Endophytic Microbial Communities Of Congeneric Native and Invasive *Lantana* (*Verbenaceae*) Species in India

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ABSTRACT

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Plant invasion has emerged as a major focus of research in ecology and invasion biology as it poses a serious threat to the integrity of natural ecosystems by displacing native plant communities and promoting the formation of monocultures in newly colonized habitats. Although numerous theories have been advanced to explain the success of invasive species, the role of plant-microbe interactions remains as one of the most understudied aspects of invasion biology. Endophytic microbes play an important role in maintaining plant health, adaptability, and ecological interactions. In this study, I investigated the diversity, composition, and abiotic stress tolerance of bacterial and fungal endophytes associated with *Lantana camara*, an invasive plant species, and *Lantana indica*, its native congener, co-occurring in India. Using culture-independent amplicon sequencing (16S rRNA and ITS region of nuclear rDNA), culture-dependent isolation methods and abiotic stress tolerance assays, I examined the invasive and native plants to identify their distinct bacterial and fungal communities and the tolerance of fungi to abiotic stressors. Amplicon sequencing demonstrated *Ascomycota* to be the dominant phyla and *Talaromyces* as the dominant fungal genus in both *L.* camara and *L.indica*, while the bacterial communities were dominated by the family *Clostridiaceae* in *L. camara* and *Pseudomonadaceae* in *L. indica.* The native plants showed higher bacterial diversity and evenness compared to invasive plants suggesting a long-term adaptation to local biotic and abiotic conditions, whereas fungal communities showed no difference emphasizing that the endophytic fungi share similar community composition and functional adaptation. Culture dependent isolation of endophytic fungi revealed *Ascomycota* to be the dominant phyla. Both culture dependent and culture independent methods were compared to show how they can complement each other due to various bias adhered to these techniques. Abiotic stress tolerance assays on selected fungal isolates demonstrated species specific stress tolerance. *Aspergillus spp*. showing high salinity (2.5M NaCl) and drought (15% PEG) tolerance, while *Macrophomina phaseolina* remained viable at 45 °C. These results suggest that endophytes can potentially support host survival under abiotic stress, likely facilitating plant growth. Overall, the thesis highlights that the endophytic communities differ between invasive and native *Lantana* species, and specific endophytes show stress resilience *in vitro*. Understanding such microbial associations is essential in uncovering mechanisms underlying plant adaptation and invasion process.

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CONTRIBUTIONS OF AUTHORS

The research projects presented in this thesis is my original work. I am the principal author of all chapters in this thesis. I contributed in designing the experiments, carrying them out, analyzing the resulting data, and writing each of the four chapters. Dr. Dayanandan provided guidance and supervision throughout the research process and Dr. R.Umashaanker helped to develop the concepts of the project and contributed to the revision. Each chapter in this thesis has been prepared in the format of a manuscript intended for submission to peer-reviewed journals.

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ABBREVATIONS

IAP - Invasive Alien Plant

EF - Endophytic Fungi

PDA - Potato Dextrose Agar

PDB - Potato Dextrose Broth

ITS - Internal Transcribed Spacer

QIIME2 - Quantitative Insights Into Microbial Ecology 2

OTU - Operational Taxonomic Unit

ASV - Amplicon Sequence variant

UNITE - Unified System For DNA Based Fungal Species Linked To Taxonomy

NMDS - Non-metric Multidimensional Scaling

PEG - Polyethylene Glycol

NaCl - Sodium Chloride

CTAB - Cetyl Trimethyl Ammonium Bromide

DNA - Deoxyribonucleic Acid

RNA - Ribonucleic Acid

BLASTn - Basic Local Alignment Search Tool for nucleotides

dNTP - Deoxynucleoside Triphosphate

SRA - Sequence Read Archive

HSP - Heat Shock Protein

Chapter 1

Introduction

1.1 Plant invasion and its importance

Biodiversity loss is an irreversible consequence of anthropogenic global change and remains as one of the most critical environmental concerns. Biological invasion is the second-largest contributor to biodiversity loss (Sax & Gaines, 2008; Oduor, 2013). Importantly, plant invasion is one of the most significant global threats to natural ecosystems impacting both flora and fauna. In particular, the spread of invasive alien plants (IAPs) poses a significant threat to native plant species by outcompeting them, disrupting ecosystems leading to monocultures in its new range (Rai & Singh, 2020). According to Dietz and Edwards (2006), plant invasion is a multi-phase process that includes introduction, colonization, naturalization, and impact. Invasive alien plant species can alter the abundance or diversity of the ecosystem in an invaded environment (Daehler & Strong 1994; Wilcove et al., 1994; Huenneke et al., 1990; Daehler, 2003). With their ability to flourish and proliferate aggressively in non-native environments, invasive plants pose significant ecological problems. The rapid degradation of natural habitats has significantly accelerated the rate of biological invasions over the past century (Schei, 1996). To better understand and track the development of plant invasion, scientists are looking at invasive plants genetically to find invasive genes, expression and regulatory patterns, and invasiveness genotypes (Mable, 2019). The IAPs are often generalists with a broad tolerance to ecological conditions. They are highly plastic and can alter fundamental and realized niches of the native community (Berg and Ellers 2010).

1.2 What is an invasive alien plant?

Any plant species that expands beyond its natural range is defined as an alien plant, and the one which causes ecological or economic harm (Richardson et al., 2000) is known as an alien invasive plant. Invasive alien plants are widely considered to be one of the serious threats to global biodiversity since they establish monocultures in new habitats. Plant invaders of natural ecosystems are also termed ‘environmental weeds’ (Richardson et al., 2000). They are spread intentionally or unintentionally by humans, birds, animals, and other pollinators. The alien species are considered invasive only when they tend to grow rapidly, reproduce quickly, and spread aggressively, with the potential to cause devastating effects on native flora and fauna, thereby affecting the biodiversity of the ecosystem. They affect biodiversity, directly or indirectly. The direct threats account for out-competing native species, utilizing nutrients or other resources, causing or carrying disease, and preventing the growth of the young native seedling. Indirect threats occur when native food sources are lost or replaced, leading to shifts in the food web (Rai & Singh, 2020).

1.3 Stages of plant invasion

The process of plant invasion is typically described through four interrelated stages: transport, establishment, spread, and impact (Figure 1). This conceptual framework helps us understand how alien plant species progress from introduction to becoming invasive, and what ecological or anthropogenic factors influence each step (Blackburn et al., 2011; Pyšek et al., 2012).

1.3.1 Transport

The first stage involves the transport of plant propagules (seeds, spores, or vegetative parts) from their native range to a new environment. Sometimes this can occur intentionally through ornamental or agricultural purposes, or unintentionally, via containments, animal fur, bird excretion, or water (Mack *et al*., 1996; Hulme, 2009). Many invasive species were originally introduced as ornamentals or for economic purposes but later escaped cultivation and colonized new ranges (Richardson et al., 2000).

1.3.2 Establishment

Once introduced, the propagule germinate, grow, and reproduce in the new environment. Successful establishment depends on the match between the plant's traits and the abiotic and biotic conditions of the habitat (Sakai et al., 2001). Factors such as reproductive strategy, phenotypic plasticity, tolerance to local environmental stressors, and escape from natural enemies play critical roles in this stage (Levine et al., 2004; Catford et al., 2009).

1.3.3 Spread

After establishment, invasive species expand their population size and geographic range. There are different dispersal mechanisms such as wind, water, or animal movement, and human activities (Wilson *et al.*, 2009; Blackburn *et al*., 2011). The habitat and availability of suitable resources such as temperature, nutrients, water, etc also influence the rate and extent of spread (Richardson *et al.*, 2000).

1.3.4 Impact

The impacts can be ecological, such as reducing native biodiversity, altering ecosystem processes (e.g., nutrient cycling, wild fire), and displacing native plant communities; economic loss, such as reducing crop yields or increasing management practices and affecting human or animal health (Pyšek *et al.*, 2012). Invasive plants often form monocultures, modify soil conditions, and engage in novel interactions (e.g., allelopathy or enhanced mutualisms) that amplifies the ecological imbalance (Klironomos, 2002; Callaway & Ridenour, 2004).

1.4 The concept of "Endophytic Microorganisms"

The word "endophyte" was coined by De Barry in 1866 to describe organisms that live within plant tissues (Wilson, 1995). Etymologically, "phyte" means "plant" and "endo" signifies "within." According to recent studies, the evidence suggests that approximately 300,000 plant species are in existence, with endophytes constituting the obvious majority (Smith et al. 2008). Indeed, microbial endophytes, particularly those of the fungal and bacterial variety, have been identified in the majority of studied plant species. Partida-Martínez and Heil (2011) argue that an endophyte-free plant is an atypical state in nature; in the absence of endophytes, plants would be vulnerable to environmental stressors and experience a reduction in their resistance against infections (Timmusk et al. 2011). Initially, endophytes were defined as microorganisms that colonize living plant tissues (e.g. leaves, stem, roots, seeds, trunk, flowers and fruits) causing asymptomatic and undetected infections solely within the plant, without exhibiting any disease symptoms, either throughout their entire life cycle or a portion thereof (Figure 1.4). Consequently, irrespective of taxonomic or infection cycle considerations, the term "endophyte" encompasses both the spatial location and the infection strategy utilized by a particular fungus or bacterium (Wilson, 1995). Nevertheless, the contemporary descriptions of endophytes have become more refined, focusing on their categorization into fungal and bacterial types and delineating their relationships as either strict or facultative association (Rosenblueth and Martínez-Romero, 2006, Khare et al., 2018).

1.5 Different categories of endophytes

In general, two primary categories of endophytes have been identified, which are distinguished by their ecological functions, taxonomy, plant hosts, and evolutionary relationships (Rodriguez et al 2009). The clavicipitaceous endophytes are fungal endophytes that (C-endophytes) infect specific grasses, while the non-clavicipitaceous endophytes include microorganisms such as bacteria, archaea, and viruses, in addition to fungi. (NC-endophytes) can be isolated from asymptomatic tissues of nonvascular plants, ferns and their allies, conifers, and angiosperms (Rodriguez et al 2009).

1.5.1 Clavicipitaceae endophytes

Clavicipitaceous endophytes constitute a limited group of phylogenetically related fungi, typically challenging to culture *in vitro* (Bischoff & White, 2005). They are predominately associated with grasses and vertically transmitted (Devarajan & Suryanarayanan, 2006). This group of fungi are well known as symbiotic and free-living fungi. For example *Epichloë*, *Claviceps, Cordyceps* and *Balansia* are endophytes that are reportedly found in grasses family (Leuchtmann *et al*., 2000; Bacon & White, 2016). Individuals within this group, classified under the order *Hypocreales*, and are recognized as pathogens, their ability to generate bioactive compounds, and some are known as saprotrophs, or endophytes (Spatafora et al., 2007; Rodriguez et al., 2009). *Epichloë* species colonize grass during cool-seasons. Many clavicipitaceous endophytes are known to protect their host from insect herbivores (Rodriguez et al., 2009).

1.5.2 Non-Clavicipitaceous endophytes

These endophytes consist of a wide variety of fungi that can be distinguished into distinct functional categories based on their ecological functions and life cycles. Non-clavicipitaceous endophytes colonise the aerial tissues of plants without showing symptoms, thereby residing within these tissues for at least a portion of their life cycle. These endophytes are primarily restricted to the phylum *Ascomycota* and their anamorphs. They infect a wide range of plant hosts and are transmitted horizontally. They are classified into ecological roles such as commensals (Deckert *et al*., 2001), mutualists (Redman *et al*., 2002; Arnold *et al*., 2003), and latent pathogens (Gonthier *et al.*, 2006; Suryanarayanan & Murali, 2006). Their functions include inducing systemic resistance to hosts (Vu *et al*., 2006), outcompeting pathogens for resources and space (Rodriguez *et al*., 2009), and producing antifungal compounds (Schulz *et al*., 1999) and cell wall-solubilizing enzymes (Cao *et al*., 2009).

1.6 Advancements in molecular and genomic methods for studying endophytic diversity

Traditionally, endophytes were obtained from surface-sterilized sections of plant tissue cultured in a nutrient-rich media. According to "Great Plate Anomaly" stated by Staley and Konopka in 1985, microbes viewed under a microscope cannot thrive in a laboratory condition, since they are non-viable or viable but non-culturable (Epstein, 2009). However, in recent times, culture-independent methods have been used to shed light on a wide variety of nonviable and nonculturable endophytes using the whole-genome sequencing of endophytic communities, and marker genes such as internal transcribed spacer regions (ITS1 and ITS2) for fungi, and ribosomal rRNA gene (16S rRNA) sequencing for bacteria (Kandel et al., 2017). The study of the host-microbiome relationship makes extensive use of omics approaches such as transcriptomics and genomes. Metagenomics provides comprehensive evidence of microbial diversity and their functional abilities by analysing the genetic composition of microbial communities in their natural environments. Contrarily, transcriptomics delves into the analysis of gene expression patterns within a particular organism or community, offering insights into the genetic components that are active at any given moment (Qi et al., 2023).

1.7 The importance of endophytic diversity in plant health and ecology

They are ubiquitous, colonizing a wide range of plant species, and have been successfully isolated from almost every plant investigated so far (Nair & Padmavathy, 2014). Endophytes play an important role in the overall health of plants. They confer tolerance and resistance during abiotic and biotic stressors while fostering improved plant growth (Oukala *et al.*, 2021). Although research on plant endophyte interactions has advanced considerably, the mechanisms by which endophytes alleviate the environmental stresses remain elusive. Studies indicate that plants actively perceive and associate with particular microbial partners to establish beneficial symbiotic relationships

(Hardom *et al*., 2008; Oukala *et al*., 2021). The long-term association between endophytes and host plants has driven genetic changes that promote niche-specific adaptation. Such adaptations involve the acquisition of plant-derived DNA sequences, enabling certain endophytes to synthesize phytochemicals that are similar to their host plants (Zhang *et al*., 2006). Successful colonization of endophytes depends on a compatible interaction between the host plant and the microbe. Upon initial entry, the endophyte is recognized by the host, initiating a molecular cross-talk through the exchange of signaling compounds (Khare *et al*., 2018).

1.8 The genus "*Lantana*"

Lantana is a member of the *Verbenaceae* family, found in more than 60 countries. With about 100 species, the genus Lantana is mostly found in Latin America, with a smaller number in Africa and Asia (Kannan *et al*. 2013). *Lantana camara*, a hybrid species of tropical America often known as wild or red sage, is the most widely distributed *Lantana* (Figure 1.1). The widespread distribution demonstrates its ecological resilience and capacity to flourish in a variety of environmental settings (Broughton 2000; Day *et al*. 2003; Kannan *et al*. 2013). *Lantana* has been documented across a wide geographic range in India, from the Central Himalayas to the southernmost regions, where it has been reported to displace numerous native plant species (Kannan *et al*., 2013). As described by Parsons and Cuthbertson in 2001, *Lantana camara is* ubiquitous and invasive with a global presence affecting pastures and native forests. This hardy plant thrives in open, sunny spaces and has been known to invade forest plantations and riparian zones. Although *L.camara* was initially brought to India in the early nineteenth century as an aesthetic plant, it now spreads widely over the country (Thakur *et al*. 1992). It grows in discrete clusters or dense thickets, displacing more desirable plants. Gentle and Duggin in 1997, studied the probable role of plant invasion and control of ecosystems. Mallik and colleagues (1998) observed that certain perennial species of *Lantana* release allelochemicals into the soil. This allelopathic behavior contributes to the formation of dense, localized invasions that reduce habitat suitability for native flora and fauna, thereby posing significant threats to nature reserves and biodiversity hotspots and (Sundaram & Hiremath, 2012) The congeneric counterpart *L.indica*, is a wild shrub often known as "Indian Lantana" is indigenous to the India and Asian subcontinents (Figure 1.2). It is extensively dispersed in tropical and subtropical regions. This plant is characterized by its light purple or white coloured small flowers and opposite, whorled, pubescent leaves, the stem growing up to 1-2 meters in height. It is commonly used in Ayurveda medicine for the treatment of malaria, rheumatism and tetanus (Akhtar *et al*., 2006).

1.9 The potential roles of endophytes and importance in understanding plant invasiveness

Plant invasions are driven by dynamic interactions between biotic and abiotic factors. Among the biotic factors, endophytes of invasive plants are one of the major aspects that require attention. Generally, endophytes are known to help plants and have unique survival strategies during biotic and abiotic stresses. A key factor in plant symbiosis is host tolerance to stressful conditions

(Rodriguez *et al*., 2004; Marquez *et al*., 2007), help growth (Ernst *et al*., 2003) and defence (Bailey *et al*., 2006). Endophytic fungi, which inhabit the internal tissues of plants are an intriguing aspect, yet overlooked ecological paradox. They are important in plant symbiosis, regulating host stress tolerance, plant defense, growth, and biodiversity. According to recent estimates of fungal diversity, more than 90% of fungal species and endophytes are unknown (Chadha et al., 2015; Blackwell, 2011). Nevertheless, there is a significant lack of knowledge about the variety, geographic distribution, and host specificity of endophytes of invasive and native plants. The symbiotic relationship between endophytes and their plant hosts has been seen to have mutualistic effects, which significantly contribute to several ecological functions. It is plausible that invasive plants possess altered endophytic communities compared to native species, thereby influencing the composition and dynamics of recipient plant communities. As a result, endophytes can help reduce both biotic and abiotic stress through their interactions with invasive species, ultimately affecting native plant populations. Microorganisms can influence both plant growth and health in positive, negative, or neutral ways. For example, many invasive plant species harbor distinct microbiomes compared to their native counterparts, potentially conferring a competitive advantage in particular environments (Qi et al., 2023). This highlights the importance of exploring the largely understudied domain of endophytic fungi and bacteria in both invasive and native plants to better understand the intricate interactions that explains invasion success.

1.10 Theories on Global Plant Invasion Success: A Microbial Perspective

The question of what makes plant invasions successful remains a major challenge in invasion biology. Numerous hypotheses explain invasion success by focusing on plant traits, propagule pressure, and environmental filters. However, microbial communities play a key part and haven't been studied much. A microbiome perspective, particularly on plant endophytes can offer novel insights into the process of invasion (Traveset & Richardson, 2014; Hardoim et al., 2015). Microbial partners can modulate plant health, fitness, and competitive ability, at different stages of the invasion process from establishment to spread. Therefore, addition of microbial interaction to plants and its ecosystem attributes, would give us a better understanding of invasion dynamics. In the following section, I discuss major invasion hypotheses pertaining to the role of microbes.

1.10.1 Biotic Resistance Hypothesis

The biotic resistance hypothesis states that an environment with diverse native communities resist invasion of alien plants by occupying niche spaces and efficiently utilizing the resources (Elton, 1958; Beshai *et al*., 2023). This resistance can extend to microbial competition, where the native microbiome, including antagonistic bacteria and fungi, can outcompete or inhibit the pathogen. Conversely, reduced microbial diversity due to disturbance or low native richness allows invaders and their associated beneficial microbes to exploit the plant health (Kowalchuk *et al.*, 2002).

1.10.2 Island Susceptibility Hypothesis

Islands are often considered invasion-prone due to low species diversity and evolutionary isolation (Vitousek, 1988; Kueffer *et al.*, 2010). This applies not only to plant communities but also to the microbial symbionts of plants. The lack of microbial antagonists or native microbiomes on islands can facilitate invader success by reducing pathogen loads or allowing novel mutualists to thrive unchecked.

1.10.3 Invasional Meltdown Hypothesis

The invasional meltdown hypothesis suggests that introduced species has a positive feedback over other introduced species and facilitate one another's establishment potentially through shared microbiota or microbial feedbacks (Simberloff & Von Holle, 1999; Simberloff 2006). Soil microbes facilitate other invaders by altering soil microbial composition in a way that benefits subsequent invaders. However, this is context-dependent. The allelopathic impact of the plant Acacia and Eucalyptus species has been shown to reduce the germination of co-occurring exotics, due to microbial suppression or phytotoxic compounds that alter soil microbial communities (Riveiro *et al*., 2024), contradicting the invasional meltdown theory.

1.10.4 Novel Weapon Hypothesis

The novel weapon hypothesis states that invaders produce allelochemicals to suppress the native plant species (Callaway & Ridenour, 2004). These biochemical compounds can influence the microbial communities by inhibiting native mutualists that are beneficial to the invader (Inderjit *et al.*, 2011). For instance, fungal endophytes of *Centaurea stoebe* have shown to enhance the allelopathic potential of invasive species in both greenhouse and in field indicating a synergistic effect between endophytic fungi and allelochemical production (Aschehoug *et al*., 2014). However, distinguishing between allelopathy and phytotoxicity is critical for such interactions.

1.10.5 Enemy Release Hypothesis

The enemy release hypothesis suggests that invasive species gain a competitive advantage in their new environments by escaping their natural enemies, such as herbivores and pathogens (Keane & Crawley, 2002). The absence of soil-borne pathogens or microbial antagonists can significantly enhance plant fitness. For instance, *Centaurea maculosa* in North America benefits from reduced native soil pathogens compared to its native range in Europe (Callaway et al., 2004). Endophyte profiles also shift in invaded ranges, with invaders often harboring fewer pathogenic fungi and more mutualistic or neutral taxa (Sun & He, 2010; Shaerin et al., 2018), supporting enemy release at the microbial level.

1.10.6 Enhanced Mutualism Hypothesis

According to enhanced mutualism theory, the invasive species forms more effective or novel mutualisms in the invaded range, enhancing growth and competitiveness (Reinhart & Callaway, 2006). For example, invasive plants often associate with generalist endophytes or plant growth-promoting microbes (PGPMs) in their new range, which provide benefits such as stress tolerance,

nutrient mobilization, and hormone production (Farhana et al., 2018). *Chromolaena odorata* alters the soil microbiome by enriching *Fusarium semitectum* in its rhizosphere, inhibiting native species (Mangla et al., 2008) supporting microbes enhanced mutualism theory.

1.11 The Missing Link - Endophytes

Although existing theories focus largely on visible ecological patterns and interactions, microbial communities especially endophytes are fundamental but an overlooked player. These microbes contribute to nutrient uptake, stress resilience, secondary metabolite production, and pathogen defense (Lengrand et al., 2024). Studies showing invasive plants modifying soil microbial communities to their benefit, establishing positive feedback to promote dominance (Bozzolo & Lipson, 2013) together these microbial dimensions enrich the existing invasion hypotheses. Integration of plant invasion ecology and microbiome promises a more integrative framework for understanding and predicting plant invasion success. In this regard, it is utmost important to acknowledge the study of microbial interactions as an essential component in the process of comprehending the invasiveness of alien plants. By incorporating the dynamics of plant-microbe relationships into current theoretical frameworks, I will be able to contribute to a more integrated framework of biodiversity, community assembly, and ecosystem resilience, thereby advancing a comprehensive understanding of plant invasions.

Despite most of the studies of endophytes in invasion ecology, comparative studies between congeneric native and invasive plant species remain limited. A deeper understanding of how endophytic microbial communities differ between these closely related taxa could shed light on their roles in facilitating invasion success. This study investigates the composition and potential ecological functions of endophytes in a native vs. invasive *Lantana* species, aiming to reveal whether endophytic associations contribute to plant invasiveness.

1.12 The research questions and objectives of the study

1. Do native and invasive plants have distinct fungal and bacterial endophytes?
2. Do native and invasive plants have tissue specific fungal and bacterial endophytes?
3. Do fungal endophytes have abiotic stress tolerance?

- **Objective 1 –** Characterize endophytic bacterial and fungal communities in co-occurring native and invasive *Lantana* species through comparative amplicon sequencing
- **Objective 2 –** Characterize culturable fungal endophytes in co-occurring congeneric native and invasive *Lantana* Species through morphological and molecular analyses
- **Objective 3 –** Investigate abiotic stress tolerance of endophytic fungi isolated from congeneric native and invasive *Lantana* species

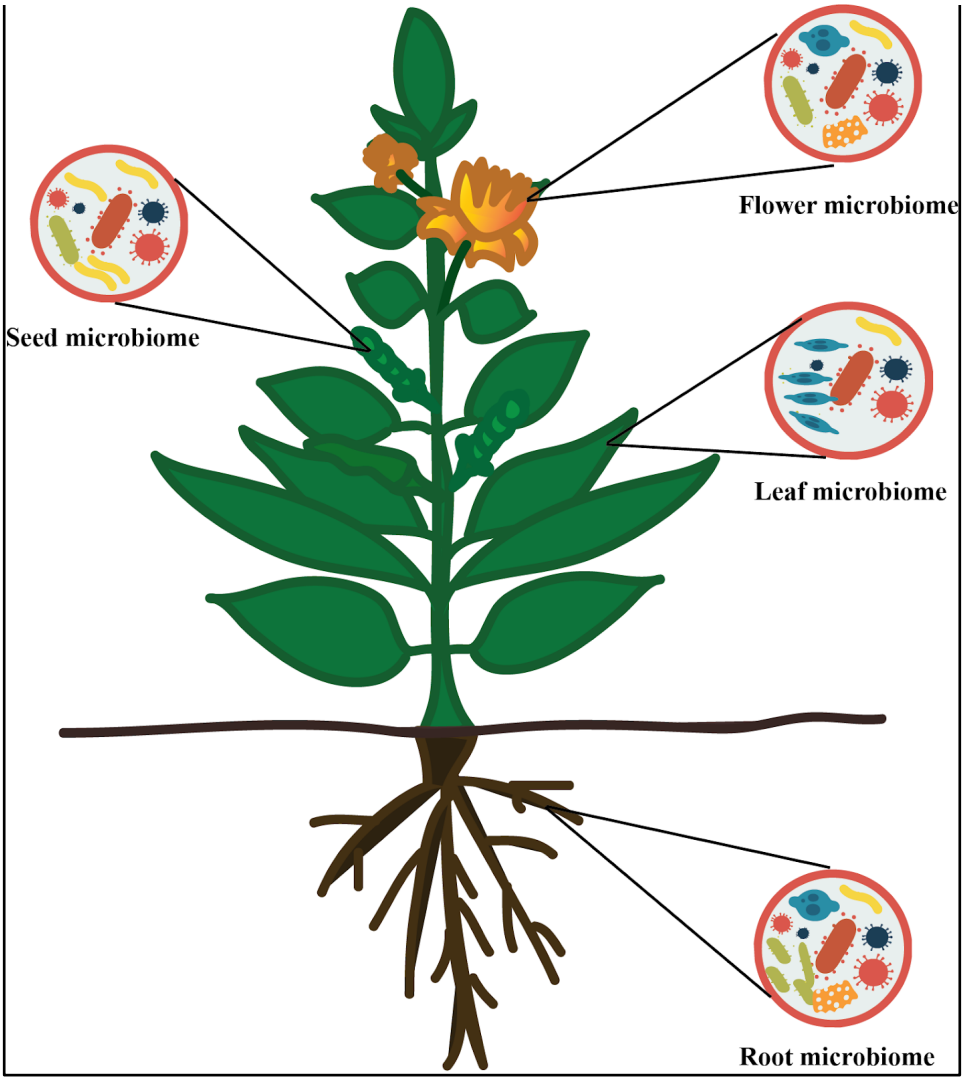


Figure 1.1: Illustration depicting the diverse microbiome associated with its host plant



Figure 1.2: The invasive plant *Lantana camara*



Figure 1.3: The native plant *Lantana indica*

Chapter 2

Characterize endophytic bacterial and fungal communities in co-occurring native and invasive *Lantana* species through comparative amplicon sequencing

2.1 Abstract

I characterized the bacterial and fungal endophytes present in invasive *Lantana camara* and non-invasive *Lantana indica* to determine whether they harbor distinct, host-specific endophytic communities. *Lantana camara* is an invasive plant species introduced to India from Central and South America, while *Lantana indica* is a native, non-invasive species that co-occur with *Lantana indica*. I sequenced the 16S rRNA gene and ITS regions using the amplicon metagenome to identify the bacterial and fungal endophytes respectively. I assessed the alpha diversity of fungal and bacterial endophytes using the Shannon index, and evaluated beta diversity with NMDS ordination based on Bray - Curtis dissimilarities. The results showed *Talaromyces* was the dominant fungal genus in both *L.* camara and *L. indica*, while the bacterial communities were dominated by the family *Clostridiaceae* in *L. camara* and *Pseudomonadaceae* in *L. indica.* The native plants showed greater bacterial diversity and evenness compared to invasive plants, whereas fungal communities did not show significant differences. My findings suggest that the environment plays a key role in shaping the endophyte communities. The native species showed higher diversity and evenness, reflecting their long-term adaptation to local biotic and abiotic conditions. In contrast, the invasive plant species showed dominance of specific bacterial and fungal taxa, supporting resource exploitation in the invaded region.

