; Glynou et al., 2016; Dasila et al., 2020).

Additionally, diseased *G. robusta* trees harbored a greater diversity of fungal species compared to healthy ones, corroborating findings from previous studies. This indicates *G. robusta*'s role as a host to a wide array of fungal species.

Conclusion

The investigation revealed notable variations in both the abundance and species composition

of endophytic fungi among the different sampling sites. *Pestalotiopsis* emerged as the most prevalent genus, followed by *Botryosphaeria* and *Phoma*, while *Xylaria* exhibited the lowest abundance. Notably, the Hawassa-Yirgalem Main road site exhibited the highest diversity, contrasting with the Meneharia-South Star road, which was identified as the least diverse sampling location.

Significant differences in the abundance of endophytic fungi were observed among the sampling sites, with the Hawassa-Yirgalem road showing the highest number of isolates and the Haik Dar-Gabriel road displaying the lowest. The observed variation could be attributed to differences in site-specific conditions, such as pollution resulting from traffic activities.

Seasonal variation exhibited a consistent trend, indicating higher counts of endophytic fungal isolates during the dry season across all sites compared to the wet season. This suggests a direct influence of seasonal changes on the species composition and abundance of endophytic fungi associated with leaf tissue. Additionally, there was variation in abundance and species composition between midrib and leaf blade samples, with higher numbers of endophytic fungi recovered from midrib leaf samples.

Differences in the abundance of endophytic fungi were also noted concerning the location of leaves within the crown (bottom, middle, and top), although no definitive evidence supported a specific affinity for a particular crown location. Consequently, it is inferred that seasonal variation, diseased and healthy-looking leaves, leaf parts (crown), midrib, and leaf blade influence the diversity and abundance of endophytic fungi associated with *G. robusta* trees growing in roadside plantations in Hawassa city.

While the study revealed general trends in the influence of seasonal variation, local habitat conditions, leaf positions within the crown, and specific leaf locations on the species composition and abundance of leaf endophytic fungal assemblages in *G. robusta* trees, a longer study duration would have increased the dataset and confidence in the conclusions drawn. Therefore, it is recommended that future studies expand to encompass additional localities, increase sampling frequency, and include more seasons to provide a more comprehensive dataset. This expanded dataset could address questions regarding the richness of some genera in species compared to their abundance at the species level.

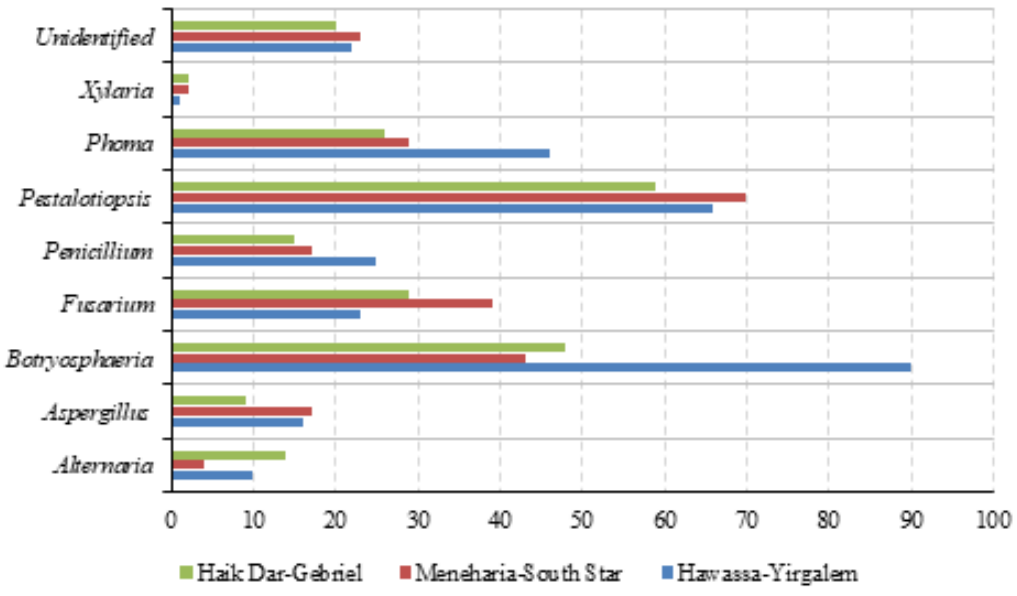

Fig 1. Endophytic fungal genera and their constituent isolates from the three sampling sites