2.2 Introduction

Exotic plant invasions pose a major threat to global biodiversity (Schei, 1996). Their rapid spread and habitat disruption has accelerated over the past century, yet the ecological process underlying invasion remains insufficiently understood, limiting effective management (Adair & Groves, 1998). Invasion success is often linked to complex interactions between biotic and abiotic factors, with endophytes as key contributors. Initially, endophytes were defined as microorganisms that colonize living plant tissues, causing asymptomatic and undetected infections solely within the plant, without exhibiting any disease symptoms, either their entire life cycle or a portion thereof. Nevertheless, the contemporary descriptions of endophytes have become more refined, focusing on their categorization into fungal and bacterial types and delineating their relationships as either obligate or facultative with the host plant (Khare *et al*., 2018). They are ubiquitous, colonizing a wide range of plant species, and have been successfully isolated from almost every plant investigated so far (Dhanya N and S. Padmavathy, 2014).

Traditionally, endophytes were obtained from surface-sterilized sections of plant tissue cultured in a nutrient-rich media. According to the ‘Great Plate Anomaly’ proposed by Staley and Konopka

(1985), many microbes observable under a microscope fail to grow under laboratory conditions, as they are either nonviable or viable but nonculturable (Epstein, 2009). However, in recent times, culture-independent methods have been used to explore a wide variety of non-viable and non-culturable endophytes. Whole-genome sequencing, sequencing of internal transcribed spacer regions (ITS1 and ITS2) for fungi, and 16S rRNA gene for bacteria are widely used for the culture-independent methods (Kandel *et al*., 2017). Among these, next-generation sequencing (NGS) platforms such as Illumina have transformed microbial studies by allowing high-throughput sequencing of marker genes (e.g., ITS, 16S rRNA) (Degnan *et al.*, 2011). This approach revealed precise taxonomic resolution, functional potential, and provided detailed comparisons across hosts and environments, as demonstrated in this study.

Here, I compare the endophytic bacterial and fungal communities of the invasive *Lantana camara* and its native congener *Lantana indica* using amplicon sequencing. This chapter aims to (1) assess the alpha and beta diversity of bacterial and fungal endophytes in *Lantana camara* and *Lantana indica*, (2) identify taxonomic and community-level differences between the two plant hosts, and (3) evaluate whether microbial community composition reflects patterns associated with plant invasion success. By evaluating microbial diversity, composition, and potential functional roles, we aim to shed light on how endophytes differ between two congeneric plants with contrasting qualities.

2.3 Materials & Methods

2.3.1 Biological material

The plants were collected from Billigirirangan Hills, or BR Hills, situated at the convergence of the Western Ghats and the Eastern Ghats, in Karnataka, India (Priti *et al.*, 2016). The hill is covered by a combination of deciduous and evergreen woods. As one of the largest natural biodiversity hotspots, it harbors a rich assemblage of both native and invasive plant species, making it a critical site for ecological studies. The diversity of endophytic bacteria and fungi was assessed in both the above-ground (leaf & stem) and below-ground (root) parts of the invasive and non-invasive plant genera, namely *Lantana camara* and *Lantana indica,* respectively. Plants were collected in triplicate from five different sites to avoid bias (Figure 2.1 & Table 2.1). Each site was selected where the native and invasive plants co-occurred within an area of approximately 10 × 10 metres. From each site, three individuals per species were collected (i.e., 3 *L. camara* and 3 *L. indica* plants per site), resulting in a total of six plants per site and 30 individual plants in total across all five sites. The plants were collected aseptically, transported to the laboratory in polyethylene bags, and promptly processed.

2.3.2 Extraction of total endophytic microbiome

The leaf, stem (the aboveground), and root (the belowground), 1.0 g each from three replicates, were excised. The plants were washed thoroughly using sterile distilled water (50 mL) to remove

any external debris. The washed explants were surface-disinfected to remove epiphytic microbes by washing them in 1M sodium hypochlorite solution (NaOCl) for 2.0 min and 70% ethyl alcohol for 30 sec, followed by four serial washings in sterile distilled water for 30 sec under aseptic conditions. An explant from each tube was plated out on a potato dextrose agar medium for a sterility check. The surface-disinfected leaf, stem, and root explants were subjected to endophytic microbiome extraction by macerating in a pre-sterilized pestle-mortar in a laminar airflow cabinet. Surface-sterilized plant tissues (root, stem, and leaf) were ground into fine powder using liquid nitrogen. The finely powdered tissue was transferred to 1.5 ml sterile Eppendorf tubes. The total genomic DNA of the plant tissues was extracted using the cetyltrimethylammonium bromide (CTAB) method (Rajendrakumar *et al*., 2008) with few modifications.

2.3.3 Metagenome Library Preparation

Fungal diversity was assessed using the Internal Transcribed Spacer (ITS) region with specific primers, ITSF (5′-TTGGTCATTTAGAGGAAGTAA-3′) and ITSR (5′-GCTGCGTTCTTCATCGATGC-3′). For bacterial diversity, the 16S rRNA V3–V4 region was amplified using primers 16SF (5′-AGAGTTTGATGMTGGCTCAG-3′) and 16SR (5′-TTACCGCGGCMGCSGGCAC-3′). Amplicons were purified, indexed with Illumina adapters (8 PCR cycles), repurified, and quantified with the Qubit dsDNA High Sensitivity Assay Kit. Purified amplicons were sequenced using Illumina MiSeq platform using the 2×300 paired-end (PE) kit targeting the ITS region for fungi and the V3–V4 region for bacteria. Raw base call (BCL) data from the sequencer was demultiplexed to generate FASTQ files. Raw sequences from the FASTQ files were checked for the quality using FastQC (v0.11.9), ensuring that only high-quality reads were used for subsequent analyses. The reads were merged and de-multiplexed to obtain unique representative sequences. These representative sequences were then assigned as Amplicon Sequence Variants (ASVs) using a 97% similarity threshold. Fungal ASVs were taxonomically assigned using the UNITE ITS database, and bacterial ASVs were classified using the Greengene 16S database. QIIME2 (Bolyen et al., 2019), an open-source software for microbiome research, was used to perform downstream analyses. All samples were rarefied to the lowest sequencing depth to ensure even sampling effort and valid diversity comparisons

2.3.4 Data analysis:

Microbial community analysis was performed using the microeco R version 4.3.1 package (Liu et al., 2021). The different taxonomic compositions and relative abundances were visualized using stacked bar plots and hierarchical clustering using the trans_abund class function based on the ggplot2 package. Differences in alpha diversity were estimated using the trans_alpha function between the native (*Lantana indica*) and invasive (*Lantana camara*) plants, and their significance were assessed using the Wilcoxon rank-sum test method was chosen because it is a non-parametric test and does not assume normal distribution and effectively compare median diversity values between two independent groups. Alpha diversity indices such as Shannon and Chao1 were used

where Shannon index captures both species richness and evenness, reflecting community heterogeneity, and Chao1 estimates total richness by accounting for rare taxa, providing insight into rare microbial diversity (Daly et al., 2018). Beta diversity was assessed using the trans_beta function to determine Non-metric Multidimensional Scaling (NMDS) based on Bray-Curtis dissimilarity matrices, to visualize complex multivariate community differences in lesser dimensions, effectively showing dissimilarities in species composition between host plants (Anderson, 2001, Anderson et al., 2011). PERMANOVA (Permutational Multivariate Analysis of Variance) was performed using 999 permutations to statistically test whether the microbial community composition differs significantly between native and invasive hosts, the tissue types and different sites based on dissimilarity matrices using (adonis2) function in vegan package (Anderson, 2001, Anderson et al., 2011). The community overlap between plant hosts was determined using the trans_venn class function to develop a Venn diagram to display the proportion of shared and unique amplicon sequence variants (ASVs). To evaluate host or environmental filtering between the two hosts, phylogenetic clustering was assessed by the Nearest Taxon Index (NTI) that quantifies terminal phylogenetic clustering indicating selection for closely related taxa within communities and Net Relatedness Index (NRI) that measures overall phylogenetic relatedness across the tree to infer whether host filtering or environmental selection drives community assembly in R using the picante package. Positive values indicate phylogenetic clustering, and negative values show over dispersion and zero values show randomness (Kembel et al., 2010). Functional profiles of bacterial communities were predicted using the tool PICRUSt2 (Douglas et al., 2020), to infer putative metabolic and functional pathways from 16S rRNA data, linking taxonomic data to ecological functions. The functional outputs were used to compare predicted metabolic potentials across samples. The FUNGuild annotation tool (Nguyen et al., 2016), was used to assign fungal ASVs to ecological guilds such as pathotrophs, saprotrophs, or symbiotrophs, thus enabling interpretation of the trophic roles and potential ecological functions of fungal endophytes. It is a curated database that classifies fungal ASVs, to infer the ecological roles of fungal taxa. Taxonomic assignments were used as input to interpret their trophic modes.

2.4 Results:

2.4.1 Diversity of endophytic bacteria and fungi:

The size of the sequenced library averaged between 12290 and 97699 for endophytic bacteria and 31359 and 169549 for endophytic fungi.In total, 820,728 and 826,745 bacterial ASVs were identified in *Lantana camara* and *Lantana indica*, respectively, while 927,361 and 592,231 fungal ASVs were detected in *L. camara* and *L. indica*. The ASV of endophytic bacteria was slightly higher when compared to fungi in *L. indica,* whereas in *L. camara* both endophytic bacteria and fungi were found to be nearly equal in abundance. Hence, both bacterial and fungal samples were rarefied based on the lowest sequencing depth to avoid bias and compare the microbial diversity across all samples. The rarefaction curves for all analyzed samples reached a clear asymptote (Figure 2.2 & Figure 2.16), showing sequencing depth sufficient to capture the overall diversity

across samples.

2.4.2 Bacteria:

A total of 17 bacterial phyla, 24 classes, 59 orders, 97 families, and 150 genera were taxonomically identified from the amplicon sequence variant (ASVs) library. Among these, *Proteobacteria* was the most dominant phylum in both the invasive species (*Lantana camara*) and its native counterpart (*Lantana indica*), followed by *Firmicutes* and *Bacteroidota* (Figure 2.8). At the genus level, *Clostridiales* and *Enterobacterales* were more prevalent in *L. camara*, while *Pseudomonas* was the dominant genus in *L. indica*.

Alpha diversity was estimated at the genus level using the Shannon index and Chao1 indices. The Shannon index showed a statistically significant difference in microbial diversity between *L. camara* and *L. indica* ($p = 0.025$) (Figure 2.3), whereas Chao1 indicated no significant difference in species richness ($p > 0.05$) (Figure 2.4). Beta diversity was estimated using NMDS based on Bray-Curtis dissimilarity. PERMANOVA showed no significant difference in bacterial community composition between the invasive *Lantana camara* and the native *L. indica* ($F = 2.30$, $R^2 = 0.1133$, $p = 0.06$; Figure 2.5). Likewise, no significant variation was observed between above- and below-ground tissues ($F = 1.45$, $R^2 = 0.2139$, $p = 0.18$; Figure 2.6) or among sampling sites ($F = 2.48$, $R^2 = 0.3977$, $p = 0.43$; Figure 2.7).

Despite the overall similarity, *L. camara* exhibited a higher relative abundance of *Enterobacterales* and *Clostridiaceae* (Figure 2.10) in both above and below-ground tissues, whereas *Pseudomonas* remained dominant across all tissues in both hosts (Figure 2.9). Additionally, the relative abundance of these genera varied across different sites (Figure 2.11). In terms of unique taxa, *L. camara* and *L. indica* hosted 22.6% and 37.1% unique bacterial communities, respectively, with about 40.2% of ASVs shared between them (Figure 2.12).

Indicator species analysis revealed significant tissue-specific associations (Table 2.2). *Comamonadaceae* was found to be a key indicator in the overall microbiome of *L. indica* (IndVal = 0.894, $p = 0.037$), while in *L. camara*, *Unassigned_2* (IndVal = 0.958, $p = 0.016$) and *Enterobacteriaceae* (IndVal = 0.894, $p = 0.043$) were significantly associated with aboveground tissues, suggesting a potential role in shaping the aerial endophytic community of the invasive host.

Host/environment filter analysis showed both *L. camara* and *L.indica* show positive NRI (Figure 2.13) and NTI (Figure 2.14) values, indicating phylogenetic clustering of bacterial endophytes. Notably, *L. camara* exhibits slightly higher NRI and NTI scores than *L. indica*, suggesting stronger host filtering or environmental selection in the invasive species, particularly at fine (NTI) and broad (NRI) phylogenetic scales.

The PICRUSt2 analysis showed different functional profiles between *Lantana camara* and

Lantana indica. *L. indica* showed higher abundances with pathways like ILEUSYN-PWY, VALSYN-PWY, PWY-5101, PWY-3781 (amino acid biosynthesis), PWY-5667 (terpenoid biosynthesis), PWY-5973 (nucleotide biosynthesis), and PWY-7111 (cell wall synthesis), indicating enhanced host metabolism. In contrast, *L.camara* was enriched with NONOXIPENT-PWY and FASYN-ELONG-PWY, related to pentose phosphate metabolism and fatty acid elongation, suggesting possible adaptation to abiotic stress and production of allelochemicals. These findings reflect host-specific microbial functional differentiation (Figure 2.15).

2.4.3 Fungi:

A total of 9 phyla, 31 classes, 83 orders, 188 families, and 292 genera of endophytic fungi were taxonomically identified from the ASV library. Among the phyla, *Ascomycota* was found to be the dominant in both *Lantana camara* and *Lantana indica*, followed by *Basidiomycota* (Figure 2.22). At the genus level, *Talaromyces* was the most abundant in both hosts, while *Fusarium* was more prevalent in *L. camara* and *Psathyrella* in *L. indica* (Figure 2.23). *Talaromyces* was consistently dominant across aboveground and belowground tissues (Figure 2.24) and across all the collection sites (Figure 2.25). However, the relative abundance of other genera varied by tissue type and location.

Alpha diversity was assessed using the Shannon index and Chao1 estimator. They showed no significant differences between the two plant species (p = 0.31 and p = 0.20, respectively), indicating comparable fungal richness and diversity (Figures 2.17 & Figures 2.18). Beta diversity analysis based on Bray-Curtis dissimilarity showed no significant variation in fungal community composition between the invasive *Lantana camara* and the native *L. indica* (F = 2.30, R^2 = 0.1133, p = 0.25; Figure 2.19). Similarly, no significant difference was observed between the above- and below-ground tissues (F = 1.45, R^2 = 0.2139, p = 0.30; Figure 2.20). However, a near-significance was detected across sampling sites (F = 2.48, R^2 = 0.3977, p = 0.057; Figure 2.21).

Approximately 51.8% of fungal taxa were shared between the two hosts, while *L. camara* and *L. indica* harbored 26.6% and 21.6% unique taxa, respectively (Figure 2.26). Indicator species analysis showed that several taxa are significantly associated with specific tissues of *L. camara* (Table 2.3). In the overall fungal community, *Wojnowiciella sp.* (IndVal = 0.889, p = 0.040), *Phaeosphaeria sp.* (IndVal = 0.888, p = 0.040), and *Stigmatomyces sp.* (IndVal = 0.872, p = 0.042) were found to be significant indicators. In aboveground tissues, *Phaeosphaeriaceae gen.* (IndVal = 0.996, p = 0.007) and *Phaeosphaeria sp.* (IndVal = 0.894, p = 0.043) showed strong associations. In the belowground tissues, *Colletotrichum sp.* (IndVal = 0.997, p = 0.039) and *Mycena sp.* (IndVal = 0.894, p = 0.047) were found to be significantly enriched, indicating tissue-specific fungal colonization patterns in the invasive host.

Host/environment filter analysis showed both *L.camara* and *L.indica* show positive NRI (Figures 2.27) and NTI (Figures 2.28) values, showing phylogenetic clustering of fungal endophytes. Like endophytic bacteria, *L. camara* exhibits slightly higher NRI and NTI scores than *L. indica*,

suggesting stronger host filtering or environmental selection in the invasive species.

The presence of saprotrophs and pathotrophs in both *L. camara* and *L. indica* suggests functional specialization shows a more functionally diverse and less specialized fungal community, possibly reflecting a more generalized microbial interaction strategy (Figure 2.29).

2.5 Discussion

This chapter focuses on the diversity and community composition of endophytic bacteria and fungi present in the invasive *Lantana camara* and its native congener *Lantana indica* to understand whether microbial community assembly varies across co-located species and contributes to invasion success. Amplicon-based metagenomic sequencing was employed, considering that a large proportion of microbial taxa remain unculturable under standard laboratory conditions (Staley and Konopka, 1985; Epstein, 2009). Despite comparable environmental conditions across sampling sites, distinct microbial assemblages were observed between the two host plants. Bacterial communities in both species were dominated by *Proteobacteria*, followed by *Firmicutes* and *Bacteroidota*, aligning with previous findings in plant microbiomes (Wu et al., 2021; Gola et al., 2023). Interestingly, the invasive plant exhibited a higher relative abundance of *Enterobacterales* and *Clostridiaceae*, suggesting functional adaptation toward plant growth promotion and stress tolerance. Both taxa are known endophytic bacteria involved in nitrogen fixation, hormone production, and abiotic stress mitigation (Compant et al., 2010; Hardoim et al., 2015; Figueiredo et al., 2020). Notably, *Enterobacter* spp. was first recognized as beneficial endophytes due to their ACC deaminase activity, which alleviates ethylene-induced stress, while *Enterobacter* sp. SA187 enhances plant growth and salt tolerance through rhizosphere-mediated mechanisms (Liu et al., 2022). Similar host-specific differentiation in bacterial communities has also been reported in seagrasses, where native and invasive species exhibit distinct but overlapping microbiomes, reflecting both environmental adaptation and functional needs (Aires et al., 2021). In contrast, native *L. indica* was enriched with *Pseudomonas*, a genus associated with generalist mutualisms and often found prevalent in native plant microbiomes (Janardhan and Vijayan, 2012). Alpha diversity indices revealed that bacterial communities in *L. indica* possessed significantly higher Shannon diversity, while species richness estimated by Chao1 was also greater in the native host but not significant. This pattern suggests a more even distribution of bacterial taxa in native plants, reflecting long-term adaptation to local biotic and abiotic factors. Invasive *L. camara* appeared to be enriched by *Enterobacterales* and *Clostridiaceae*, with the rest occurring at low abundance of generalist bacteria, supporting previous findings that invasive species selectively recruit microbial partners (Elsheikh et al., 2021; Dawson & Schrama, 2016). Comparable patterns have been observed in *Prosopis* congeners, where invasive *P. juliflora* exhibited higher abundance of antimicrobial-related species in its rhizosphere microbiome, likely an adaptive microbial response aiding invasion success (Kaushik et al., 2021). Beta diversity analysis based on Bray-Curtis's dissimilarity showed a partial difference in bacterial community composition between the two plant species, though it was not statistically significant. The R^2 values suggest that hosts

showed about 11% of the variation, above and below ground tissues around 21%, and different sites nearly 40%. Together, they show differences in community structure, that both host and environmental factors shape the bacterial community composition. Approximately 40.2% of bacterial ASVs were shared, while 22.6% and 37.1% were unique to *L. camara* and *L. indica*, respectively. This suggests that both plants recruit a core microbiome from the shared environment; however, they also harbor host-specific microbial taxa (Reinhart & Callaway, 2006; Kato-Noguchi & Kato, 2025). Such host specificity and overlap are consistent with findings in invasive seagrasses, where native and non-native hosts share environment-related OTUs but differ functionally in their core microbial communities (Aires et al., 2021). Indicator species analysis revealed significant tissue-specific associations (Table 2.2). *Comamonadaceae* was identified as a key indicator in the overall microbiome of *L. indica*, while in *L. camara*, *Unassigned_2* and *Enterobacteriaceae* were significantly associated with aboveground tissues, suggesting a potential role in shaping the aerial endophytic community of the invasive host. The presence of such indicator taxa highlights tissue-level microbial specialization, indicating that certain endophytes are preferentially recruited by specific tissues to support host adaptation. Similarly, indicator species analysis of the fungal community showed that several taxa were significantly associated with specific tissues of *L. camara*. *Wojnowiciella*, *Phaeosphaeria*, and *Stigmatomyces* were key fungal indicators in the overall community, while *Phaeosphaeriaceae* and *Phaeosphaeria* were closely associated with aboveground tissues. In contrast, *Colletotrichum* and *Mycena* were more prominent in belowground tissues. These associations indicate strong tissue-specific fungal colonization patterns, suggesting that different fungal endophytes contribute to specialized ecological roles within distinct plant compartments of the invasive host. Fungal communities showed no differences in the diversity and community composition. While *L. camara* harbored a numerically greater number of fungal ASVs, this cannot imply that invasive plants harbor greater numbers of fungi because it depends on the sequencing depth and the sequencing methods. The alpha diversity indices (Shannon and Chao1) did not differ significantly between the two plant hosts, implying a conserved fungal diversity across species, likely shaped more by environmental filtering than host specificity (Saikkonen et al., 2000; Christian et al., 2016). The dominance of *Ascomycota*, particularly the genus *Talaromyces*, across both plant species and tissue types supports previous reports that certain fungal endophytes are generalists (Pandey, 2024). However, *Fusarium*, known for its dual role as both symbiont and pathogen, was more abundant in the invasive host, highlighting the potential for functional divergence (Schulz and Boyle, 2005). Similar trends were found in *Tabebuia* species, where *T. chrysantha* harbored a significantly richer and more diverse fungal and bacterial community compared to *T. billbergii* (Llacsa et al., 2019). The alpha and beta diversity were similar between the hosts, different tissue types and the sites, the R^2 values indicate that both host and environmental factors play a role in shaping the microbial communities. The site differences were prominent in fungi, suggesting that fungal assembly is more responsive to environmental conditions. There are notable proportions of ASVs that are host-specific (26.6% in *L. camara*, 21.6% in *L. indica*), while 51.8% were shared. These results show that fungal endophyte communities are shaped by both host identity and shared environmental

factors (U'Ren et al., 2019). Indicator species analysis revealed associations of *Phaeosphaeriaceae* with aboveground and *Colletotrichum* with belowground tissues of *L. camara*, suggesting tissue-specific colonization patterns that confer selective advantages to the invasive plant. This pattern aligns with observations that leaf and root-associated microbes often differ markedly in both diversity and function across native and invasive species, reflecting their distinct physiological demands (Aires et al., 2021). The co-occurrence of unique and shared taxa, particularly among bacterial endophytes, suggests that invasive plants selectively enrich microbes that promote stress resilience or resource acquisition, traits critical for establishment and spread (Bulgarelli et al., 2013; Sun and He, 2010). Moreover, as shown by Fang et al. (2023), native plants can alter the endophyte assembly and growth of invasive species under climatic stress, suggesting that microbial recruitment in *L. camara* might also be climate responsive. While fungal endophytes appeared more influenced by environmental homogeneity, bacteria exhibited clearer host-driven patterns, possibly due to their faster turnover and metabolic plasticity (Müller et al., 2016). Notably, the studies by Bickford et al. (2018) have shown that belowground endophytes alone do not fully explain performance differences between native and invasive plants. However, in this study, evidence was found that specific bacterial taxa were differentially enriched in invasive tissues, suggesting a functional component to microbial recruitment. This supports the hypothesis that microbial interactions contribute to the success of invasive species by modulating ecological interactions (Hawkes et al., 2005; Klironomos, 2002). Similar observations were found in *Phragmites* lineages, where both native and non-native types influenced soil bacterial composition differently but did not significantly differ in plant responses, suggesting subtle microbial roles in invasion processes (Martin et al., 2021). The host filtering results imply that *L. camara* selectively recruits phylogenetically related bacterial endophytes, due to specific plant traits or stress-adaptive requirements. Phylogenetic clustering has been linked to environmental filtering, where closely related taxa with similar traits are selected (Webb et al., 2002). Such patterns in invasive species support the “enhanced mutualist hypothesis” (Reinhart & Callaway, 2006), where invasive plants filter for beneficial endophytes that promote growth or stress tolerance. The top 10 predicted metabolic pathways from PICRUSt2 analysis reveal functional differences between endophytic communities of *Lantana camara* and *Lantana indica*. *L. indica* showed higher abundance of amino acid, terpenoid, nucleotide, and cell wall biosynthesis pathways, suggesting enhanced microbial support for nutrient metabolism and host interaction. In contrast, *L. camara* exhibited enrichment in pathways related to pentose phosphate metabolism and fatty acid elongation, linked to stress adaptation and carbon metabolism. These shifts highlight distinct microbial functional profiles between the native and invasive species, indicating plant-microbe co-adaptation, an observation consistent with functional enrichment in antimicrobial and stress-related pathways in invasive *Prosopis* (Kaushik et al., 2021). To conclude this chapter, both *L. camara* and *L. indica* hosted diverse endophytic communities, the bacterial diversity was more distinctly structured between the two species, and the fungal communities shared similar diversity and functional adaptation. The results suggest that *L. camara* gains a competitive advantage through its diverse yet phylogenetically clustered fungal community, potentially enhancing its adaptability across

environments in this study. As initially expected, the congeners with different invasive statuses exhibited variation in their microbiome composition; however, the results also indicate that the environment plays a dominant role in shaping microbial recruitment in both species. At the same time, the selective enrichment of specific bacterial and fungal indicator taxa in the invasive host shows a degree of microbial selection and specialization, potentially contributing to the ecological success of *L. camara*.

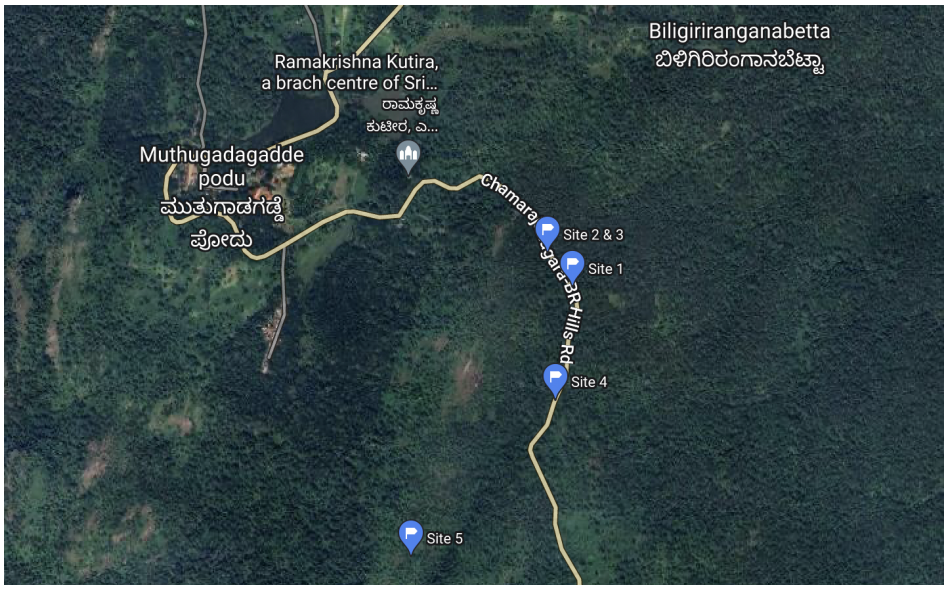


Figure 2.1: Geographic location of the sampling sites in Biligirirangan Hills, Karnataka, India, showing the areas where *Lantana camara* and *Lantana indica* were collected

Site	Geographical location	Distance (m)
#1	239º SW 11º58’49 N 77º9’6 E	0
#2	250º W 11º58’53 N 77º9’3 E	150
#3	34º NE 11º58’53 N 77º9’3 E	20
#4	287º W 11º58’36 N 77º9’4 E	530
#5	250º W 11º58’18 N 77º8’47 E	760

Table 2.1: Geographic coordinates of the sampling sites located in Biligirirangan Hills, Karnataka, India, along with their inter-site distances

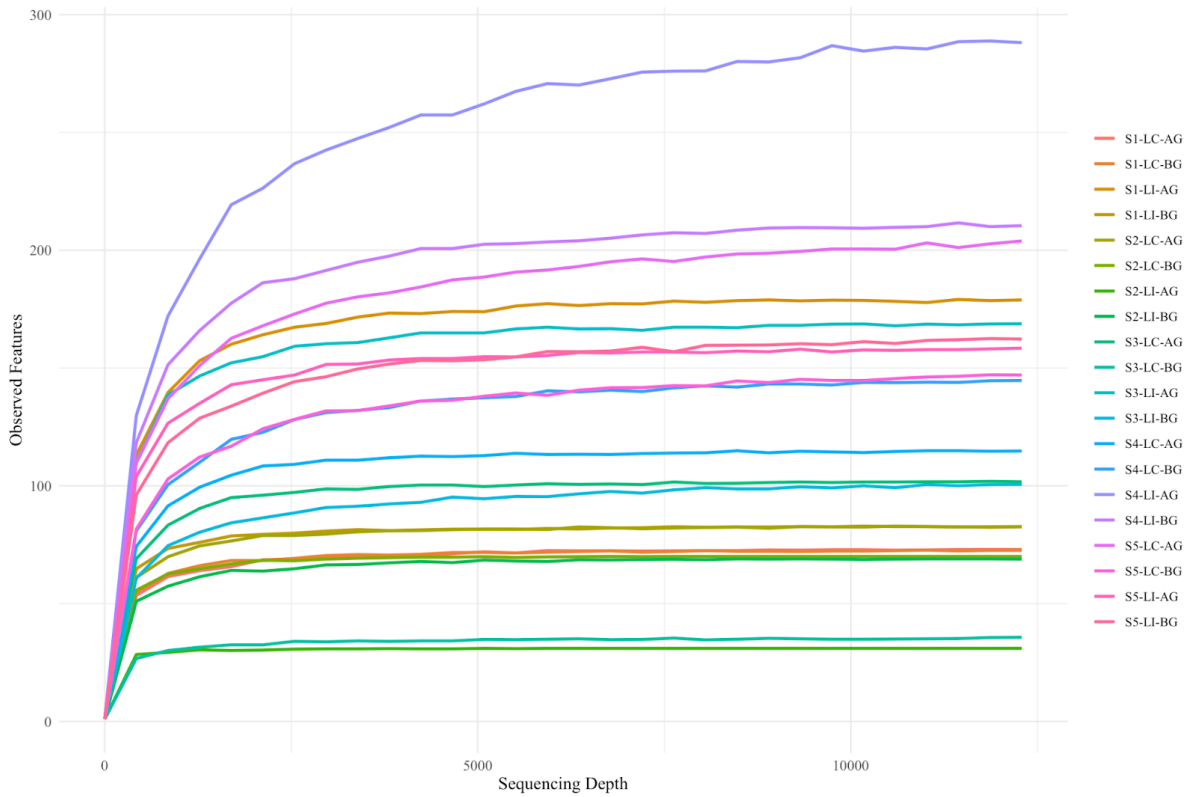


Figure 2.2: Rarefaction curve showing the observed features of endophytic bacterial communities across all 20 samples of *Lantana camara* and *Lantana indica*

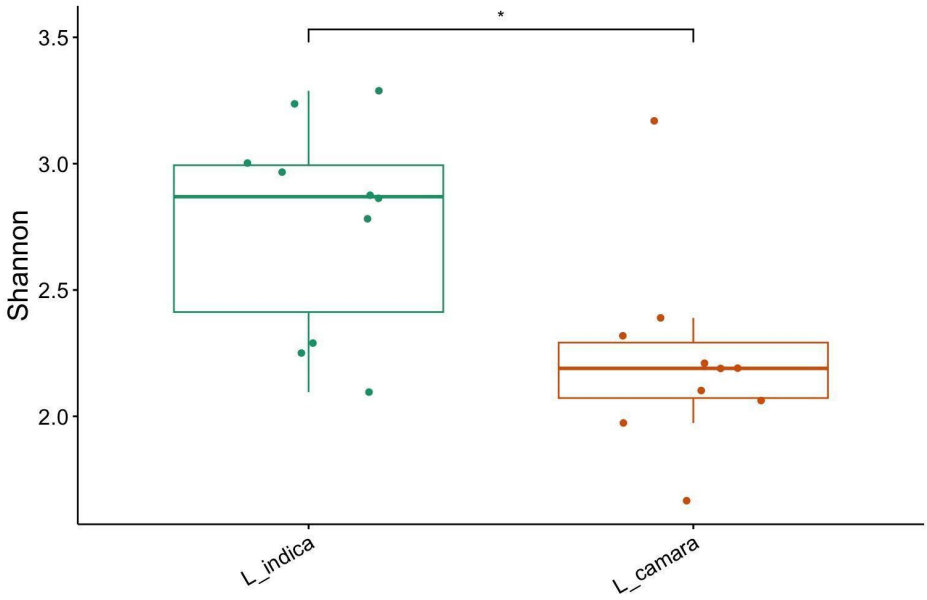


Figure 2.3: Boxplot showing the Shannon diversity index of bacterial communities associated with *Lantana camara* and *L. indica*. The plot represents the differences in bacterial alpha diversity between the invasive and native hosts, indicating differences in community richness and evenness

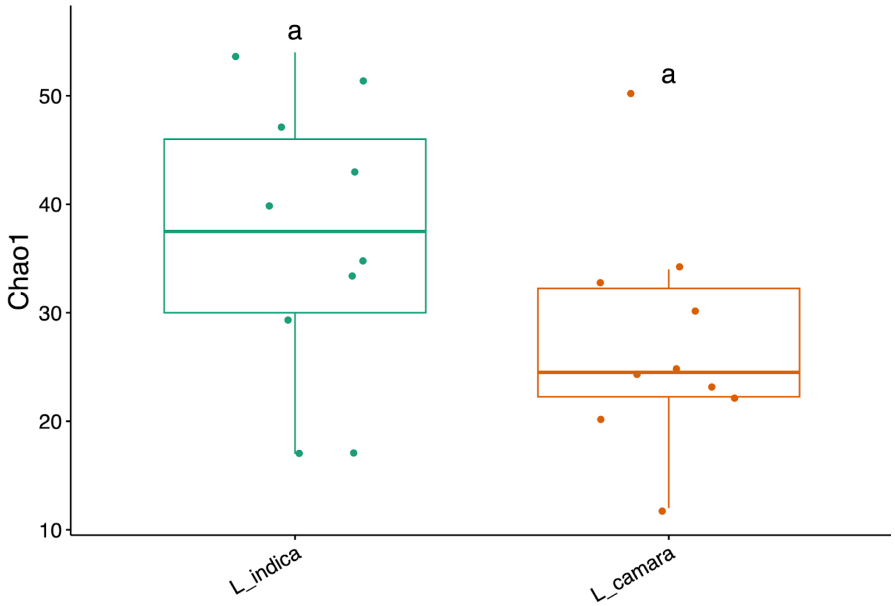


Figure 2.4: Boxplot showing the Chao1 richness estimate of bacterial communities associated with *Lantana camara* and *L. indica*. The plot represents differences in estimated species richness

between the invasive and native hosts, estimating the total number of bacterial taxa, including rare species, providing insight into the overall richness of the communities associated with the invasive and native hosts

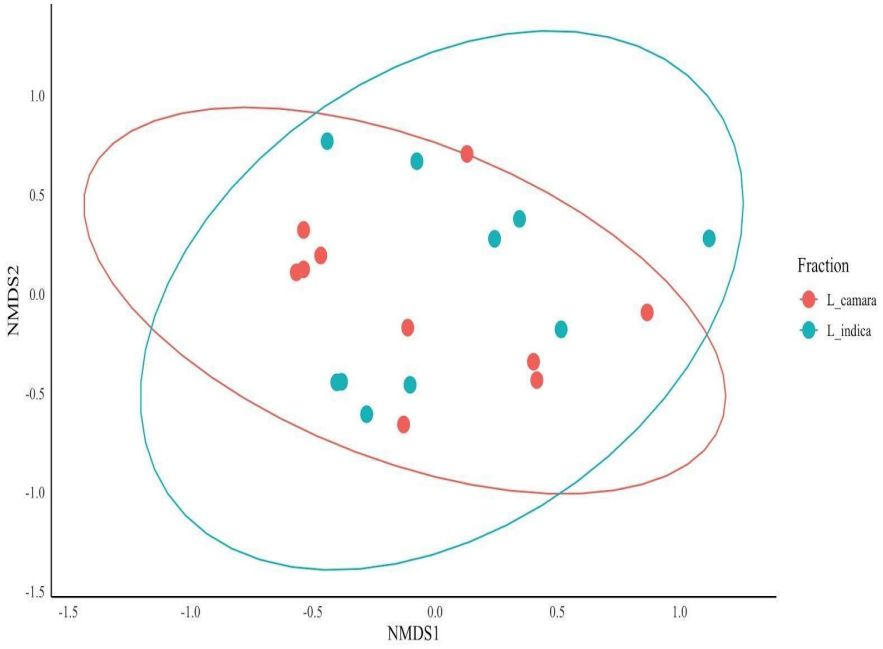


Figure 2.5: Non-metric multidimensional scaling (NMDS) ordination illustrating the bacterial community composition in *Lantana camara* and *Lantana indica*, calculated using Bray-Curtis dissimilarity

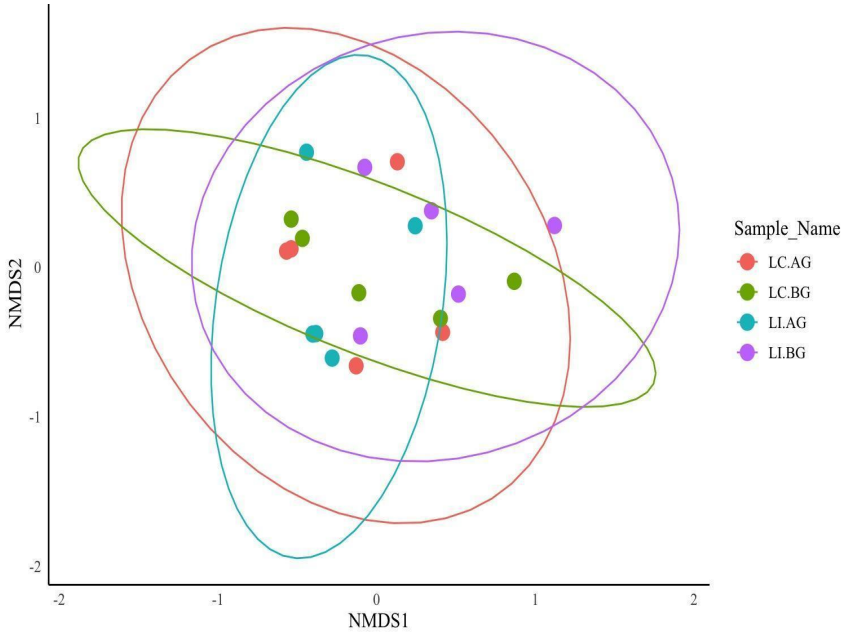


Figure 2.6: Non-metric multidimensional scaling (NMDS) ordination illustrating the bacterial community composition across above-ground and below-ground tissues of *Lantana camara* and *Lantana indica*, calculated using Bray–Curtis dissimilarity

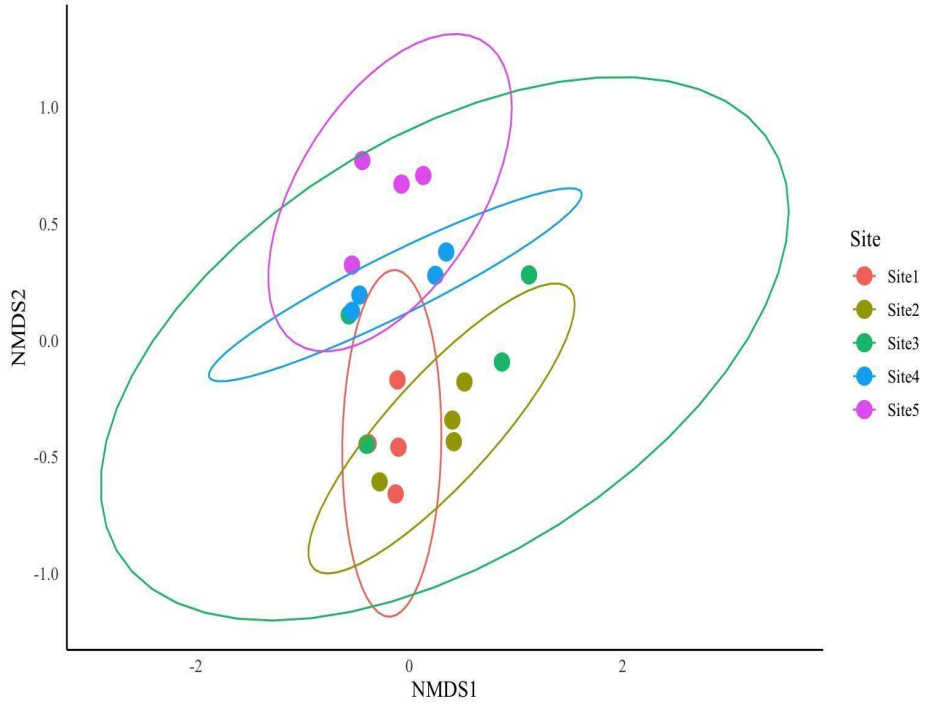


Figure 2.7: Non-metric multidimensional scaling (NMDS) ordination illustrating the variation in bacterial community composition across different collection sites, calculated using Bray–Curtis dissimilarity

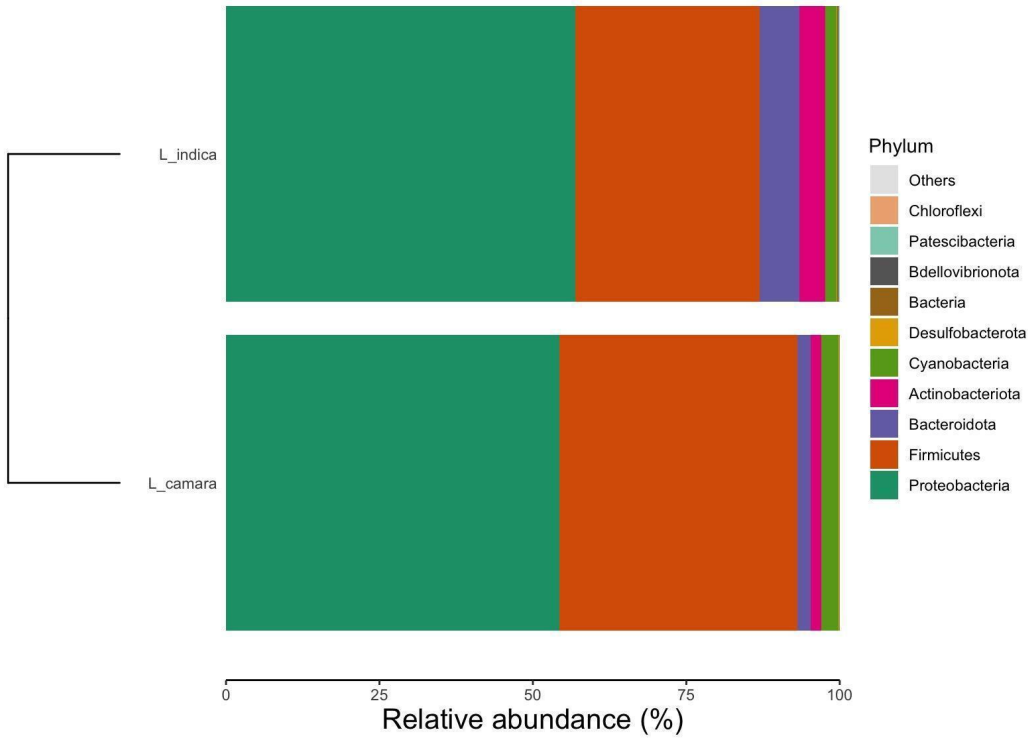


Figure 2.8: The bar plot illustrating the mean relative abundance (%) of the ten most dominant bacterial phyla associated with *Lantana camara* and *Lantana indica*. The bars represent the average proportion of each phylum within the bacterial community of the respective host plant. The samples were clustered according to similarities in community composition, highlighting distinct and shared bacterial assemblages between the invasive (*L. camara*) and the native (*L. indica*) species

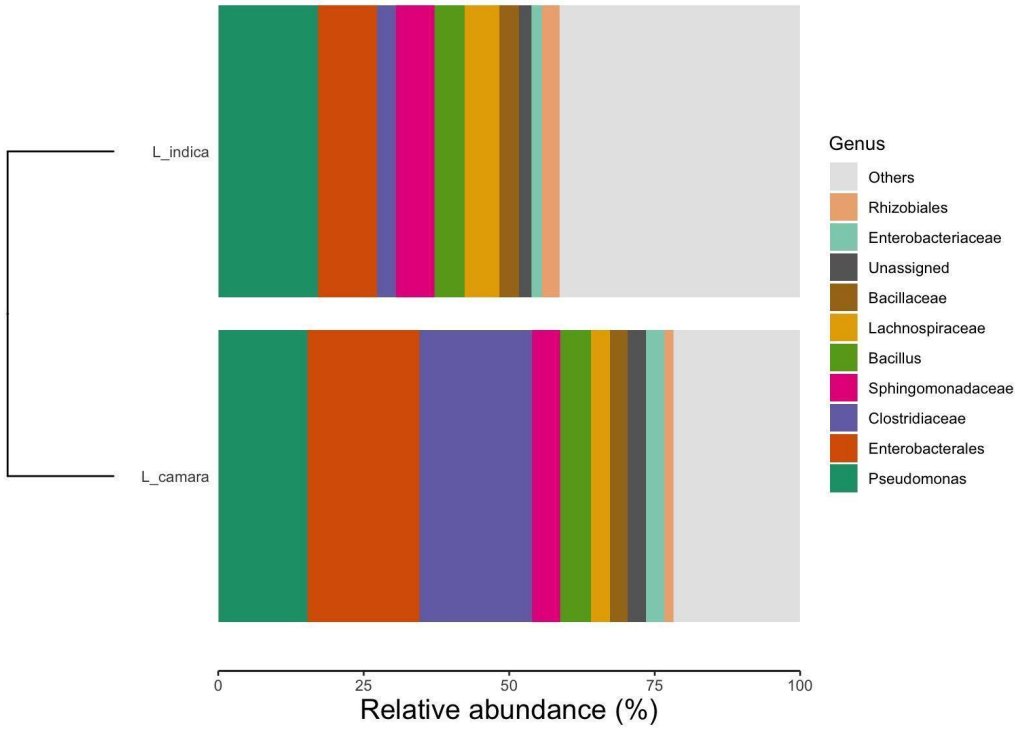


Figure 2.9: The bar plot illustrating the mean relative abundance (%) of the ten most dominant bacterial genera associated with *Lantana camara* and *Lantana indica*. The bars represent the average proportion of each genera within the bacterial community of the respective host plant. The samples were clustered according to similarities in community composition, highlighting distinct and shared bacterial assemblages between the invasive (*L. camara*) and the native (*L. indica*) species

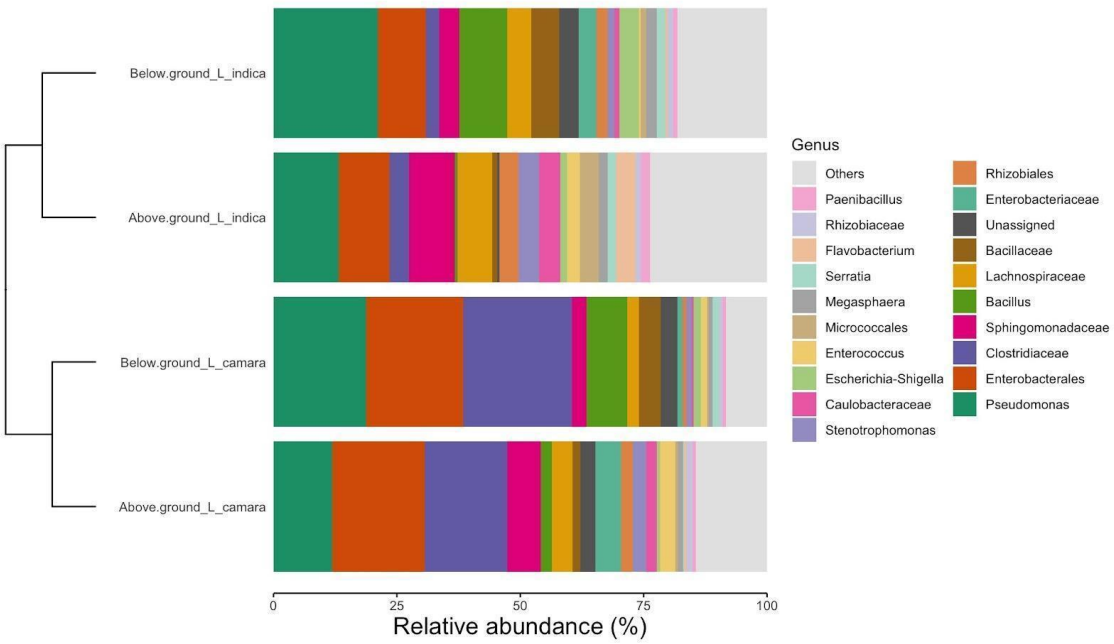


Figure 2.10: The bar plot illustrates the mean relative abundance (%) of the twenty most dominant bacterial genera associated with *Lantana camara* and *Lantana indica* across above-ground and below-ground tissues. The bars represent the average proportion of each genus within the bacterial community of the respective host plant. The samples were clustered according to similarities in community composition, highlighting distinct and shared bacterial assemblages between the invasive (*L. camara*) and the native (*L. indica*) species

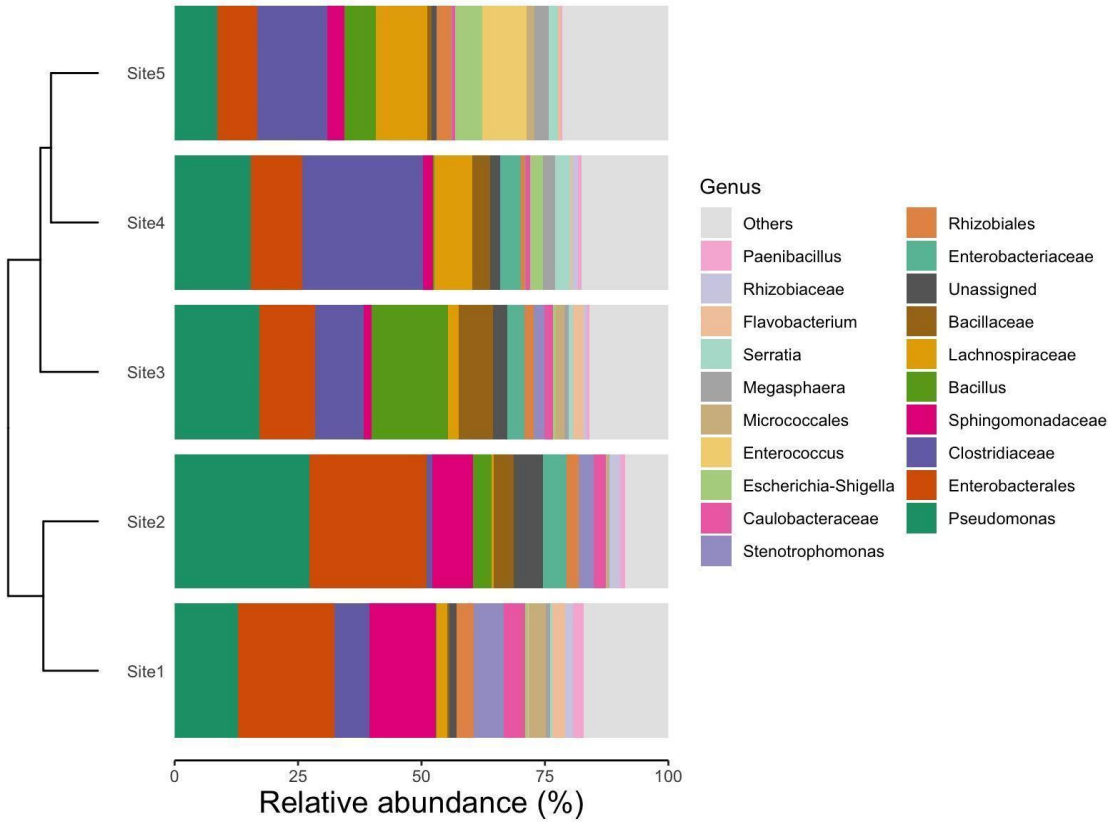


Figure 2.11: The bar plot illustrates the mean relative abundance (%) of the twenty most dominant bacterial genera associated with *Lantana camara* and *Lantana indica* across different sampling sites. The bars represent the average proportion of each genus within the bacterial community of the respective host plant. The samples were clustered according to similarities in community composition, highlighting distinct and shared bacterial assemblages between the invasive (*L. camara*) and the native (*L. indica*) species across the study sites