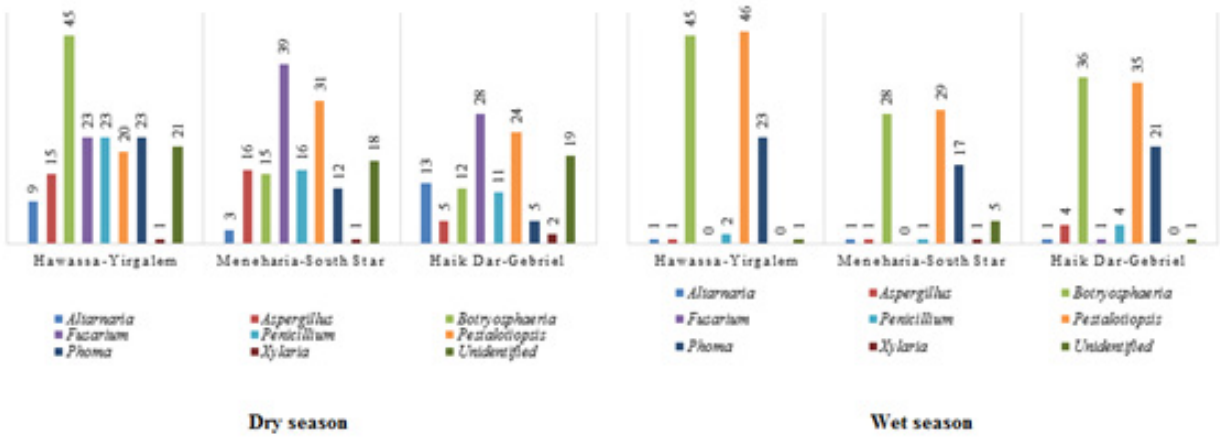

Fig 2. Distribution of endophytic fungal isolates recovered from leaves of *G. robusta* trees to sampling sites and seasons

References

Adeleke, B. S., Ayilara, M. S., Akinola, S. A., & Babalola, O. O. (2022). Biocontrol mechanisms of endophytic fungi. Egyptian Journal of Biological Pest Control, 32(1), 1-17. https://doi.org/10.1186/s41938-022-00547-1

Bajpai, A., Rawat, S., & Johri, B. N. (2019). Fungal diversity: global perspective and ecosystem dynamics. Microbial Diversity in Ecosystem Sustainability and Biotechnological Applications: Volume 1. Microbial Diversity in Normal & Extreme Environments, 83-113. https://doi.org/10.1007/978-981-13-8315-1_4

Caselli, E., Pancaldi, S., Baldisserotto, C., Petrucci, F., Impallaria, A., Volpe, L., ... & Mazzacane, S. (2018). Characterization of biodegradation in a 17th century easel painting and potential for a biological approach. PLoS One, 13(12), e0207630. https://doi.org/10.1371/journal.pone.0207630

Chitnis, V. R., Suryanarayanan, T. S., Nataraja, K. N., Prasad, S. R., Oelmüller, R., & Shaanker, R. U. (2020). Fungal endophyte-mediated crop improvement: the way ahead. Frontiers in Plant Science, 11, 561007. https://doi.org/10.3389/fpls.2020.561007

Currah, R. S., Zelmer, C. D., Hambleton, S., & Richardson, K. A. (1997). Fungi from orchid mycorrhizas. In 'Orchid biology: reviews and perspectives. VII'.(Eds J Arditti, A Pridgeon) pp. 117–170. https://doi.org/10.1007/978-94-017-2498-2_4

Dasila, K., Pandey, A., Samant, S. S., & Pande, V. (2020). Endophytes associated with Himalayan silver birch (Betula utilis D. Don) roots in relation to season and soil parameters. Applied Soil Ecology, 149, 103513. https://doi.org/10.1016/j.apsoil.2020.103513