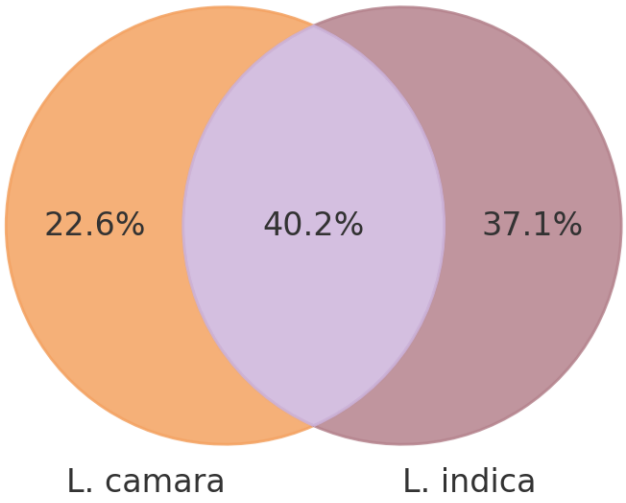


Figure 2.12: Venn diagram illustrating the distribution of endophytic bacterial ASVs between *Lantana camara* and *Lantana indica*, showing the number of unique and shared features

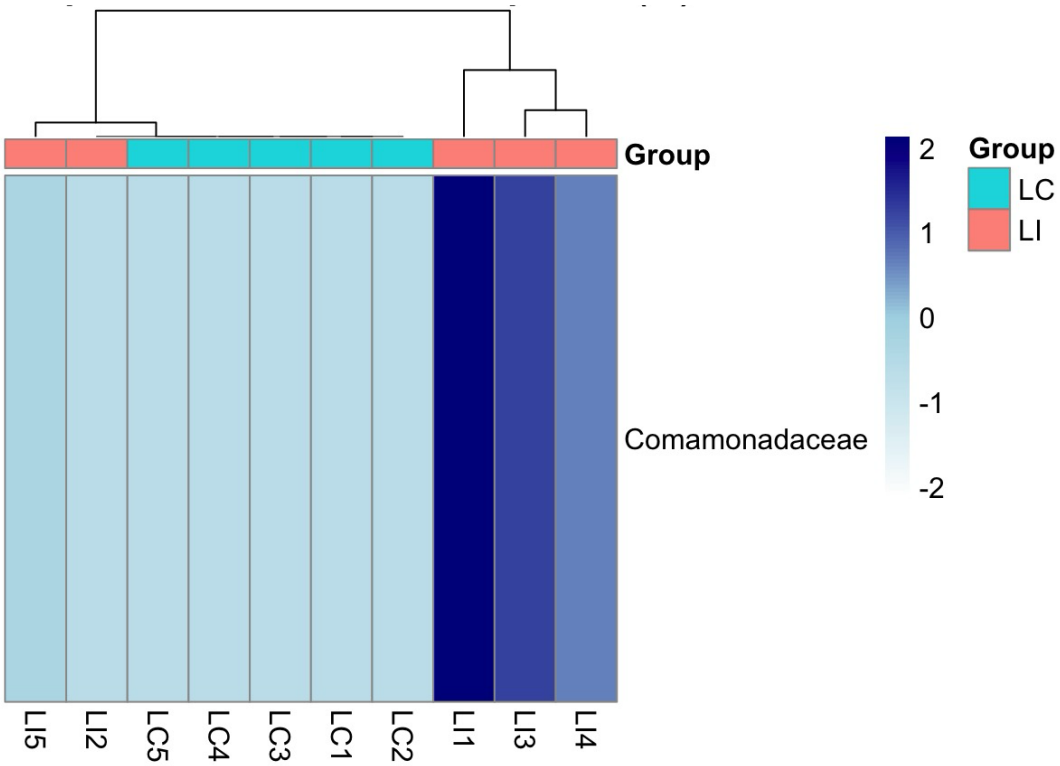


Figure 2.13: Heatmap showing the bacterial indicator taxon identified based on indicator value analysis. The heatmap displays the relative abundance pattern of *Comamonadaceae* across replicate samples of *Lantana camara* (LC) and *Lantana indica* (LI). Samples are hierarchically clustered according to community composition, illustrating a higher association of *Comamonadaceae* with *L. indica* (IndVal = 0.894, p = 0.037). The color gradient represents standardized abundance, and the grouping of samples (LC and LI) is indicated by colored bars above the heatmap

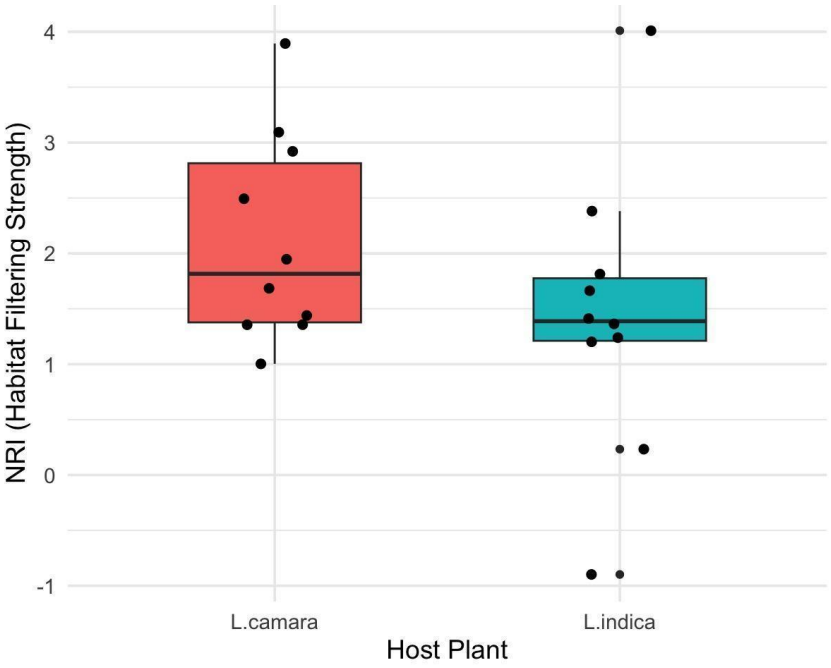


Figure 2.14. Net Relatedness Index (NRI) showing the phylogenetic clustering of bacterial endophytic communities associated with *Lantana camara* and *Lantana indica*

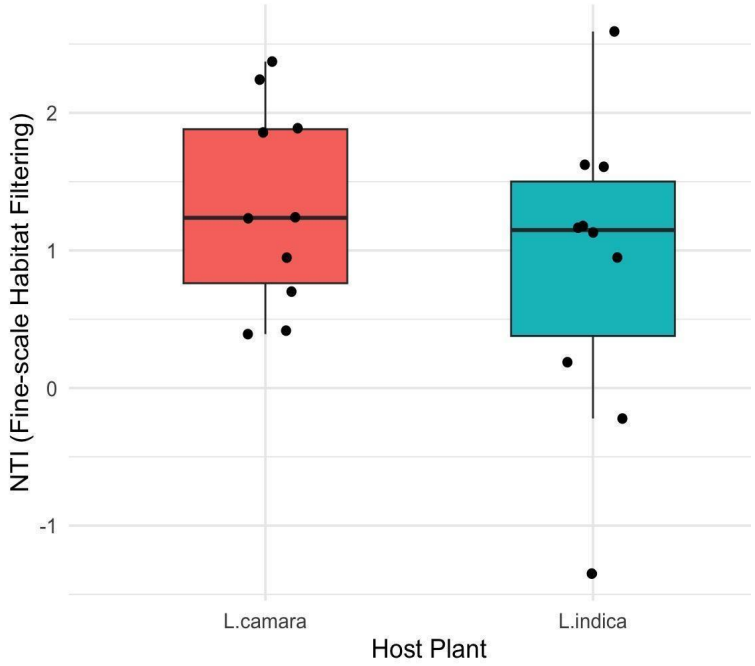


Figure 2.15. Nearest Taxon Index (NTI) showing the phylogenetic clustering of bacterial endophytic communities associated with *Lantana camara* and *Lantana indica*

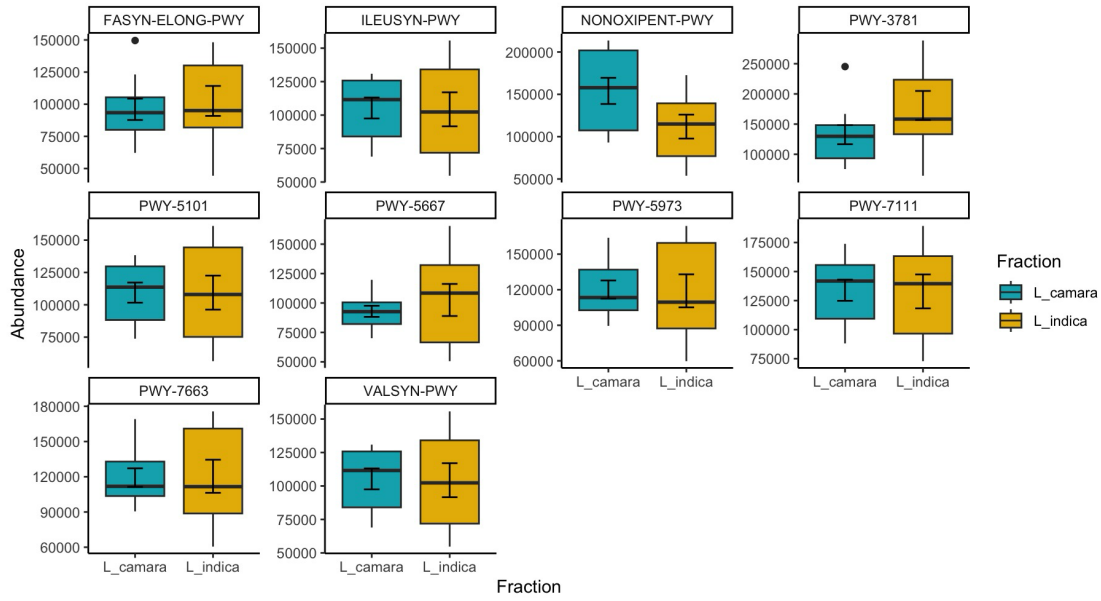


Figure 2.16: Boxplot illustrating the top 10 predicted MetaCyc metabolic pathways in bacterial endophytic communities associated with *Lantana camara* and *Lantana indica*, as inferred from PICRUSt2 functional profiling

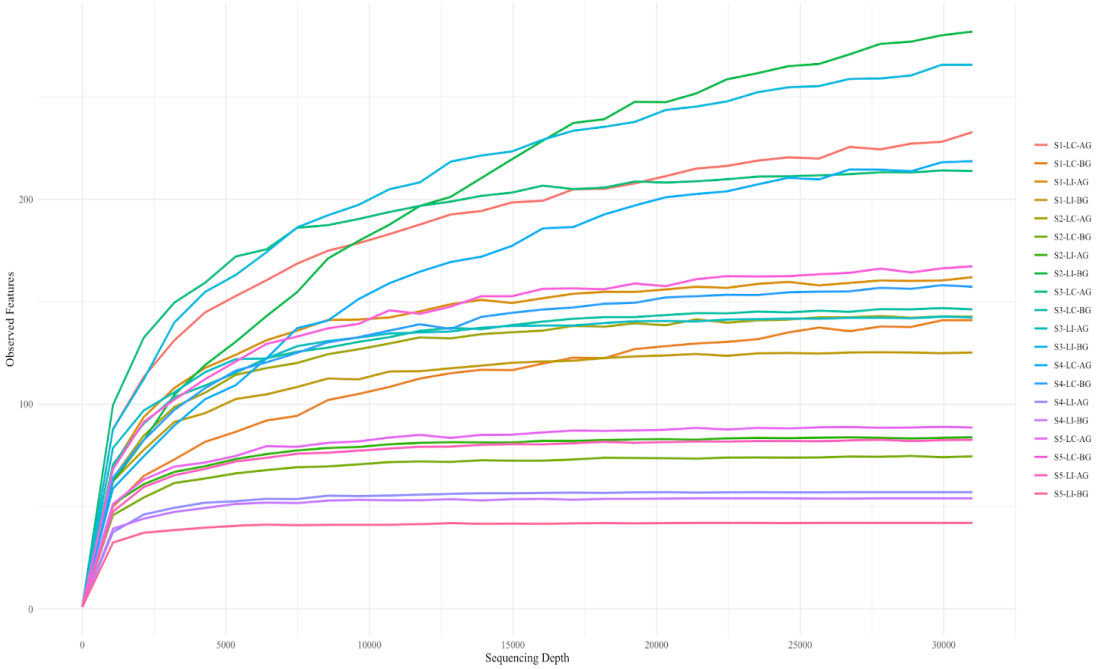


Figure 2.17: Rarefaction curve showing the observed features of endophytic fungal communities across all 20 samples of *Lantana camara* and *Lantana indica*

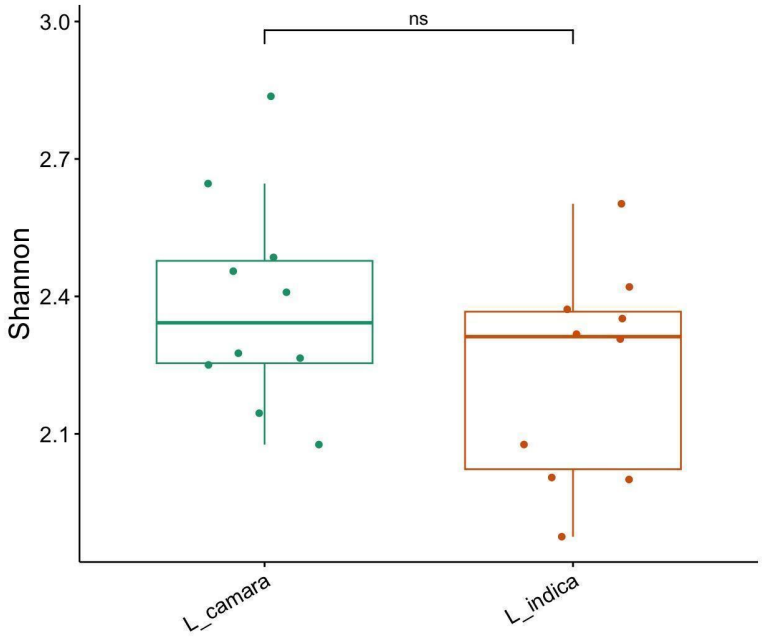


Figure 2.18: Boxplot showing the Shannon diversity index of fungal communities associated with *Lantana camara* and *L. indica*. The plot represents the differences in bacterial alpha diversity between the invasive and native hosts, indicating differences in community richness and evenness

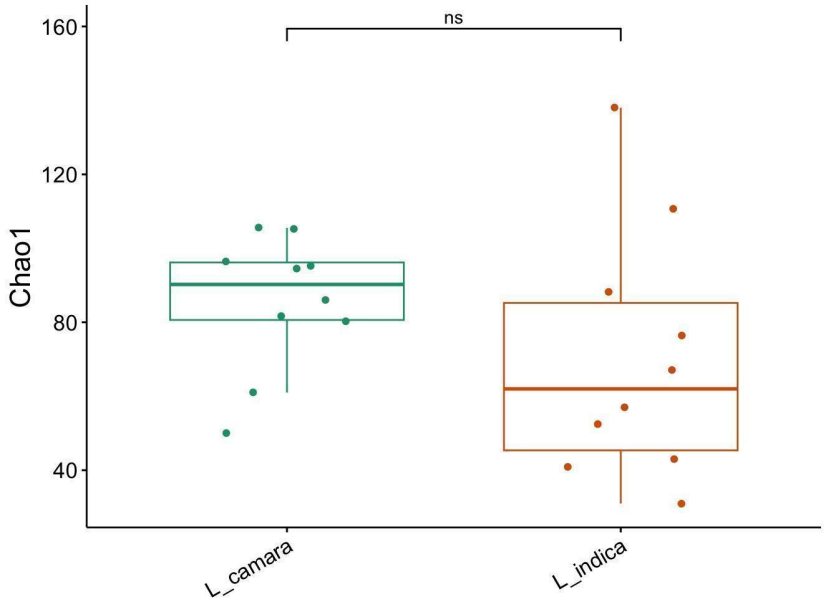


Figure 2.19: Boxplot showing the Chao1 richness estimate of fungal communities associated with *Lantana camara* and *L. indica*. The plot represents differences in estimated species richness between the invasive and native hosts, estimating the total number of fungal taxa, including rare species, providing insight into the overall richness of the communities associated with the invasive and native hosts

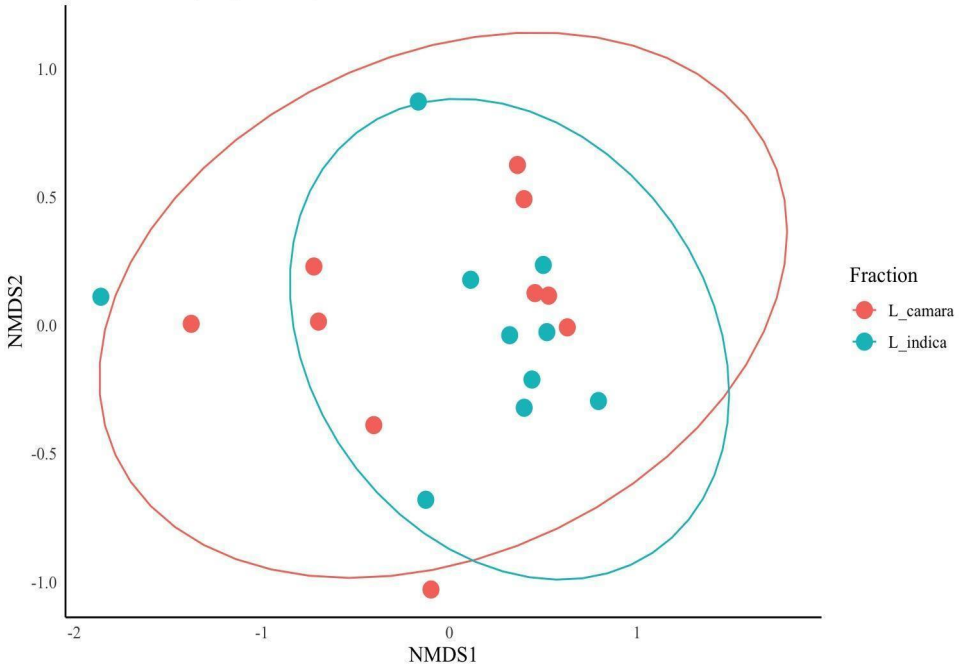


Figure 2.20: Non-metric multidimensional scaling (NMDS) ordination illustrating the fungal community composition in *Lantana camara* and *Lantana indica*, calculated using Bray-Curtis

dissimilarity

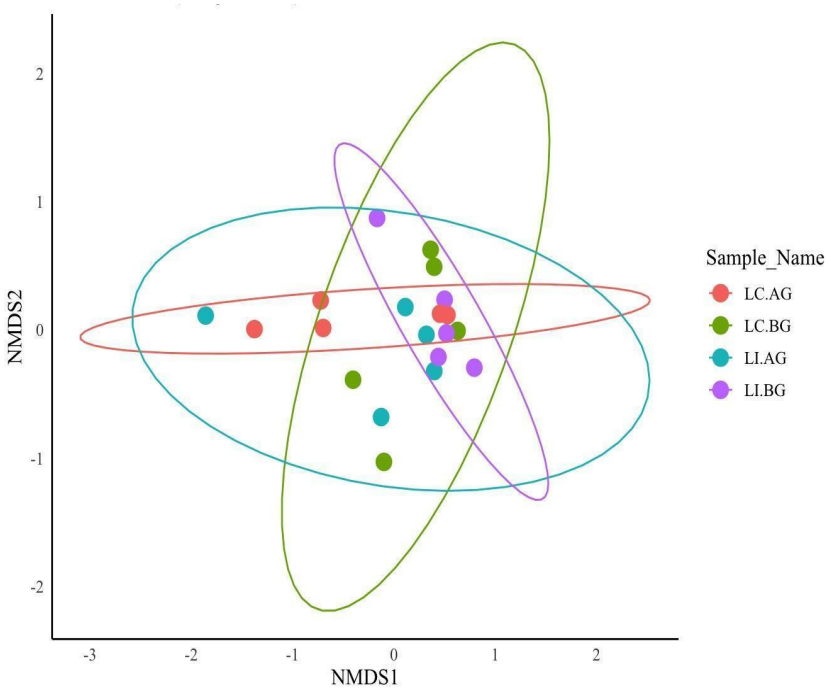


Figure 2.21: Non-metric multidimensional scaling (NMDS) ordination illustrating the fungal community composition across above-ground and below-ground tissues of *Lantana camara* and *Lantana indica*, calculated using Bray-Curtis dissimilarity

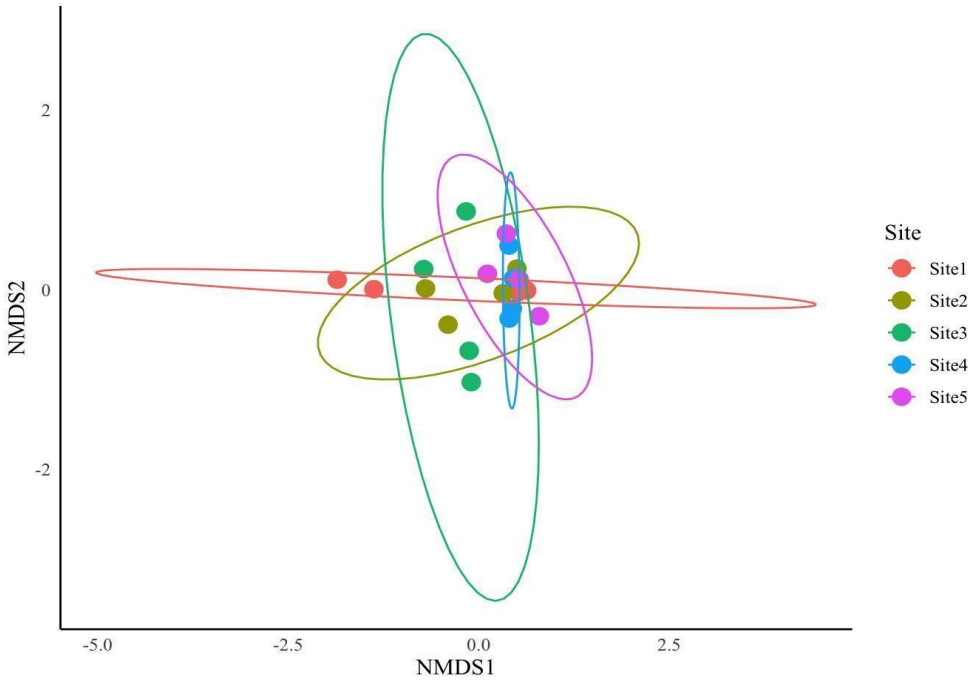


Figure 2.22: Non-metric multidimensional scaling (NMDS) ordination illustrating the variation in fungal community composition across different collection sites, calculated using Bray-Curtis dissimilarity

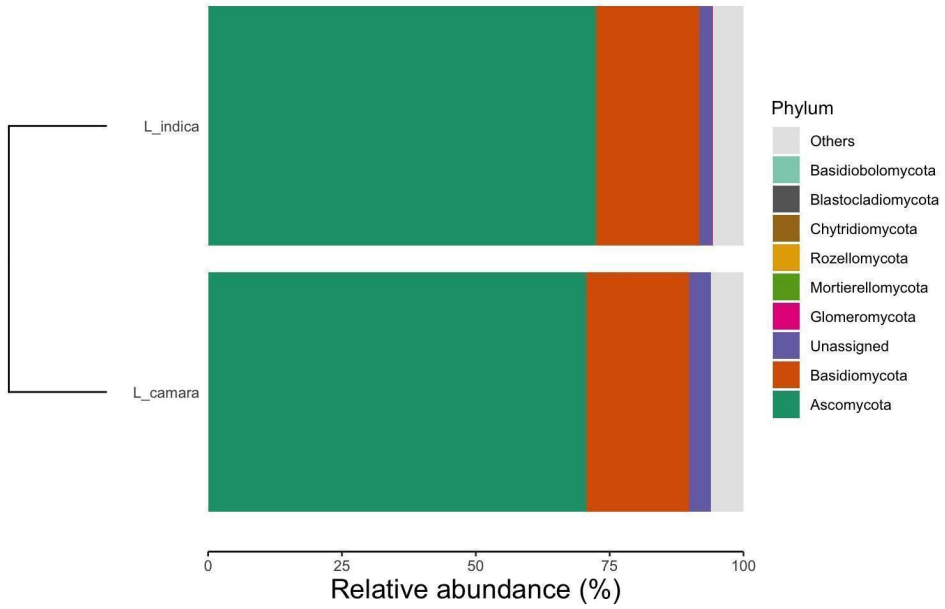


Figure 2.23: The bar plot illustrates the mean relative abundance (%) of the ten most dominant fungal phyla associated with *Lantana camara* and *Lantana indica*. The bars represent the average proportion of each phylum within the fungal community of the respective host plant. The samples were clustered according to similarities in community composition, highlighting distinct and shared fungal assemblages between the invasive (*L. camara*) and the native (*L. indica*) species

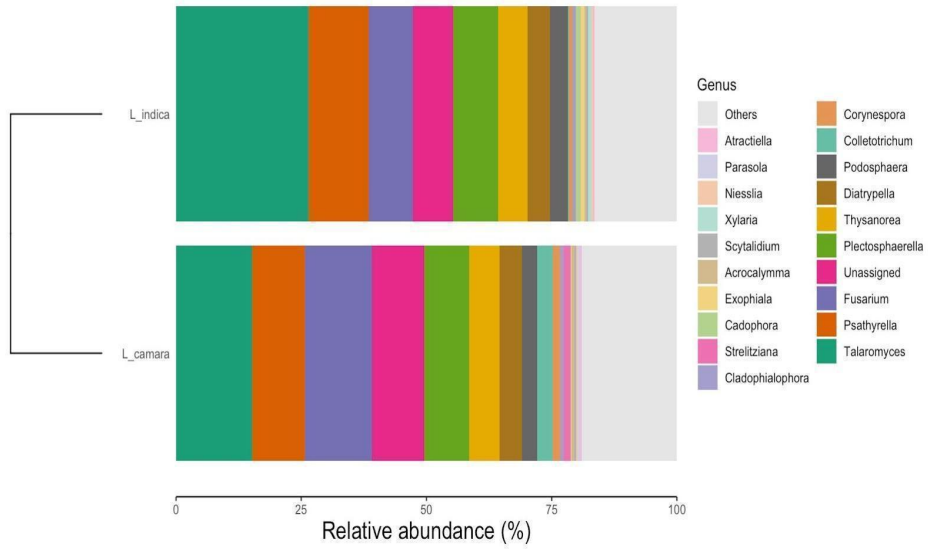


Figure 2.24: The bar plot illustrates the mean relative abundance (%) of the twenty most dominant fungal genera associated with *Lantana camara* and *Lantana indica*. The bars represent the average proportion of each genus within the fungal community of the respective host plant. The samples were clustered according to similarities in community composition, highlighting distinct and shared fungal assemblages between the invasive (*L. camara*) and the native (*L. indica*) species

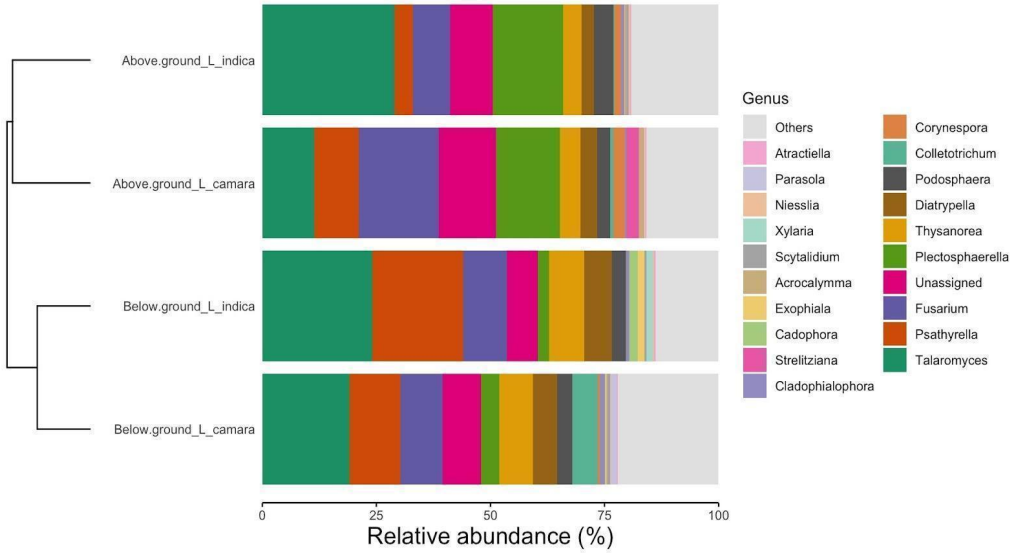


Figure 2.25: The bar plot illustrates the mean relative abundance (%) of the twenty most dominant fungal genera associated with *Lantana camara* and *Lantana indica* across above-ground and below-ground tissues. The bars represent the average proportion of each genus within the fungal community of the respective tissue type. The samples were clustered according to similarities in community composition, highlighting distinct and shared fungal assemblages between the above-ground and below-ground compartments of the invasive (*L. camara*) and the native (*L. indica*) species

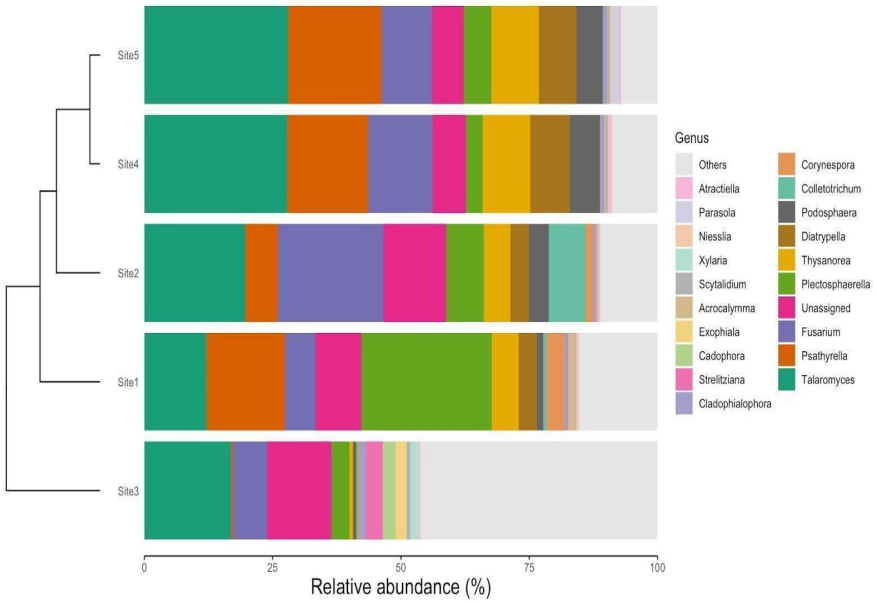


Figure 2.26: The bar plot illustrates the mean relative abundance (%) of the twenty most dominant fungal genera associated with *Lantana camara* and *Lantana indica* across different sampling sites. The bars represent the average proportion of each genus within the fungal community of the respective host plant. The samples were clustered according to similarities in community composition, highlighting distinct and shared fungal assemblages between the invasive (*L. camara*) and the native (*L. indica*) species across the study sites

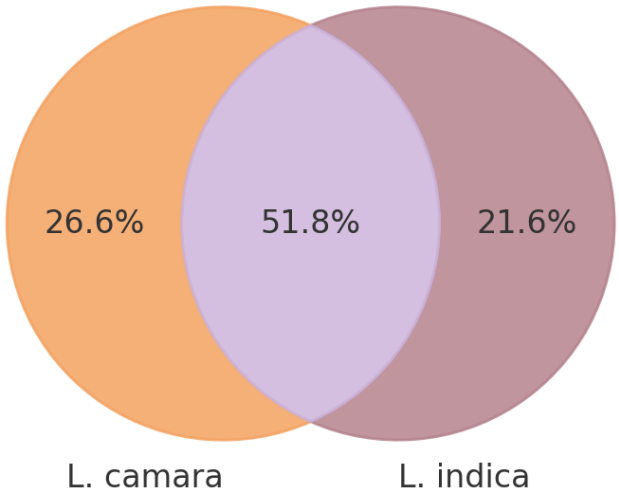


Figure 2.27: Venn diagram illustrating the distribution of endophytic fungal ASVs between *Lantana camara* and *Lantana indica*, showing the number of unique and shared features

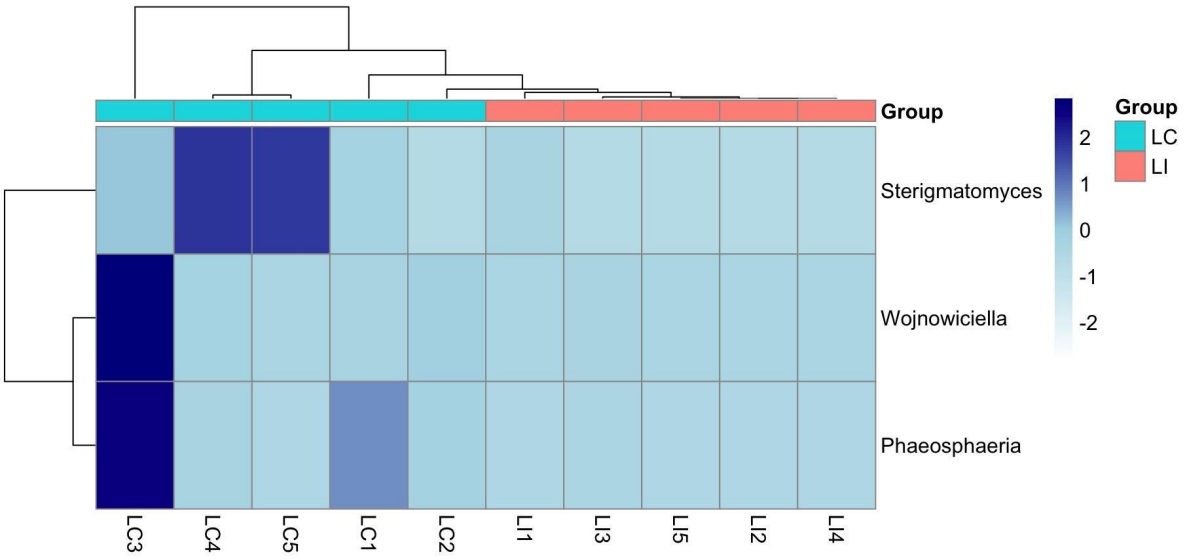


Figure 2.28: Heatmap showing fungal indicator taxa identified based on indicator value analysis. The heatmap displays the relative abundance patterns of *Wojnowiciella sp.*, *Phaeosphaeria sp.* and *Stigmatomyces sp.* across replicate samples of *Lantana camara* (LC) and *Lantana indica* (LI). Samples are hierarchically clustered according to community composition, showing higher associations of these taxa with *L. camara.* Indicator values (IndVal) and significance levels ($p < 0.05$) were as follows: *Wojnowiciella sp.* (IndVal = 0.889, $p = 0.040$), *Phaeosphaeria sp.* (IndVal = 0.888, $p = 0.040$), and *Stigmatomyces sp.* (IndVal = 0.872, $p = 0.042$). The color gradient represents standardized abundance, and the grouping (LC and LI) is indicated by colored bars above the heatmap

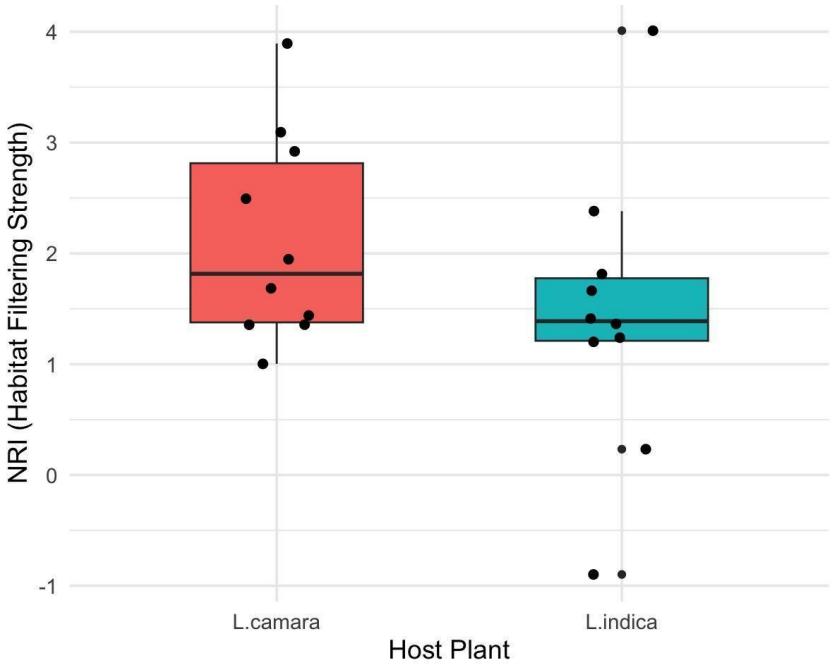


Figure 2.28: Net Relatedness Index (NRI) showing the phylogenetic clustering of fungal endophytic communities associated with *Lantana camara* and *Lantana indica*

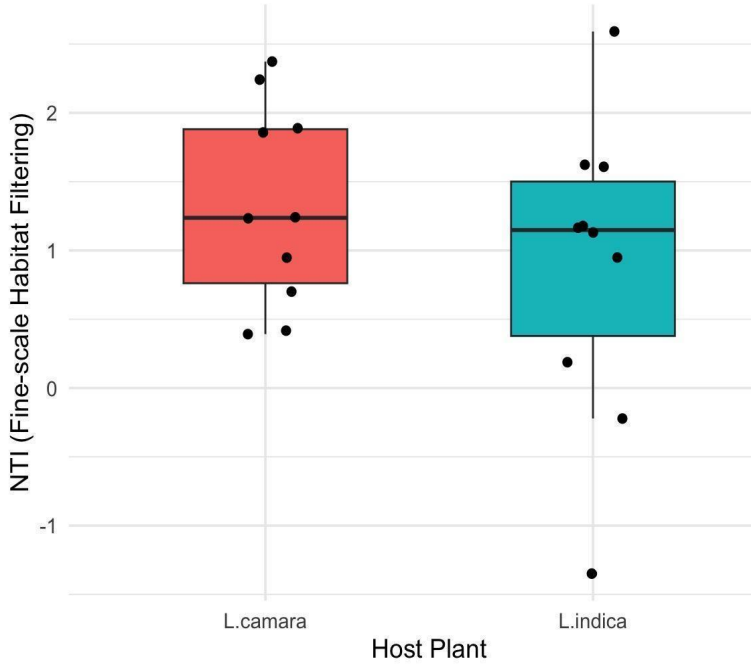


Figure 2.29: Nearest Taxon Index (NTI) showing the phylogenetic clustering of fungal endophytic communities associated with *Lantana camara* and *Lantana indica*

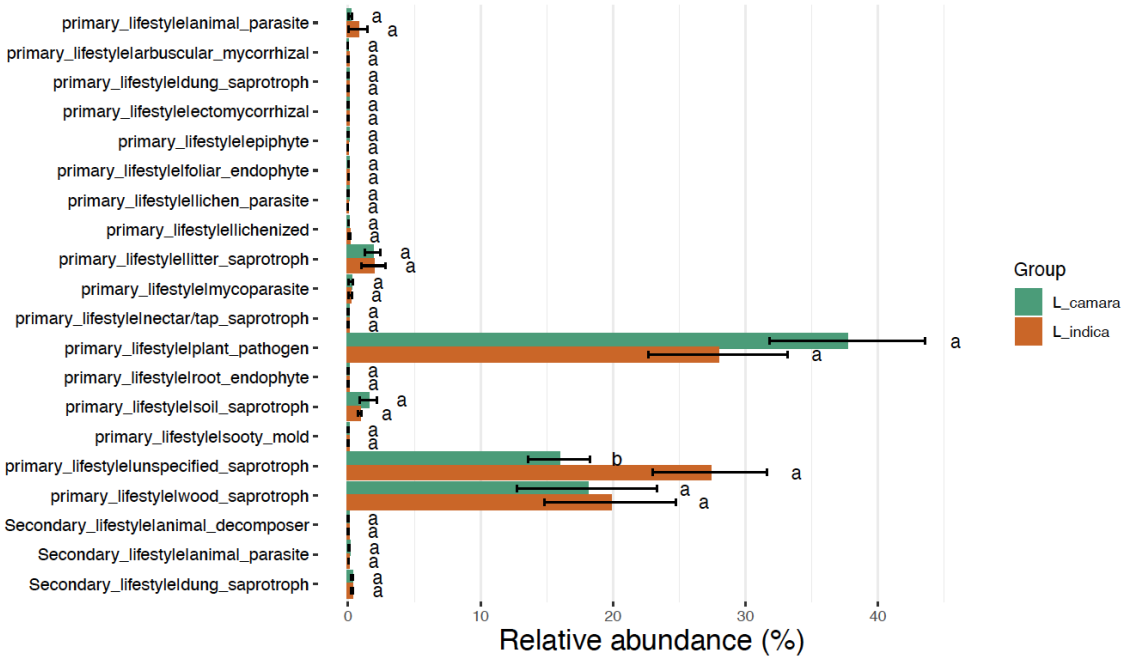


Figure 2.30: Top 20 most relative abundance of fungal functional lifestyles of endophytic fungi of *Lantana camara* and *Lantana indica*, based on FUNGuild annotation. Bars represent average proportions of each ecological guild (e.g., saprotrophs, endophytes, pathogens, mycorrhizal fungi) across replicates

Sample Name	BioSample Accession number	Description	Library	Forward reads
S1.LC.AG_16S	SAMN49904347	Endophytic bacteria of Lantana camara above ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S1-LC-AG-16S_1.fastq.gz
S1.LC.BG_16S	SAMN49904348	Endophytic bacteria of Lantana camara below ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S1-LC-BG-16S_1.fastq.gz
S1.LI.AG_16S	SAMN49904349	Endophytic bacteria of Lantana indica above ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S1-LI-AG-16S_1.fastq.gz
S1.LI.BG_16S	SAMN49904350	Endophytic bacteria of Lantana indica below ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S1-LI-BG-16S_1.fastq.gz
S2.LC.AG_16S	SAMN49904351	Endophytic bacteria of Lantana camara above ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S2-LC-AG-16S_1.fastq.gz
S2.LC.BG_16S	SAMN49904352	Endophytic bacteria of Lantana camara below ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S2-LC-BG-16S_1.fastq.gz
S2.LI.AG_16S	SAMN49904353	Endophytic bacteria of Lantana indica above ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S2-LI-AG-16S_1.fastq.gz
S2.LI.BG_16S	SAMN49904354	Endophytic bacteria of Lantana indica below ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S2-LI-BG-16S_1.fastq.gz
S3.LC.AG_16S	SAMN49904355	Endophytic bacteria of Lantana camara above ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S3-LC-AG-16S_1.fastq.gz
S3.LC.BG_16S	SAMN49904356	Endophytic bacteria of Lantana camara below ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S3-LC-BG-16S_1.fastq.gz

S3.LI.AG_16S	SAMN49904357	Endophytic bacteria of Lantana indica above ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S3-LI-AG-16S_1.fastq.gz
S3.LI.BG_16S	SAMN49904358	Endophytic bacteria of Lantana indica below ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S3-LI-BG-16S_1.fastq.gz
S4.LC.AG_16S	SAMN49904359	Endophytic bacteria of Lantana camara above ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S4-LC-AG-16S_1.fastq.gz
S4.LC.BG_16S	SAMN49904360	Endophytic bacteria of Lantana camara below ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S4-LC-BG-16S_1.fastq.gz
S4.LI.AG_16S	SAMN49904361	Endophytic bacteria of Lantana indica above ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S4-LI-AG-16S_1.fastq.gz
S4.LI.BG_16S	SAMN49904362	Endophytic bacteria of Lantana indica below ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S4-LI-BG-16S_1.fastq.gz
S5.LC.AG_16S	SAMN49904363	Endophytic bacteria of Lantana camara above ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S5-LC-AG-16S_1.fastq.gz
S5.LC.BG_16S	SAMN49904364	Endophytic bacteria of Lantana camara below ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S5-LC-BG-16S_1.fastq.gz
S5.LI.AG_16S	SAMN49904365	Endophytic bacteria of Lantana indica above ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S5-LI-AG-16S_1.fastq.gz
S5.LI.BG_16S	SAMN49904366	Endophytic bacteria of Lantana indica below ground	Genomic; Amplicon; PCR; Single; Illumina MiSeq	S5-LI-BG-16S_1.fastq.gz

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Table S1. Summary of 16S rRNA gene amplicon sequencing libraries generated from bacterial endophytes associated with *Lantana camara* (invasive) and *Lantana indica* (native). The sequences are submitted in the NCBI Sequence Read Archive (SRA) having the corresponding BioSample accession numbers provided in the table

Sample Name	BioSample Accession number	Description	Library	Forward reads	Reverse reads
S1.LC.AG_ITS	SAMN49903409	Endophytic fungi of Lantana camara above ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S1-LC-AG-ITS_1.fastq.gz	S1-LC-AG-ITS_2.fastq.gz
S1.LC.BG_ITS	SAMN49903410	Endophytic fungi of Lantana camara below ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S1-LC-BG-ITS_1.fastq.gz	S1-LC-BG-ITS_2.fastq.gz
S1.LI.AG_ITS	SAMN49903411	Endophytic fungi of Lantana indica above ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S1-LI-AG-ITS_1.fastq.gz	S1-LI-AG-ITS_2.fastq.gz
S1.LI.BG_ITS	SAMN49903412	Endophytic fungi of Lantana indica below ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S1-LI-BG-ITS_1.fastq.gz	S1-LI-BG-ITS_2.fastq.gz
S2.LC.AG_ITS	SAMN49903413	Endophytic fungi of Lantana camara above ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S2-LC-AG-ITS_1.fastq.gz	S2-LC-AG-ITS_2.fastq.gz
S2.LC.BG_ITS	SAMN49903414	Endophytic fungi of Lantana camara below ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S2-LC-BG-ITS_1.fastq.gz	S2-LC-BG-ITS_2.fastq.gz

S2.LI.AG_ITS	SAMN49903415	Endophytic fungi of Lantana indica above ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S2-LI-AG-ITS_1.fastq.gz	S2-LI-AG-ITS_2.fastq.gz
S2.LI.BG_ITS	SAMN49903416	Endophytic fungi of Lantana indica below ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S2-LI-BG-ITS_1.fastq.gz	S2-LI-BG-ITS_2.fastq.gz
S3.LC.AG_ITS	SAMN49903417	Endophytic fungi of Lantana camara above ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S3-LC-AG-ITS_1.fastq.gz	S3-LC-AG-ITS_2.fastq.gz
S3.LC.BG_ITS	SAMN49903418	Endophytic fungi of Lantana camara below ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S3-LC-BG-ITS_1.fastq.gz	S3-LC-BG-ITS_2.fastq.gz
S3.LI.AG_ITS	SAMN49903419	Endophytic fungi of Lantana indica above ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S3-LI-AG-ITS_1.fastq.gz	S3-LI-AG-ITS_2.fastq.gz
S3.LI.BG_ITS	SAMN49903420	Endophytic fungi of Lantana indica below ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S3-LI-BG-ITS_1.fastq.gz	S3-LI-BG-ITS_2.fastq.gz
S4.LC.AG_ITS	SAMN49903421	Endophytic fungi of Lantana camara above ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S4-LC-AG-ITS_1.fastq.gz	S4-LC-AG-ITS_2.fastq.gz
S4.LC.BG_ITS	SAMN49903422	Endophytic fungi of Lantana camara below ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S4-LC-BG-ITS_1.fastq.gz	S4-LC-BG-ITS_2.fastq.gz
S4.LI.AG_ITS	SAMN49903423	Endophytic fungi of Lantana indica above ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S4-LI-AG-ITS_1.fastq.gz	S4-LI-AG-ITS_2.fastq.gz
S4.LI.BG_ITS	SAMN49903424	Endophytic fungi of Lantana indica below ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S4-LI-BG-ITS_1.fastq.gz	S4-LI-BG-ITS_2.fastq.gz
S5.LC.AG_ITS	SAMN49903425	Endophytic fungi of Lantana camara above ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S5-LC-AG-ITS_1.fastq.gz	S5-LC-AG-ITS_2.fastq.gz

S5.LC.BG_ITS	SAMN49903426	Endophytic fungi of Lantana camara below ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S5-LC-BG-ITS_1.fastq.gz	S5-LC-BG-ITS_2.fastq.gz
S5.LI.AG_ITS	SAMN49903427	Endophytic fungi of Lantana indica above ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S5-LI-AG-ITS_1.fastq.gz	S5-LI-AG-ITS_2.fastq.gz
S5.LI.BG_ITS	SAMN49903428	Endophytic fungi of Lantana indica below ground	Genomic; Amplicon; PCR; Paired; Illumina MiSeq	S5-LI-BG-ITS_1.fastq.gz	S5-LI-BG-ITS_2.fastq.gz

Table S2: Summary of ITS gene amplicon sequencing libraries generated from fungal endophytes associated with *Lantana camara* (invasive) and *Lantana indica* (native). The sequences are submitted in the NCBI Sequence Read Archive (SRA) having the corresponding BioSample accession numbers provided in the table

Chapter - 3

Characterize culturable fungal endophytes in co-occurring congeneric native and invasive *Lantana* Species through morphological and molecular analyses

3.1 Abstract

Endophytic fungi are an integral part of the plant microbiome. They often contribute to host fitness through stress mitigation, growth facilitation, and defense. Despite their recognized ecological significance, their functional role in plant invasion dynamics remains less explored. In this chapter, I investigated the culturable endophytic fungi colonizing two congeneric species *Lantana camara*, a globally invasive species, and *Lantana indica*, its native counterpart. The aim of this chapter is to elucidate the differences in fungal endophyte communities that could contribute to invasion success. In total, 228 fungal endophytes were isolated from different plant tissues (leaf, stem, and root). Among them, 24 representative morphotypes from *L. camara* and 32 from *L. indica* were molecularly identified using ITS rDNA sequencing. *Ascomycota* was found to be the predominant phyla found in both *L.camara* and *L.indica.* The genus *Fusarium* was the most abundant genus in both native (47.2%) and invasive (38.8%) plants followed by *Diaporthe* (32.2%) and *Trichoderma* (10%) in *L. camara*, and *Corynespora* (15.7%) and *Diaporthe* (12.5%) in *L. indica*. The alpha diversity metrics were used to estimate the diversity, the richness and evenness between the native and invasive species, beta diversity analysis were performed using NMDS based on Bray Curtis dissimilarity to show the differences in community composition. The findings suggest that the total diversity of fungal endophytes and their community composition does not vary substantially. Nonetheless, this chapter underscores the importance of culture dependent approaches for exploring the potential functional roles of fungal endophytes in invasion ecology.

3.2 Introduction

Invasive plant species have substantial ecological and economic consequences on native ecosystems. Mutualisms can strongly influence invasion success (Callaway *et al*. 2011). Evidence suggests that the competitive ability of certain invasive plant species can be enhanced through associations with mutualistic organisms (Soares *et al.*, 2016). Gaining insight into such determinants that contribute to the invasiveness of certain plant species will improve our understanding of invasiveness and also provide useful insights to promote plant health and conservation efforts. Endophytes engage in beneficial interactions with their host plants, enhancing growth and improving resistance to environmental stresses (Knoch *et al*., 1993). They also contribute to host defense by increasing tolerance to herbivory and pathogenic fungal attacks (Faeth & Fagan, 2002). Fungal endophytes, non-pathogenic organisms that reside inside plant tissues, have been identified as possible drivers of the proliferation of invasive plants. Host plant

adaptation to biotic and abiotic stressors is thought to be influenced by endophytic fungal colonization (Aly *et al*., 2010). Studies show a lack of mutualistic fungus which restricts the growth of invasive species. For instance, the earliest attempts to establish pine trees (*Pinus sp*.) in the southern hemisphere but the introduction of suitable ectomycorrhizal fungi enabled their establishment (Pringle *et al*., 2009). Conversely, fungal infections hinder the establishment and expansion of invasive plant populations (known as the Biotic Resistance Hypothesis), preventing them from becoming established or reaching high enough numbers to outcompete native species (Shearin *et al*., 2018). Previous research has shown a possible connection between the variety of endophytes and the ability of invasive plants to invade new habitats. Despite the efforts to identify consistent patterns, the outcomes of species interactions with respect to mutualism-parasitism gradient remain unpredictable, which emphasises the importance of ecological context in shaping their evolutionary roles (Agrawal *et al*., 2007). Hence, This research explores the variety of fungal endophytes found in both the invasive *Lantana camara* and the native *Lantana indica* to understand the interaction. The main objective of this work is to evaluate the fungal endophytic communities associated with *L. camara* to show unique diversity patterns in comparison to those of *L. indica*.

3.3 Materials & Methods

3.3.1 Study area & Sampling:

Lantana camara the invasive plant, and its congener *Lantana indica* the native plant, growing together within a spatial range of 10 x 10 metres were collected from five different geographical locations from Biligiriranga Hills situated at the convergence of the Western Ghats and the Eastern Ghats in Karnataka, India (Priti et al., 2016) (Figure 2.1 & Table 2.1). From each site, three individuals per species were collected (i.e., 3 *L. camara* and 3 *L. indica* plants per site), resulting in a total of six plants per site and 30 individual plants in total across all five sites. The plant samples were collected The plants were collected aseptically, transported to the laboratory in polyethylene bags, and promptly processed. Samples were collected in sterile plastic bags, transported to the laboratory, and stored at 4°C before endophyte isolation.

3.3.2 Isolation of fungal endophytes:

Each plant was washed with plenty of running tap water. Using a sterile scalpel, root, shoot, and leaf tissues were excised and subsequently washed with sterile distilled water to remove the debris. Plant materials were surface-sterilized by immersion in 70% (v/v) ethanol for 3 minutes, followed by treatment with 0.5% (v/v) sodium hypochlorite for another 3 minutes, and then rinsed three times with sterile distilled water (Arnold *et al*, 2003; Pandey *et al*., 2020) with few a modifications. After surface sterilization, the plant tissues were cut approximately to 1mm size using sterile scissors. The explants were placed on a petri dish containing Potato Dextrose Agar (PDA) media

amended with streptomycin (0.01%). An explant from every sterilized batch of tissues was pressed on a fresh PDA plate to check the sterility of the disinfection procedure. The plates with the explants were sealed and incubated at 28 ± 2°C for 7-9 days and were observed for the emergence of mycelial growth from the cut ends of the tissue segments. The emerged fungi were transferred to a fresh PDA plate for purification. The process was repeated until the pure culture was obtained.