Delgado-Ospina, J., Molina-Hernández, J. B., Chaves-López, C., Romanazzi, G., & Paparella, A. (2021). The role of fungi in the cocoa production chain and the challenge of climate change. Journal of Fungi, 7(3), 202. https://doi.org/10.3390/jof7030202

Dhanyalakshmi, K. H., Pallavi, N., Pawar, R. K., & Nataraja, K. N. (2023). Endophyte-Mediated Crop Improvement: Manipulation of Abiotic Stress-Specific Traits. In Translating Physiological Tools to Augment Crop Breeding (pp. 355-370). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-19-7498-4_16

Donald, M. L., Bohner, T. F., Kolis, K. M., Shadow, R. A., Rudgers, J. A., & Miller, T. E. (2021). Context-dependent variability in the population prevalence and individual fitness effects of plant–fungal symbiosis. Journal of Ecology, 109(2), 847-859. https://doi.org/10.1111/1365-2745.13510

Field, K. J., & Pressel, S. (2018). Unity in diversity: structural and functional insights into the ancient partnerships between plants and fungi. New Phytologist, 220(4), 996-1011. https://doi.org/10.1111/nph.15158

Froehlıch, J., & Petrini, O. (2000). Endophytic fungi associated with palms. Mycological Research, 104(10), 1202-1212. https://doi.org/10.1017/S095375620000263X

Ganley, R. J., & Newcombe, G. (2006). Fungal endophytes in seeds and needles of Pinus monticola. Mycological research, 110(3), 318-327. https://doi.org/10.1016/j.mycres.2005.10.005

Genevieve, L., Pierre-Luc, C., Roxanne, G. T., Amélie, M., Danny, B., Vincent, M., & Hugo, G. (2019). Estimation of fungal diversity and identification of major abiotic drivers influencing fungal richness and communities in northern temperate and boreal Quebec forests. Forests, 10(12), 1096. https://doi.org/10.3390/f10121096

Glynou, K., Ali, T., Buch, A. K., Haghi Kia, S.,

Ploch, S., Xia, X., ... & Maciá-Vicente, J. G. (2016). The local environment determines the assembly of root endophytic fungi at a continental scale. Environmental microbiology, 18(8), 2418-2434. https://doi.org/10.1111/1462-2920.13112

Guo, L. D., Hyde, K. D., & Liew, E. C. (2001). Detection and taxonomic placement of endophytic fungi within frond tissues of Livistona chinensis based on rDNA sequences. Molecular Phylogenetics and Evolution, 20(1), 1-13. https://doi.org/10.1006/mpev.2001.0942

Harrison, J. G., & Griffin, E. A. (2020). The diversity and distribution of endophytes across biomes, plant phylogeny and host tissues: how far have we come and where do we go from here?. Environmental microbiology, 22(6), 2107-2123. https://doi.org/10.1111/1462-2920.14968

Herre, E. A., Van Bael, S. A., Maynard, Z., Robbins, N. A. N. C. Y., Bischoff, J., Arnold, A. E., ... & Kyllo, D. A. (2005). Tropical plants as chimera: some implications of foliar endophytic fungi for the study of host plant defense, physiology, and genetics. Biotic interactions in the tropics, 1011, 226-237.

I Litter, M., & Quici, N. (2010). Photochemical advanced oxidation processes for water and wastewater treatment. Recent Patents on Engineering, 4(3), 217-241. https://doi.org/10.2174/187221210794578574

Iqbal, B., Li, G., Alabbosh, K. F., Hussain, H., Khan, I., Tariq, M., ... & Ahmad, N. (2023). Advancing Environmental Sustainability through Microbial Reprogramming in Growth Improvement, Stress Alleviation, and Phytoremediation. Plant Stress, 100283. https://doi.org/10.1016/j.stress.2023.100283

Jones, R. A., & Barbetti, M. J. (2012). Influence of climate change on plant disease infections and epidemics caused by viruses and bacteria. CABI Reviews, (2012), 1-33. https://doi.org/10.1079/PAVSNNR20127022