3.3.3 Morphological identification of fungal endophytes

The fungal isolates were purified and then grouped into operational taxonomic units (OTUs) according to their cultural traits and reproductive structures, using established identification manuals by Booth (1971) and Sutton (1980). The selection of colonies was based on phenotypic traits like colony colour and morphology. The plating process was repeated 2-3 times to achieve pure cultures for further analysis. The purified fungal isolates were preserved in sterile water in cryovials at 4 degrees Celsius. Lacto-phenol cotton blue-staining was done to examine the mycelial and spore structures of the endophytic fungi under a light microscope.

3.3.4 Molecular identification of fungal endophytes

To minimize the uncertainties in fungal identification, a comparative approach was employed by integrating identification of morphological features and molecular identification using DNA barcoding using data retrieved from the National Center for Biotechnology Information (NCBI) (Gaddeyya et al., 2012; Fernandes et al., 2015). This comprehensive strategy ensured the accurate classification and ambiguities during the identification process. Genomic DNA was extracted from purified fungal isolates grown on PDA plates using the cetyltrimethylammonium bromide (CTAB) method and stored at −20 °C. The internal transcribed spacer (ITS) region of rDNA was amplified using the universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). PCR amplification was performed in a 25 µL reaction volume containing 1 µL of template DNA (50–100 ng), with the standard master mix containing $MgCl_2$, dNTPs, Taq buffer, and Taq DNA polymerase purchased from Barcode Biosciences, Bengaluru, 0.5 µL of F & R primer (10 pmol), and nuclease-free water. The PCR conditions consisted of an initial denaturation at 94 °C for 5 min, followed by 30 amplification cycles of denaturation at 94 °C for 1 min, primer annealing at 55 °C for 1 min, and strand extension at 72 °C for 1 min. A final extension was carried out at 72 °C for 5 min to ensure complete amplification of target fragments. The expected amplicon size ranged from 500 to 550 bp. PCR products were resolved on 1% agarose gels in 1× TAE buffer, visualized under UV light using a GelDoc system, and size of the PCR product was confirmed against a 1 kb DNA ladder. Amplified products were sent to Barcode Biosciences (Bengaluru, India) for Sanger sequencing. The sequences were identified taxonomically using BLASTn at NCBI.

3.3.5 Data Organization and Statistics:

The colonization frequency (%CF), reported as a percentage for above-ground and below-ground was estimated using the method described by Kumaresan and Suryanarayanan 2011. The dominance percentage of endophytes (D) was calculated by dividing the percentage of colonized frequency (%CF) by the total number of endophytes and multiplying by 100. Relative abundances were calculated as the number of OTU's assigned to each morphotype divided by the total number of OTU's per sample. Shannon and Simpson diversity indices, evenness and richness were calculated for endophytic fungi of invasive and native plants using PAST 4.03 (PAlaeontological STatistics) data analysis package (https://past.en.lo4d.com/windows). Additionally, non-metric multidimensional scaling (NMDS) was performed using the Bray - Curtis distances method to visualize the beta diversity, employing the function "Adonis" in the R version 4.3.1 package. To assess variations in the composition of endophytic fungal communities between invasive and native plants, a permutational multivariate analysis of variance (PERMANOVA) with 999 permutations was performed (Anderson, 2001). The ecological roles of fungal taxa, were inferred using the FUNGuild tool (Nguyen et al., 2016), a curated database that classifies fungal OTUs were used. Taxonomic assignments (genus level) were used as input to interpret their trophic modes. To estimate the endophytic fungal genera obtained from both culture-dependent isolation and metagenomic sequencing data from the previous chapter the identified genera from both the methods were compiled into a presence absence matrix. An UpSet plot was generated (UpSetR package) in R to visualize the overlap and unique genera across the culture-dependent and amplicon sequencing methods. Analyzing the composition of these shared and exclusive taxa provides insight into similarities and differences between methods (Mendes et al., 2011).

3.4 Results:

A total of 228 fungal endophytes were isolated and identified from both invasive and native plant species in this study. The colonization frequency of fungal endophytes was determined to be 73.5% and 63.1% in the above-ground and below-ground plant tissues of invasive plants, respectively (Figure 3.1). The percentage of native plants was found to be 68.3% in above-ground tissues and 52% in below-ground tissues (Figure 3.1). The colonization frequency of the native and invasive plants' above-ground (p-value = 0.31 > 0.05) and below-ground (*p*-value = 0.16 > 0.05) tissues did not vary significantly, according to the t-test. The phylum Ascomycota is the most prevalent in both non-native and native plants . The fungal isolates that were categorized belonged to 9 different orders of *Ascomycota* (*Hypocreales, Botryosphaeriales, Eurotiales, Diaporthales, Pleosporales, Capnodiales, Glomerellales, Cantharellales, Xylariales*) and 1 order of *Basidiomycota* (*Polyporales*) (Figure 3.2). Invasive plants were found to host 11 different endophytic fungal species, whereas native plants hosted 15 species. In particular, 103 Operational Taxonomic Units (OTUs) were obtained from invasive plants, while 125 OTUs were obtained from native species. The genera Fusarium and Diaporthe were found to be the most prevalent at the taxonomic level in both native and invasive plants. Nevertheless, Fusarium was increasingly

isolated from native plant species. The prevalence of Fusarium, Diaporthe, and Aspergillus in the native plants was determined to be 34.6%, 28.8%, and 10.5% respectively, based on the total isolates. On the other hand, the native plants were mostly composed of the genus *Fusarium*, accounting for 49.6% of the total isolates (Figure 3.3). This was followed by *Diaporthe* and *Corynespora*, each representing 12.8% of the isolates. Endophytic fungal community composition in native and invasive plants was compared using non-metric multidimensional scaling (NMDS) based on Bray Curtis dissimilarities. The ordination showed no significant difference in fungal community structure between native and invasive plants ($p = 0.265 > 0.05$) (Figure 3.6). Based on the FUNGuild annotation the *L.camara,* showed most endophytic fungi (68.0%) that were classified as pathotroph - saprotroph - symbiotrophs with mixed lifestyles. Saprotrophs were about 24.0%, while pathotroph - saprotrophs and pathotroph - symbiotrophs each contributed to 4.0%. In *L.indica*, pathotroph - saprotroph - symbiotrophs were also dominant (65.6%). Unlike *L.camara*, it had a higher proportion of pathotroph - saprotrophs (21.9%), while saprotrophs and pathotroph - symbiotrophs were lower (6.3% each). The UpSet plot highlights (Figure 3.9) the distribution of fungal genera identified using culture-dependent and culture-independent approaches. A total of 279 genera were detected only in culture independent method, while 7 genera were uniquely recovered from culture dependent method and 14 genera were found in both the methods, representing the overlap between culture dependent and metagenomic based approaches.

3.5 Discussion:

This chapter determines the colonization frequency and community structure of culturable endophytic fungi in both the invasive *Lantana camara* and the native *Lantana indica*. Both plant species exhibited a high frequency of endophytic fungal colonization in the above and below ground plant tissues, while *L. camara* showed slightly higher colonization rates however they are not statistically significant.

Taxonomic analysis showed *Ascomycota* as the dominant fungal phylum in both hosts, with diverse orders such as *Hypocreales, Xylariales, and Diaporthales*. Notably, the diversity of fungal endophytes in *L. camara* was slightly lower than in *L. indica*, certain genera such as *Fusarium* and *Diaporthe* were prevalent in both. This result aligns with the result of amplicon sequencing of fungal endophytes from chapter 2. *Fusarium* was more frequently isolated from both the plants, a genus known for its functional plasticity ranging from plant pathogens to endophytes with growth-promoting or stress-alleviating effects (Toghueo, 2020). The genus *Diaporthe* likewise includes both pathogenic and mutualistic species, reinforcing the ecological importance (Gomes et al., 2013).

To explore the potential ecological functions of the observed fungi, FUNGuild-based trophic mode classification was used. Both *L. camara* and *L. indica* exhibited a high proportion of multi-trophic

fungi categorized as pathotroph - saprotroph - symbiotrophs (68.0% and 65.6%, respectively), suggesting that these fungal taxa possess multifunctional roles that shifts depending on the environmental context (Nguyen et al., 2016). These fungi function as beneficial mutualists under non-stress conditions and transition to pathogenic behavior when the host defense is compromised (Schulz & Boyle, 2005). Higher saprotrophic interactions contributes to the invasive success of *L.camara* by either affecting native plants or benefiting from mutualistic interactions which can potentially disrupt local ecosystems by affecting plant health and competitive dynamics (Leslie & Summerell, 2006; Michielse & Rep, 2009).

L.indica also showed a slightly higher proportion of pathotroph - saprotrophs (21.9%) and pathotroph - symbiotrophs(6.3%) compared to *L. camara* (4.0% and 4.0%, respectively). This difference indicates a more specialized or potentially antagonistic endophyte community in the native host. Conversely, the greater functional redundancy in *L.camara* reflects a microbial community with broader ecological versatility, potentially enhancing the invasive plant's adaptability to diverse environmental conditions (Pringle *et al*., 2009).

The comparison of metagenome and culture dependent endophytic fungi in this chapter is not sequence-based, as different ITS regions were used for the identification. Thus, the analysis focuses solely on the genera identified. Comparing the culture dependent and culture independent methods, both the methodologies have their biases such as unavailability of growth requirements and identification of non-sporulating fungi in culture dependent methods (Dissanayake *et al*., 2018), and in metagenome analysis, sequencing is affected by primer choice, depth, and database limitations (Al-Awadhi *et al.*, 2013). However the bias can be reduced by using both the methods to complement each other in assessing endophytic fungal diversity. Together, the findings emphasize that integrating both approaches yields a more complete view of endophytic fungal community composition.

In conclusion, culture dependent studies are important to study the functional aspects for microbes. This would be a base to study their functionality *in vitro* and *in vivo*. The endophytic fungal community composition and functional guild profiles between native and invasive *Lantana* species highlight the complexity of plant-microbe interactions in invasion ecology. These findings underscore the importance of integrating fungal functional traits into our understanding of plant invasions and suggest that certain endophytic fungi contribute to host stress tolerance, growth promotion, and competitive success.

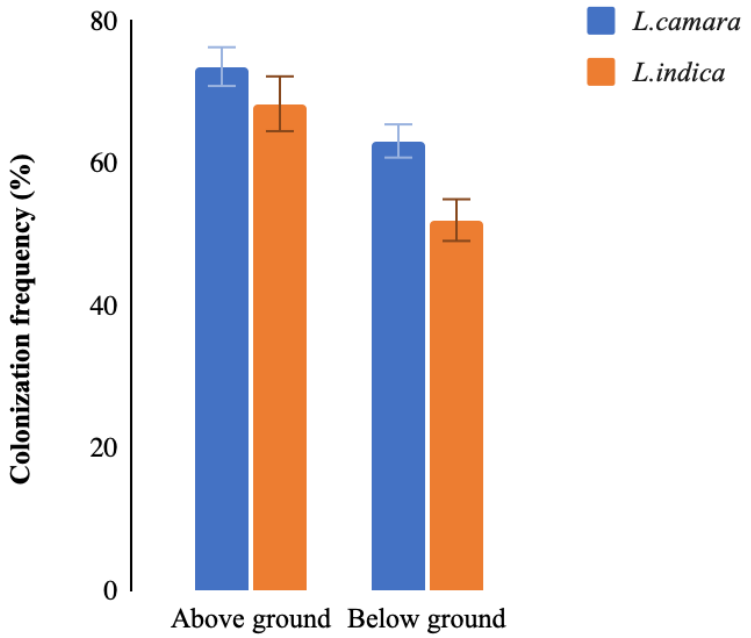


Figure 3.1: Colonization frequency of above-ground and below-ground tissue samples from invasive (*L.camara*) and native (*L.indica*) plants

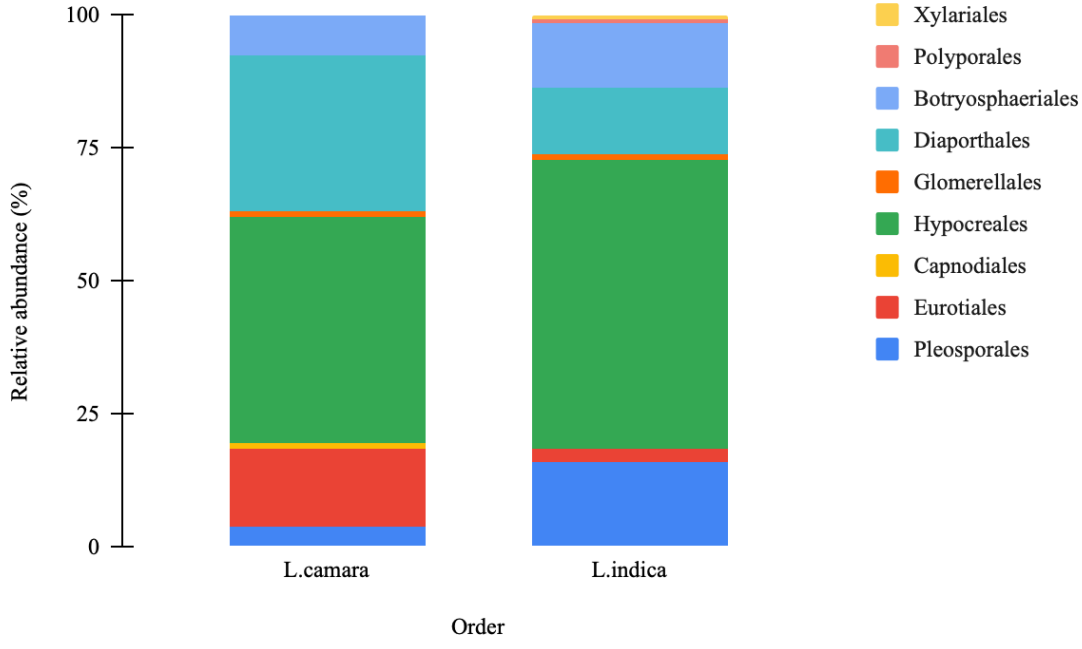


Figure 3.2: Relative abundance of endophytic fungal order associated with invasive (*L.camara*) and native (*L.indica*) plants is illustrated in the bar chart. The composition of endophytic fungal communities present in each plant species, with relative abundance expressed in percentage (%)

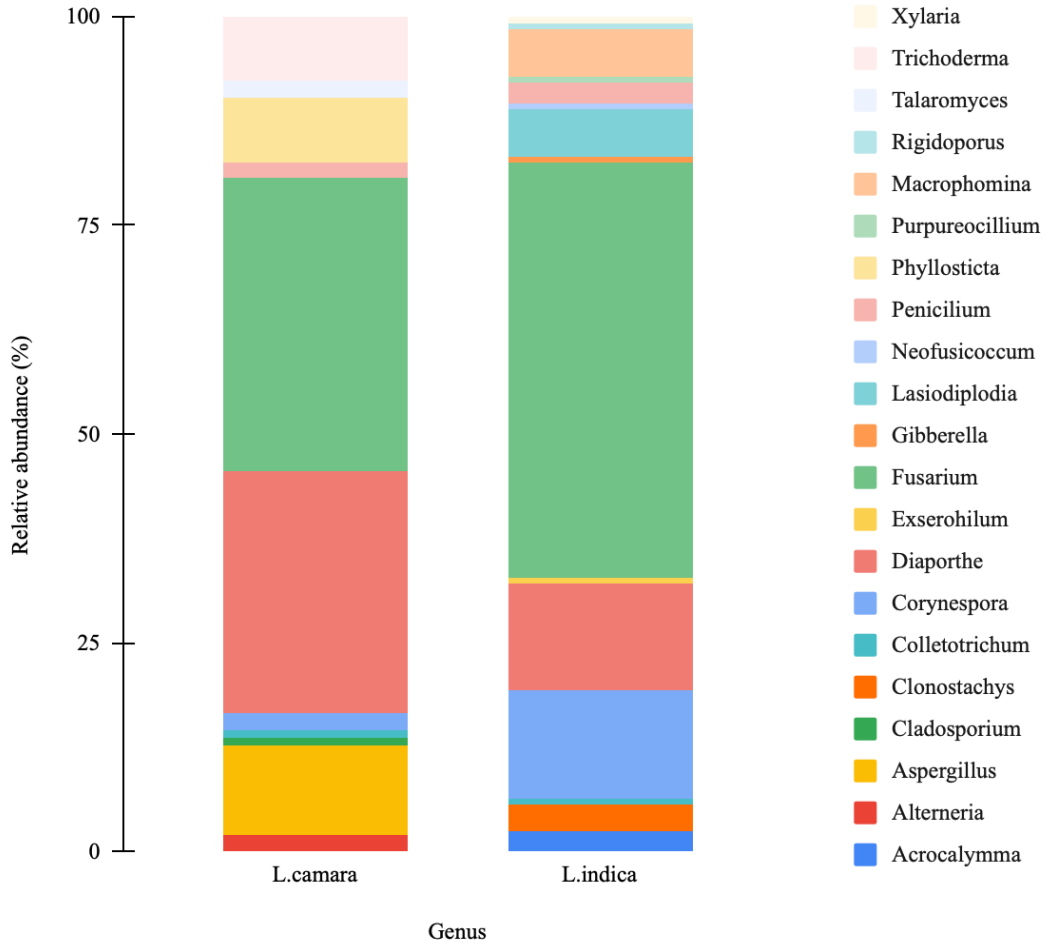
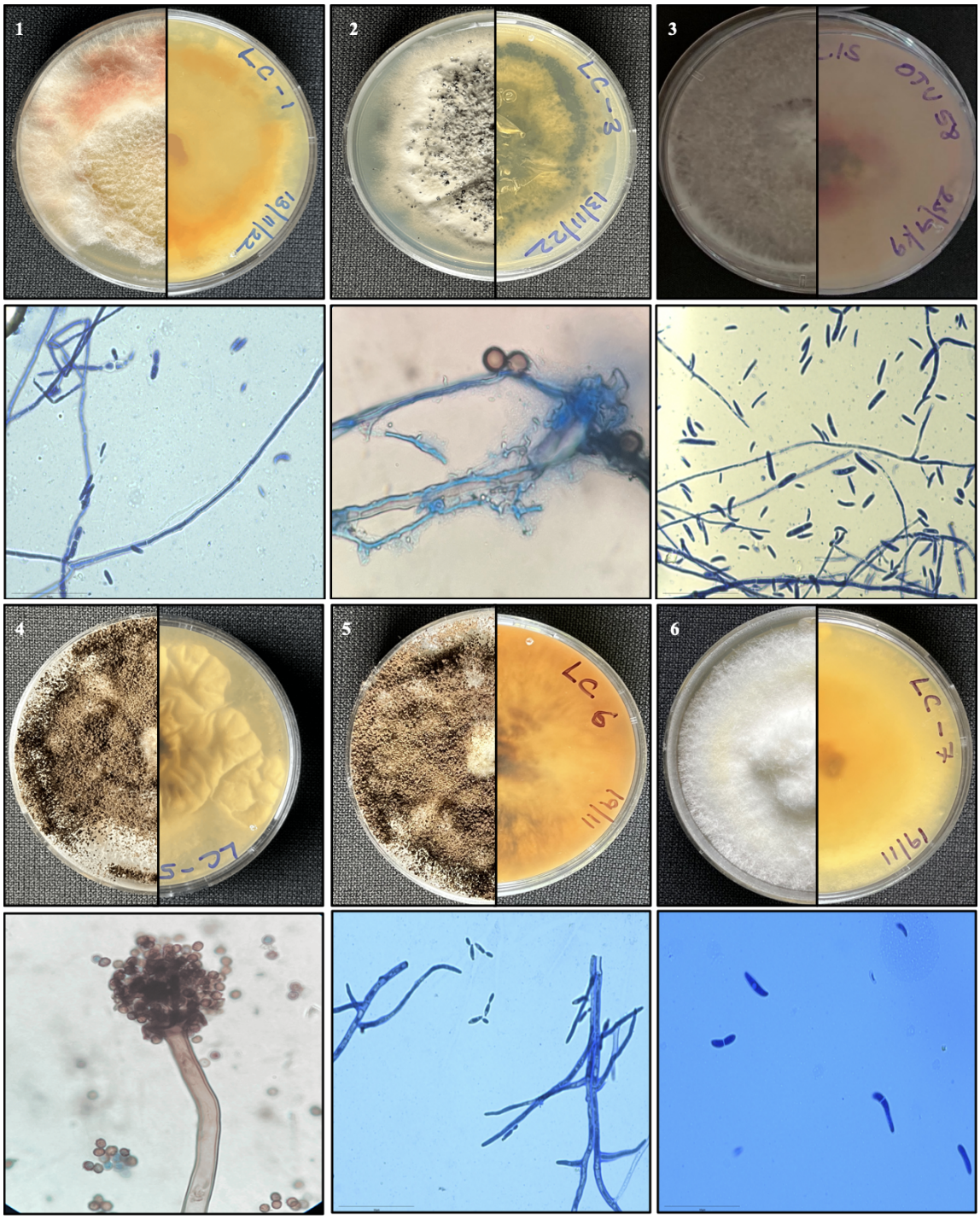
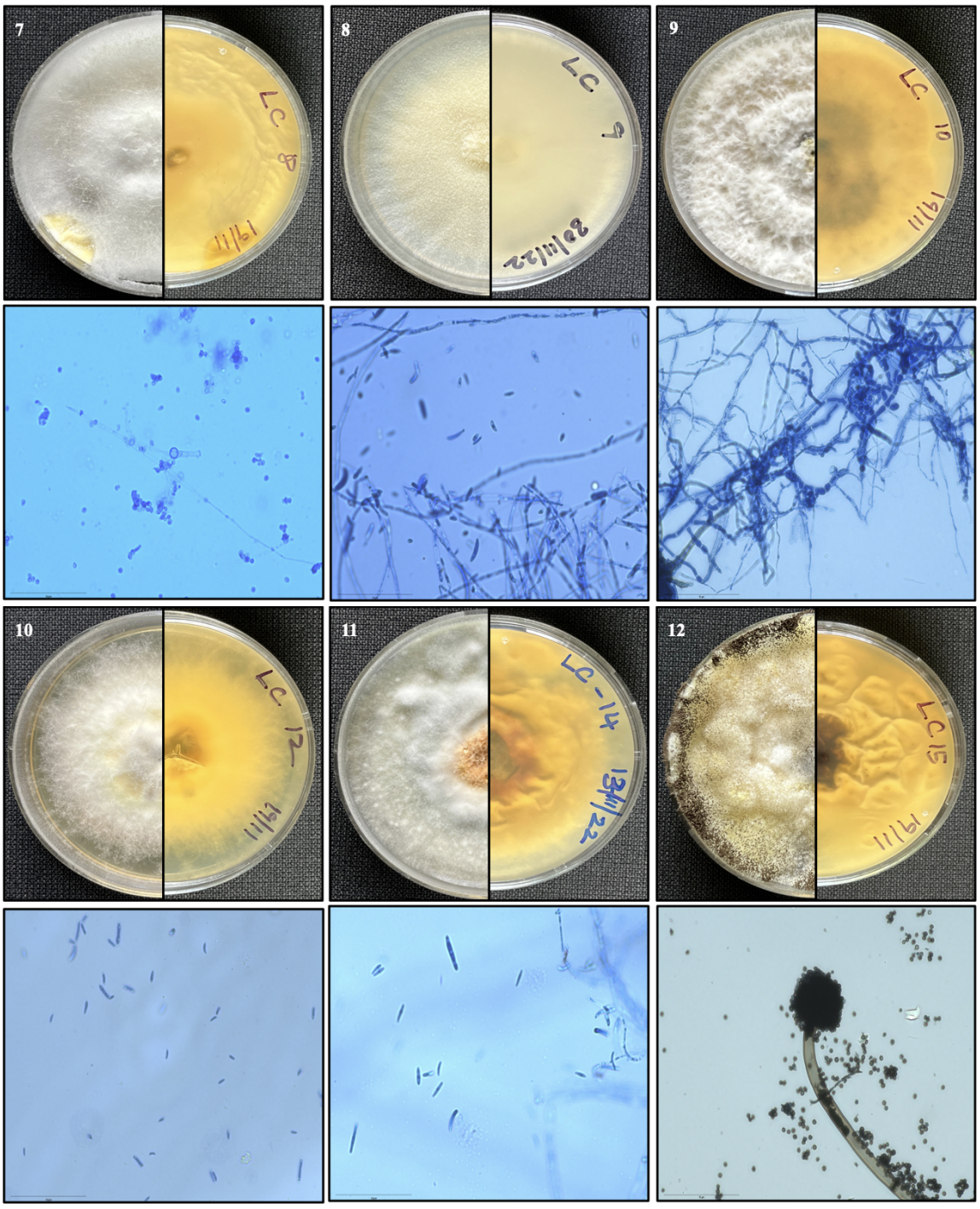
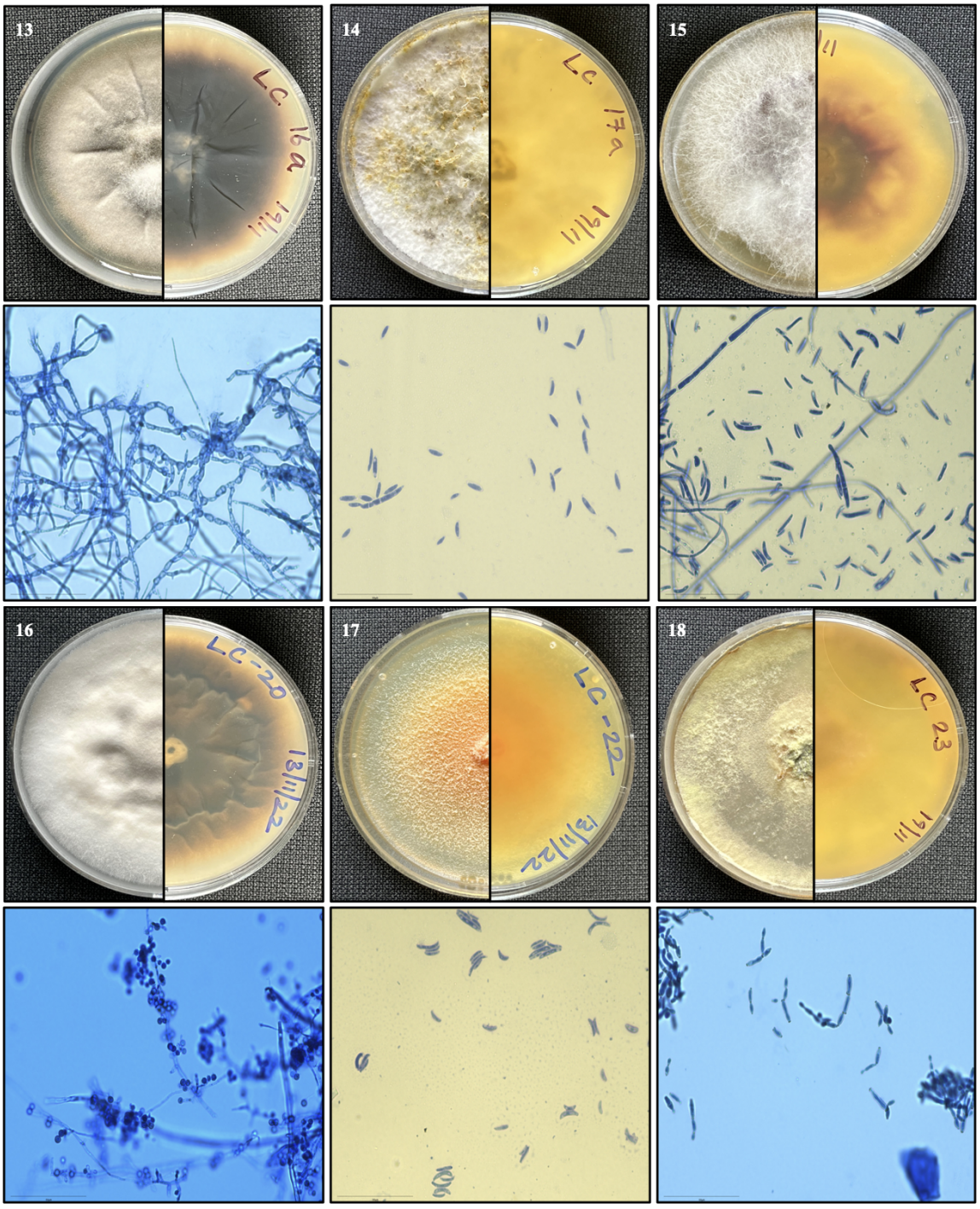
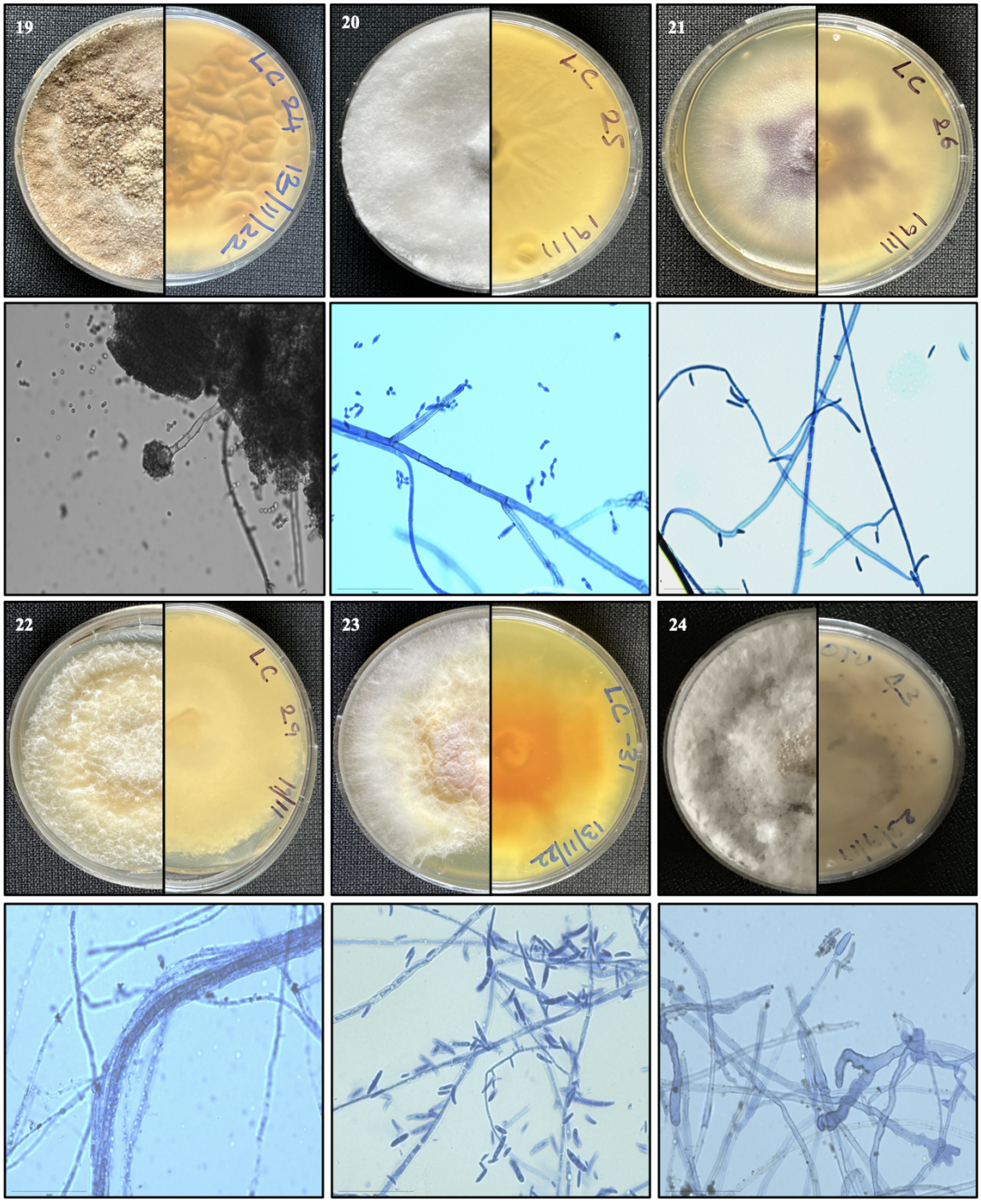


Figure 3.3: Relative abundance of endophytic fungal genera associated with invasive (*L.camara*) and native (*L.indica*) plants is illustrated in the bar chart. The composition of endophytic fungal communities present in each plant species, with relative abundance expressed in percentage (%)


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LC-1
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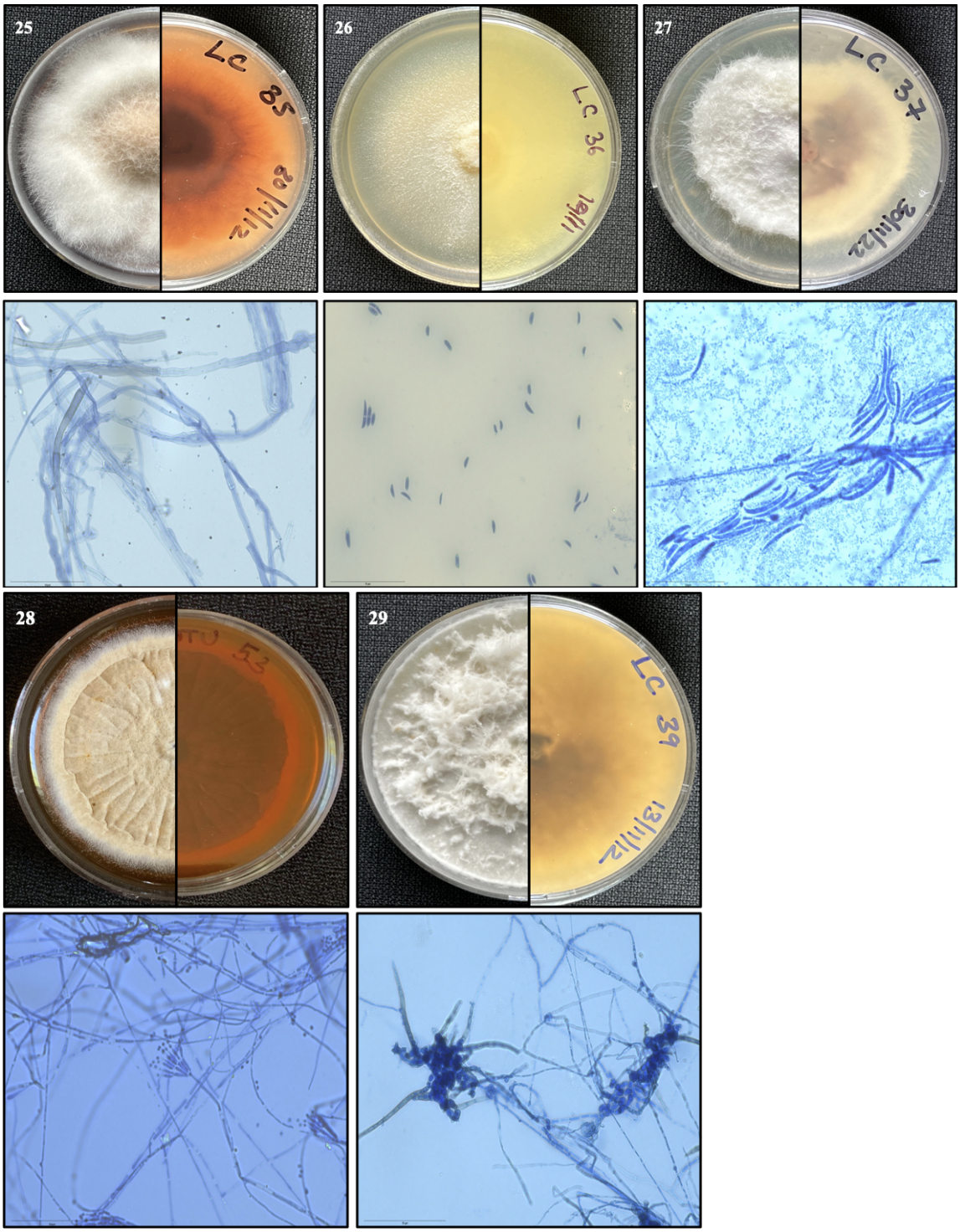
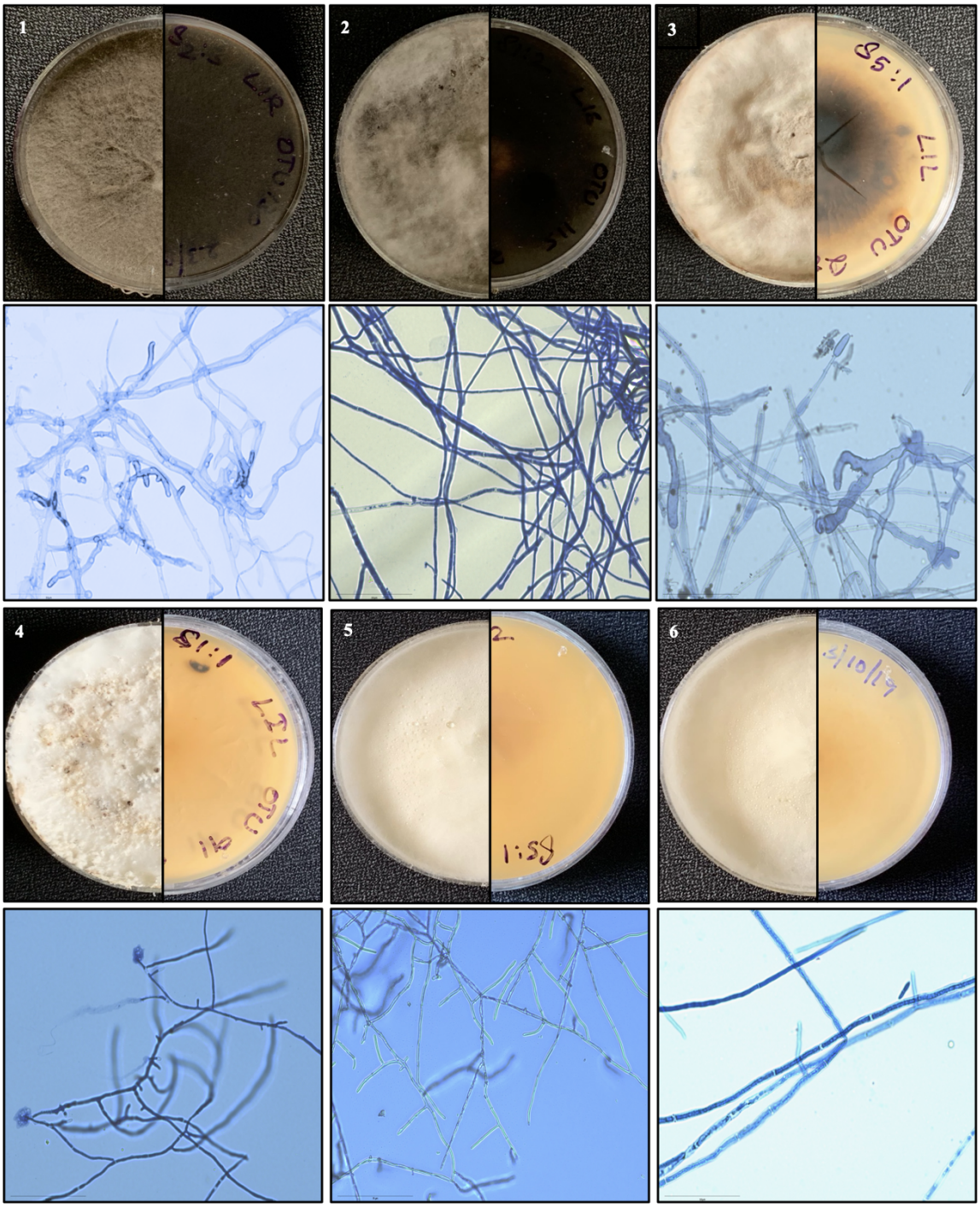
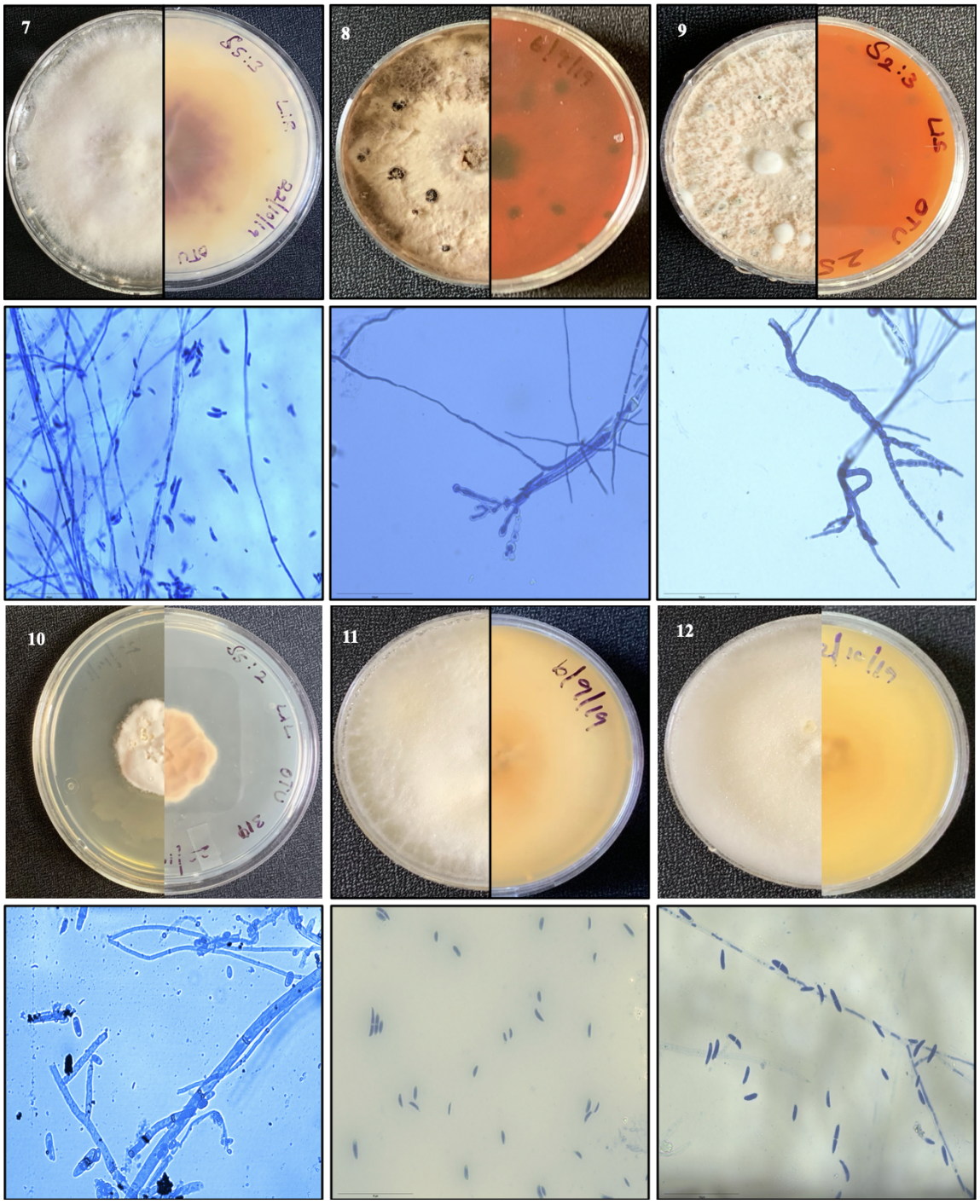
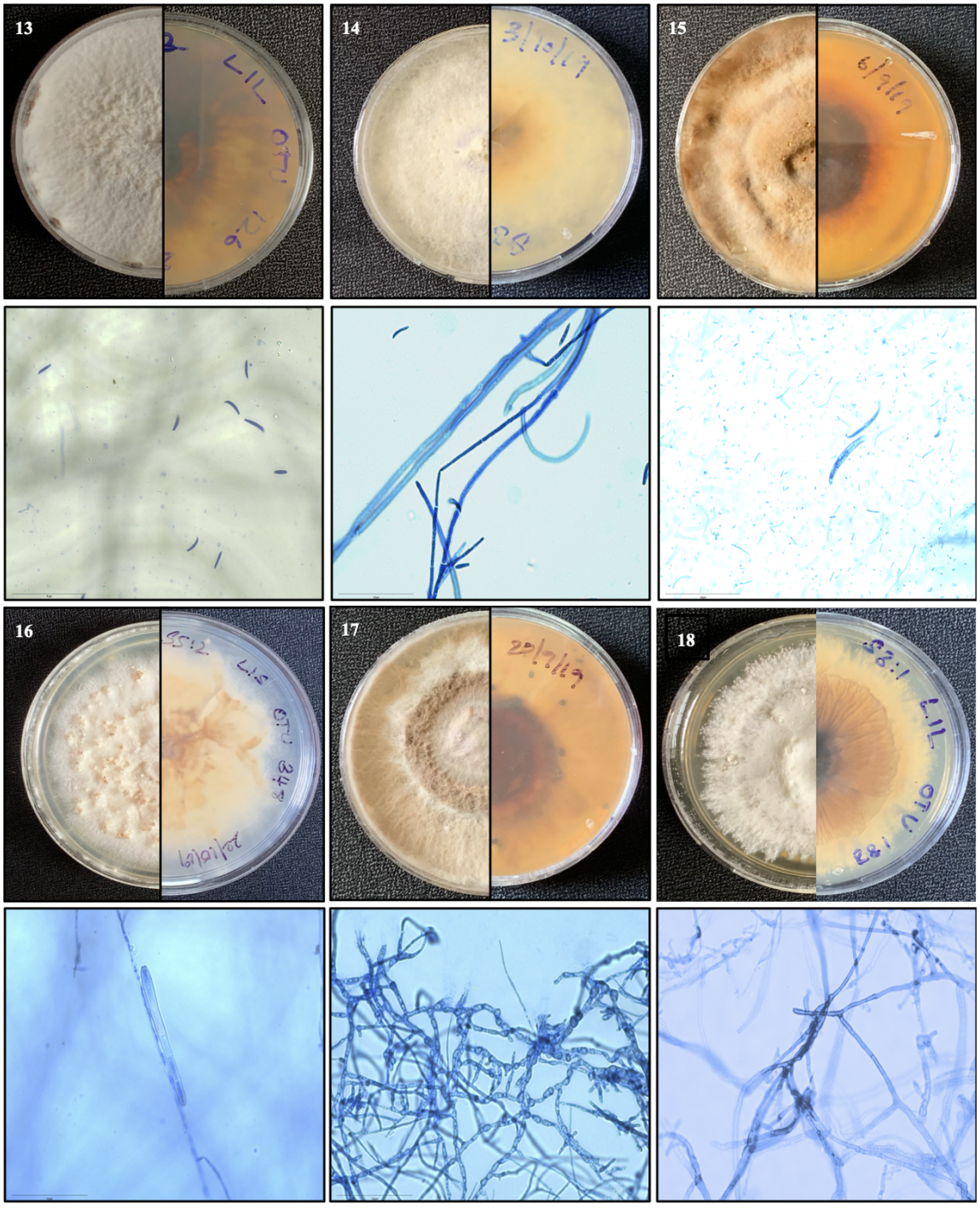


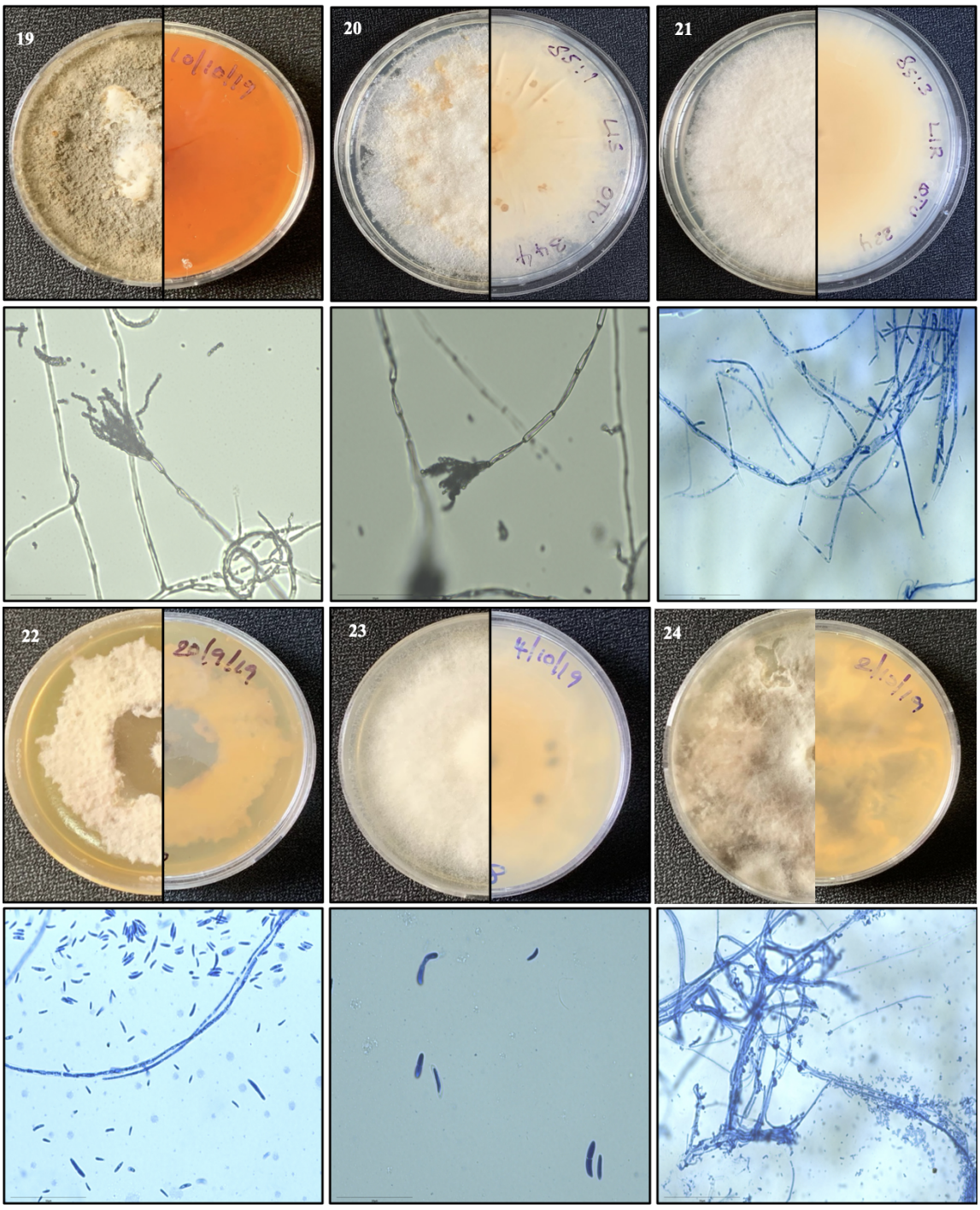
Figure 3.4: Cultural growth and microscopic structures of endophytic fungal isolates from the plant *L.camara. 1.Fusarium oxysporum, 2. Phyllosticta capitalensis, 3. Fusarium oxysporum, 4. Aspergillus sp, 5. Diaporthe sojae, 6. Fusarium thapsinum, 7. Trichoderma, 8. Fusarium inflexum, 9. Diaporthe sp, 10. Fusarium solani, 11. Talaromyces pinophilus, 12. Aspergillus niger, 13. Alternaria macrospora, 14. Fusarium solani, 15. Fusarium nirenbergiae, 16. Cladosporium*

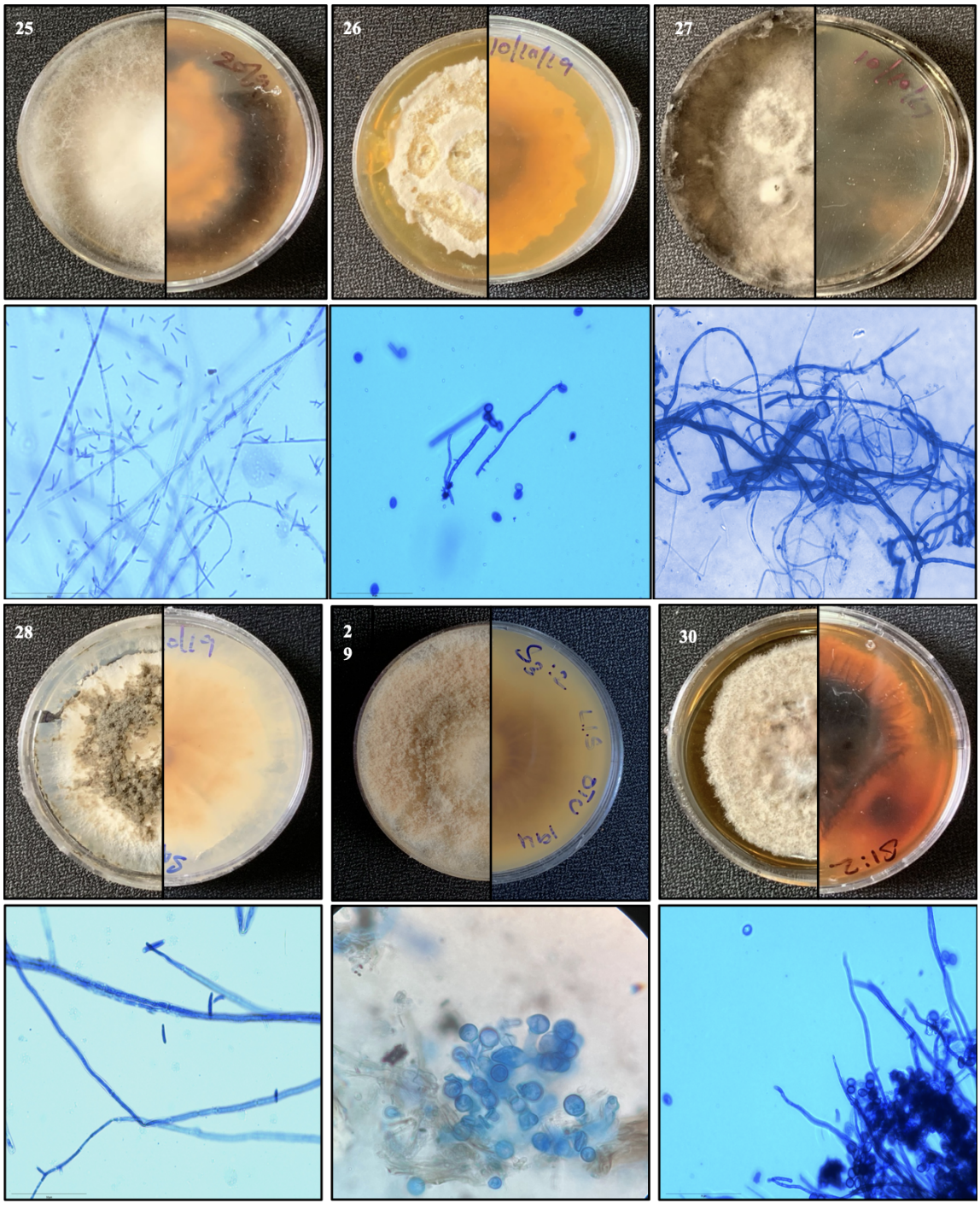
tenuissimum, 17. Fusarium sp, 18. Diaporthe melonis, 19. Aspergillus terreus, 20. Trichoderma sp, 21. Fusarium verticillioides, 22. Colletotrichum gigasporum, 23. Fusarium oxysporum, 24. Corynespora sp, 25. Colletotrichum karsti, 26. Corynespora cassiicola, 27. Fusarium fujikuroi, 28. Fusarium oxysporum, 29. Penicillium citrinum, 30. Diaporthe velutina