Lv, Y. L., Zhang, F. S., Chen, J., Cui, J. L., Xing, Y. M., Li, X. D., & Guo, S. X. (2010). Diversity and antimicrobial activity of endophytic fungi associated with the alpine plant Saussurea involucrata. Biological and Pharmaceutical Bulletin, 33(8), 1300-1306. https://doi.org/10.1248/bpb.33.1300

Mamuye, N., & Gotu, B. (2015). Statistical analysis of urban quality of life (Case study: Hawassa Town, SNNP Region, Ethiopia). American Journal of Theoretical and Applied Statistics, 4(6), 547-554. https://doi.org/10.11648/j.ajtas.20150406.26

Martins, F., Pereira, J. A., Bota, P., Bento, A., & Baptista, P. (2016). Fungal endophyte communities in above-and belowground olive tree organs and the effect of season and geographic location on their structures. Fungal ecology, 20, 193-201. https://doi.org/10.1016/j.funeco.2016.01.005

Naranjo-Ortiz, M. A., & Gabaldón, T. (2019). Fungal evolution: diversity, taxonomy and phylogeny of the Fungi. Biological Reviews, 94(6), 2101-2137. https://doi.org/10.1111/brv.12550

Ofgea, K. C., & Gure, A. (2015). Morphological diversity of fungi associated with stored grains of maize (Zea mays L.) in Shashemene and Arsi Nagelle districts, Ethiopia. IJ IS R, 15(1), 142-149.

Osono, T. (2008). Endophytic and epiphytic phyllosphere fungi of Camellia japonica: seasonal and leaf age-dependent variations. Mycologia, 100(3), 387-391. https://doi.org/10.3852/07-110R1

Pautasso, M., Schlegel, M., & Holdenrieder, O. (2015). Forest health in a changing world. Microbial Ecology, 69, 826-842. https://doi.org/10.1007/s00248-014-0545-8

Rathnayaka, A. R., Chethana, K. T., Phillips, A. J., Liu, J. K., Samarakoon, M. C., Jones, E. G., ... & Zhao, C. L. (2023). Re-evaluating Botryosphaeriales: Ancestral state reconstructions of selected characters and evolution of nutritional modes. Journal of Fungi, 9(2), 184. https://doi.org/10.3390/jof9020184

Thomas, P. A., Kuriakose, T., Kirupashanker, M. P., & Maharajan, V. S. (1991). Use of lactophenol cotton blue mounts of corneal scrapings as an aid to the diagnosis of mycotic keratitis. Diagnostic microbiology and infectious disease, 14(3), 219-224. https://doi.org/10.1016/0732-8893(91)90035-E

Tomao, A., Bonet, J. A., Castano, C., & de-Miguel, S. (2020). How does forest management affect fungal diversity and community composition? Current knowledge and future perspectives for the conservation of forest fungi. Forest Ecology and Management, 457, 117678. https://doi.org/10.1016/j.foreco.2019.117678

Vaz, A. B., Fonseca, P. L., Badotti, F., Skaltsas, D., Tomé, L. M., Silva, A. C., ... & Góes-Neto, A. (2018). A multiscale study of fungal endophyte communities of the foliar endosphere of native rubber trees in Eastern Amazon. Scientific reports, 8(1), 16151. https://doi.org/10.1038/s41598-018-34619-w

Verma, S. K., Gond, S. K., Mishra, A., Sharma, V. K., Kumar, J., Singh, D. K., ... & Kharwar, R. N. (2017). Fungal endophytes representing diverse habitats and their role in plant protection. Developments in fungal biology and applied mycology, 135-157. https://doi.org/10.1007/978-981-10-4768-8_9

Wang, Z., Solanki, M. K., Kumar, A., Solanki, A. C., Pang, F., Ba, Z. X., ... & Ren, Z. X. (2023).

Promoting plant resilience against stress by engineering root microenvironment with Streptomyces inoculants. Microbiological Research, 277, 127509. https://doi.org/10.1016/j.micres.2023.127509

Yan, L., Zhu, J., Zhao, X., Shi, J., Jiang, C., & Shao, D. (2019). Beneficial effects of endophytic fungi colonization on plants. Applied microbiology and biotechnology, 103, 3327-3340. https://doi.org/10.1007/s00253-019-09713-2