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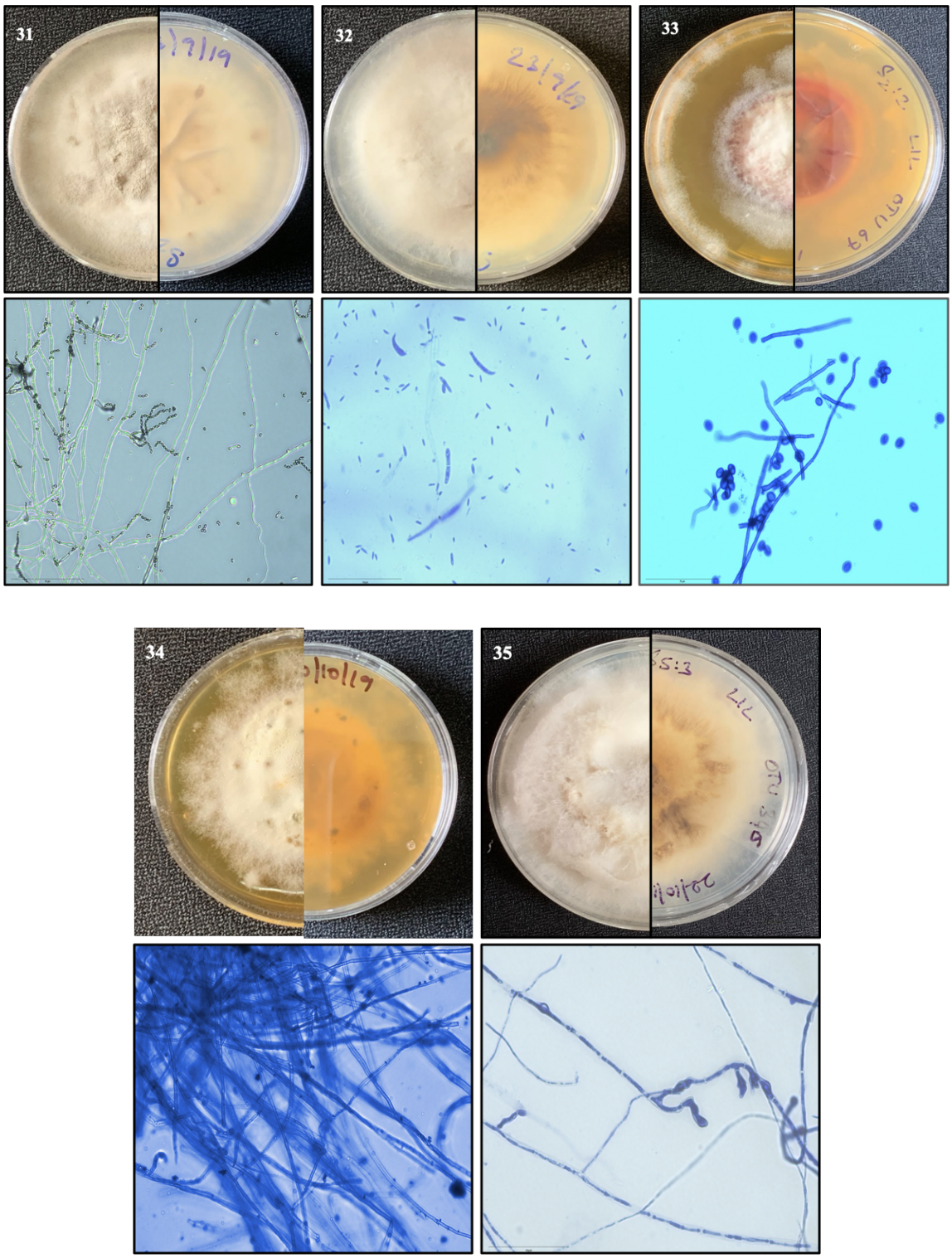


Figure 3.5: Cultural growth and microscopic structures of endophytic fungal isolates from the plant *L.indica.1.Macrophomina phaseolina, 2. Lasiodiplodia pseudotheobromae, 3. Corynespora cassiicola, 4. Fusarium sp, 5. Fusarium oxysporum, 6. Fusarium solani, 7. Fusarium oxysporum, 8. Diaporthe discoidispora, 9. Diaporthe melonis, 10. Xylariaceae sp, 11. Fusarium solani, 12.*

Fusarium oxysporum, 13. Fusarium cassiae, 14. Fusarium oxysporum, 15. Corynespora cassiicola, 16. Clonostachys rosea, 17. Diaporthe phaseolorum, 18. Penicillium shearii, 19. Penicillium sp, 20. Fusarium sambucinum, 21. Gibberella fujikuroi, 22. Fusarium thapsinum, 23. Fusarium sp, 24. Neofusicoccum parvum, 25. Exserohilum rostratum, 26. Acrocalymma vagum, 27. Lasiodiplodia theobromae, 28. Fusarium oxysporum, 29. Rigidoporus vinctus, 30. Acrocalymma sp, 31. Purpureocillium lilacinum, 32. Fusarium sp, 33. Acrocalymma medicaginis, 34. Colletotrichum nymphaeae, 35. Corynespora cassiicola

S.No.	Diversity Index	Invasive *(L. camara)*	Native *(L. indica)*	*p-value*
1	Individuals	103	125	-
2	Richness (S)	11	15	-
3	Shannon (H′)	2.17	2.42	0.18
4	Simpson (1–D)	0.85	0.89	0.222
5	Evenness (e^H/S)	0.8	0.82	0.642

Table 3.1: Diversity indices of endophytic fungal genera of invasive (*L.camara*) and native (*L.indica*) plants

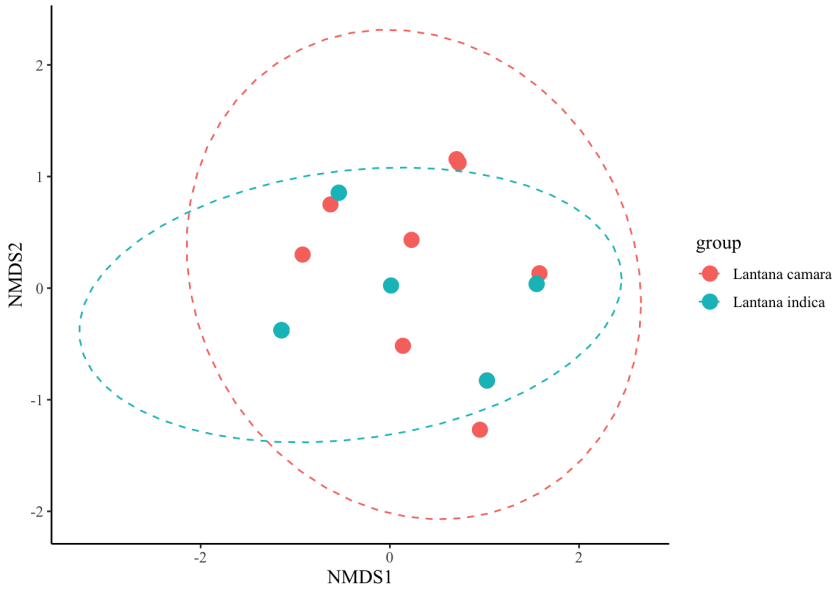


Figure 3.6: Non-metric multidimensional scaling (Bray - Curtis distances) of endophytic fungal community structure of invasive (*L.camara*) and native (*L.indica*) plants. The ordination plot illustrates the distinct endophytic fungal compositions between the native and invasive plant. The 95% confidence interval surrounding the centroid of each species group is shown by each ellipse

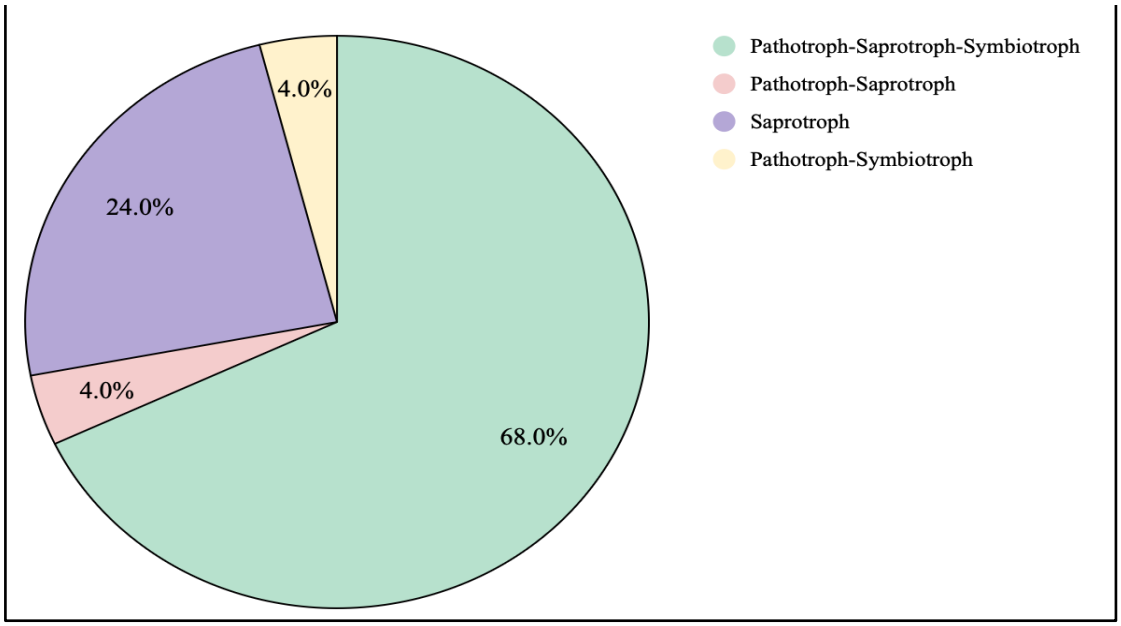


Figure 3.7: Functional lifestyles of endophytic fungi of *L.camara*, based on FUNGuild annotation

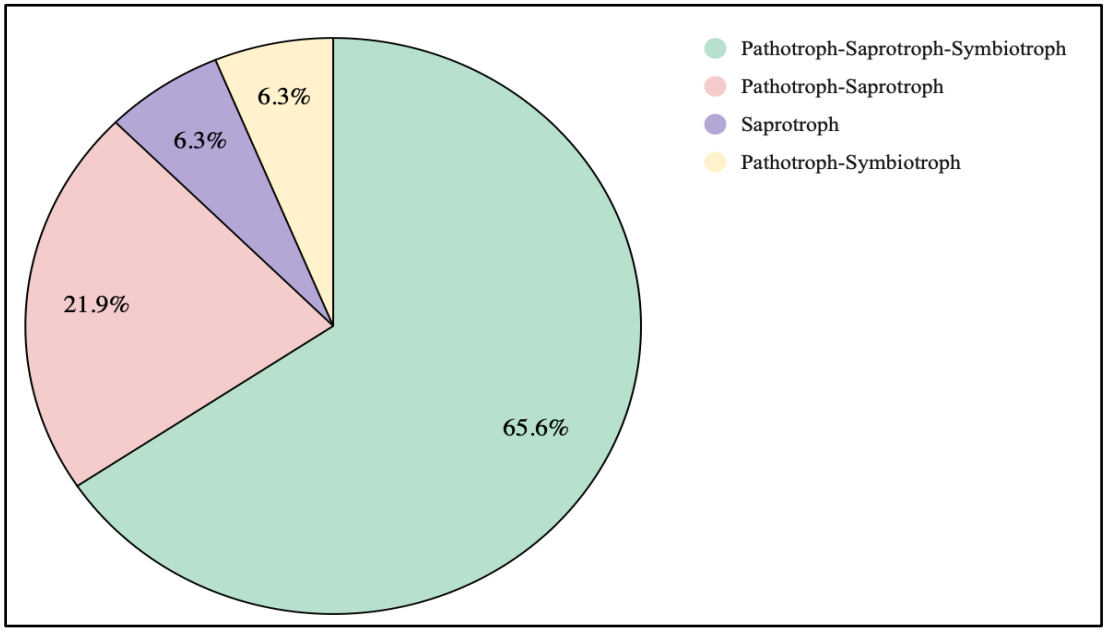


Figure 3.8: Functional lifestyles of endophytic fungi of *L.indica*, based on FUNGuild annotation

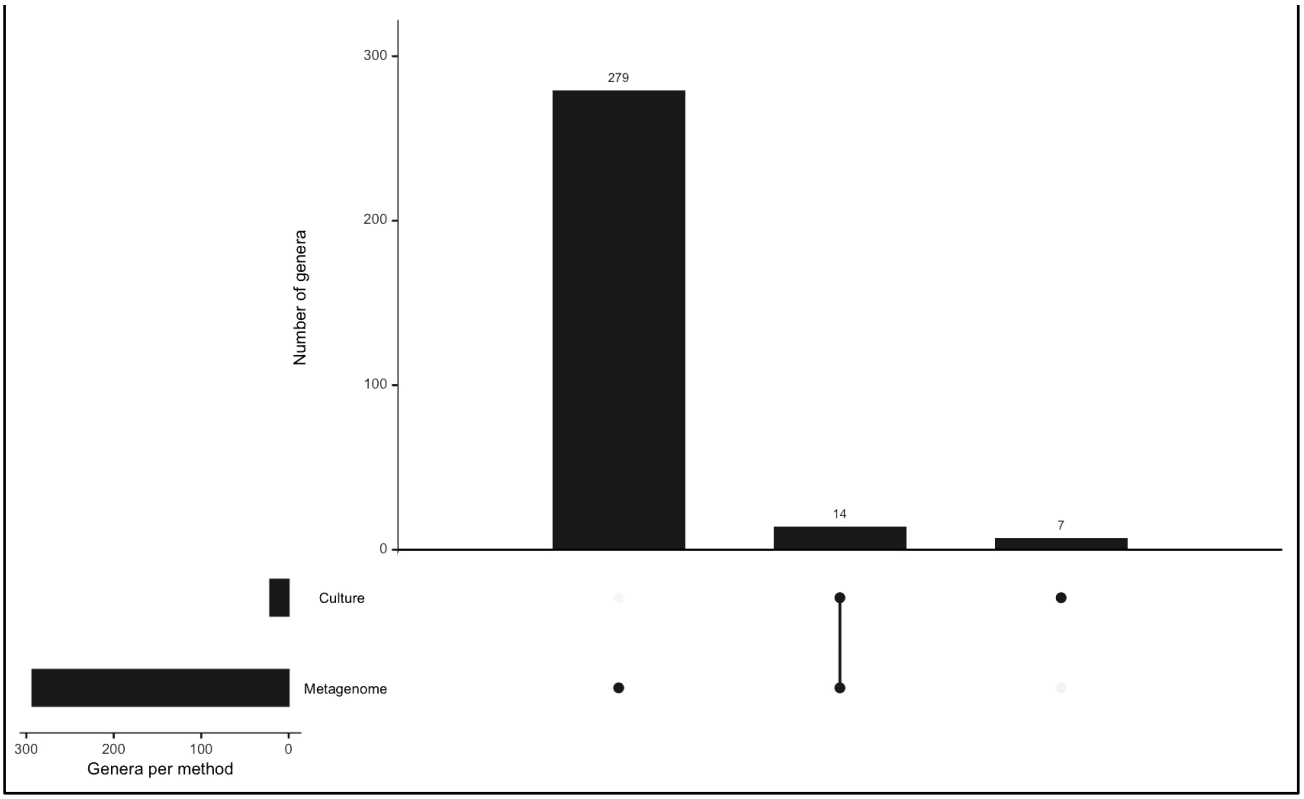


Figure 3.9: UpSet plot shows the endophytic fungal genera detected by culture-dependent and culture-independent metagenomic sequencing methods. The bar chart at the top indicates the unique and shared genera between two methods. The horizontal bars on the left shows the total number of genera recovered

Isolate ID	**Accession number (GenBank, this study)**	**Closest match in NCBI***	**Query cover (%)**	**Percentage identity (%)**	**Reference accession**
LC_1	PV888601	*Fusarium oxysporum*	99%	99.06%	MW995806.1
LC_3	PV888602	*Phyllosticta capitalensis*	99%	99.19%	MG954332.1
LC_4	PV888603	*Fusarium oxysporum*	98%	99.80%	MN559981.1
LC_5	PV888604	*Aspergillus niger*	98%	99.82%	ON973989.1
LC_6	PV888605	*Diaporthe sojae*	99%	99.64%	MT043838.1

LC_7	PV888606	*Fusarium thapsinum*	99%	99.05%	MW016514.1
LC_8	PV888607	*Trichoderma sp*	99%	95.83%	MK870565.1
LC_9	PV888608	*Fusarium inflexum*	97%	99.61%	MW855849.1
LC_10	PV888609	*Diaporthe phaseolorum*	99%	99.28%	OP002087.1
LC_12	PV888610	*Fusarium solani*	99%	99.63%	OQ784159.1
LC_14	PV888611	*Talaromyces pinophilus*	99%	99.11%	MF686811.1
LC_15	PV888612	*Aspergillus niger*	98%	89.41%	OK210353.1
LC_16	PV888613	*Alterneria macrospora*	99%	99.82%	DQ156342.1
LC_17	PV888614	*Fusarium solani*	98%	99.63%	MT638068.1
LC_19	PV888615	*Fusarium nirenbergiae*	97%	99.03%	MW016586.1
LC_20	PV888616	*Cladosporium tenuissimum*	99%	100.00%	MN429197.1
LC_22	PV888617	*Fusarium proliferatum*	99%	98.68%	OP060705.1
LC_23	PV888618	*Diaporthe sp*	99%	98.72%	FN386272.1
LC_24	PV888619	*Aspergillus terreus*	99%	99.83%	MT316343.1
LC_25	PV888620	*Trichoderma sp*	99%	98.99%	MH284378.1
LC_26	PV888621	*Fusarium verticillioides*	98%	99.61%	MN871798.1
LC_29	PV888622	*Collechotrichum*	98%	99.43%	JN198428.1
LC_31	PV888623	*Fusarium oxysporum*	96%	99.42%	MW995806.1
LC_33	PV888624	*Corynespora cassiicola*	98%	99.81%	MN393236.1

LC_35	PV888625	*Corynespora cassiicola*	99%	99.81%	MN818634.1
LC_36	PV888626	*Fusarium sp*	97%	99.80%	OQ629152.1
LC_37	PV888627	*Fusarium oxysporum*	99%	99.80%	MN538904.1
LC_38	PV888628	*Penicillium citrinum*	98%	100.00%	LC514694.1
LC_39	PV888629	*Diaporthe velutina*	99%	97.63%	MZ895503.1

Table S3: Culturable fungal endophyte isolates obtained from *Lantana camara*. The table lists the accession numbers of deposited ITS sequences in NCBI GenBank, their closest taxonomic matches based on BLASTn search against the NCBI nucleotide database

Isolate ID	**Accession number (GenBank, this study)**	**Closest match in NCBI***	**Query cover (%)**	**Percentage identity (%)**	**Reference accession**
I_1	PV875640	*Macrophomina phaseolina*	100	100	OM685088.1
I_2	PV875641	*Lasiodiplodia pseudotheobromae*	98	99.8	KY783446.1
I_3	PV875642	*Corynespora cassiicola*	93	99.81	MW300945.1
I_5	PV875643	*Fusarium oxysporum*	100	99.8	OK326823.1
I_6	PV875644	*Fusarium solani*	98	100	MK174970.1
I_7	PV875645	*Fusarium oxysporum*	99	99.23	OP199102.1
I_8	PV875646	*Diaporthe discoidispora*	99	98.9	MH371253.1
I_9	PV875647	*Diaporthe sojae*	99	98.72	FN386272.1
I_10	PV875648	*Muscodor sp.*	99	98.51	JQ760598.1
I_11	PV875649	*Fusarium solani*	99	99.63	MN602620.1
I_12	PV875650	*Fusarium solani*	99	99.62	MK158347.1

I_13	PV875651	*Fusarium sp.*	97	94.11	MW016554.1
I_14	PV875652	*Fusarium sp.*	98	99.43	MW995806.1
I_16	PV875653	*Corynespora cassiicola*	98	99.43	MK571354.1
I_17	PV875654	*Clonostachys rosea*	98	100	MT215574.1
I_18	PV875655	*Diaporthe phaseolorum*	98	98.91	OP797660.1
I_19	PV875657	*Penicillium sp.*	97	99.82	MW670522.1
I_21	PV875658	*Penicillium sp.*	99	99.28	MN153943.1
I_22	PV875659	*Gibberella fujikuroi*	97	100	MW995794.1
I_23	PV875660	*Fusarium sp.*	99	98.86	MW016515.1
I_26	PV875661	*Neofusicoccum parvum*	99	99.82	KU997548.1
I_28	PV875662	*Exserohilum rostratum*	99	99.3	MN599614.1
I_29	PV875663	*Acrocalymma vagum*	98	98.64	OP630541.1
I_30	PV875664	*Lasiodiplodia theobromae*	99	99.23	ON994668.1
I_31	PV875665	*Clonostachys rosea*	97	99.25	MW113577.1
I_32	PV875666	*Lentinus sp.*	98	99.03	MK646062.1
I_35	PV875667	*Purpureocillium lilacinum*	97	99.82	OP268323.1
I_37	PV875668	*Acrocalymma medicaginis*	98	98.82	OP113900.1
I_38	PV875669	*Colletotrichum nymphaeae*	99	99.28	OQ991246.1
I_40	PV875670	*Corynespora cassiicola*	99	99.81	MT228953.1

Table S4: Culturable fungal endophyte isolates obtained from *Lantana indica*. The table lists the accession numbers of deposited ITS sequences in NCBI GenBank, their closest taxonomic matches based on BLASTn search against the NCBI nucleotide database

Chapter - 4

Investigate abiotic stress tolerance of endophytic fungi isolated from congeneric native and invasive *Lantana* species

4.1 Abstract

I investigated the abiotic stress tolerance of endophytic fungi isolated from both congeneric invasive *(Lantana camara)* and native *(Lantana indica)* plants to explore potential contribution of fungi to abiotic stress tolerance in host plants. Abiotic stresses are a significant factor that affects plant yield and quality, inducing survival mechanisms that temporarily reduce plant growth. Here I explore the endophytic fungal responses to various abiotic factors, such as extreme temperatures, drought, and high saline conditions. Specifically, I examined whether the invasive plants harbor more endophytic fungi that are tolerant to abiotic stress compared to the native plants. This is to understand whether endophytic fungi contribute to plant adaptation to challenging environments, mitigating the impact of harsh conditions. Fungal growth decreased as levels of salt and heat stress increased, indicating a negative correlation between the intensity of stress and fungal growth. In contrast, under drought stress, the isolates showed variable responses, suggesting differential tolerance mechanisms among the fungal taxa. *Aspergillus* sp. exhibited tolerance to both salt (up to 2.5 M NaCl) and drought (up to 15% PEG), while *Macrophomina phaseolina* showed optimal growth at 40 °C and remained viable at 45 °C. This study enhances our understanding of how plant-microbe interactions contribute to the tolerance of plants, enabling them to thrive in diverse environmental conditions.

4.2 Introduction

Plants are often considered autonomous organisms, especially when it comes to the interpretation of their cellular and genetic functions. However, the plants provide an exclusive habitat or "biological niche" for various microbial communities, which often confer several advantages, including improved photosynthetic efficiency, nutrient and water consumption, and resistance to biotic and abiotic stress. Microbial endophytes are symbionts that live inside plant tissues and do not cause disease symptoms in their host plants. Fungal endophytes have been a focus of current research because of their ability to promote plant development and their advantageous roles in the responses of plants to abiotic stress.

Climatic prediction models show that rising temperatures due to climate change, along with increased soil moisture requirements by plants, intensify drought stress, resulting in lower plant output (Suzuki, 2016). Between 1980 and 2016, climate changes generated around $220 billion in agricultural losses in the United States as a result of heat and drought stress on plants (Lamaoui *et al*., 2018; Bilal *et al*., 2020). Furthermore, massive human and geologic processes have severely polluted agricultural and natural soils across the planet due to the spread of unfavorable flora and fauna. According to recent findings by IPCC regional groups, Europe and North America will be

less affected by climate change in the mid-century and end-of-century than Africa, Central America, and South America, in terms of maize and soybean production. However, Hasegawa et al. (2022) predict that all locations will encounter a blend of favorable and unfavorable outcomes. These geographical variances in mid- and late-century consequences are related to present temperature levels. Furthermore, the expansion of *Lantana*, which flourishes in tropical and subtropical locations, is anticipated to become a more serious concern in the future years. Understanding the microbial partners involved in *Lantana's* stress tolerance shed light on the etiology of its widespread distribution.

This research focuses on the vital function of endophytic fungi in invasive and native plants and how they stimulate plant tolerance to abiotic stress to survive in newer environments. In general, the growth of a plant in a given environment is primarily determined by biotic and abiotic factors (Zhang *et al*. 2022). The abiotic factors such as temperature, sunlight, water, minerals, and atmospheric conditions can cause adverse effects on plant growth and development. Notably, extreme temperature fluctuations, drought, flooding, salinity, metal exposure, and nutrient deficiencies induce distinct plant responses such as alterations in biological processes, gene expression, and cell metabolism, which in turn affect the growth and development of plants (Zhang *et al*., 2023; Zhang *et al*., 2020).

In particular, plants experience direct or indirect hyperosmotic stress in response to drought and excessive salinity (Zhang *et al*., 2022). The thermal stress can present a potential hazard of protein denaturation, which would require the production of heat shock proteins (HSPs). A considerable number of these HSPs serve as molecular chaperones, preserving proteostasis and preventing protein denaturation (Scharf J *et al*., 2012). Abiotic stresses are acknowledged as critical contributors to reduced plant yield and quality. This state of the plant can induce survival mechanisms through the inhibition of growth and development. Nevertheless, this adaptive reaction impedes overall plant development by reducing plant resistance (Zhang *et al*., 2020).

This study focuses on understanding the pivotal role of EF in both invasive and native plants, examining how these EF contribute to enhancing plant tolerance to abiotic stress, ultimately enabling plants to thrive in novel environments and thereby mitigating the challenges posed by harsh environmental conditions. The objective of this research involves screening fungal isolates specifically associated with *Lantana camara* and *Lantana indica*, invasive and native plants, respectively. These fungal endophytes were subjected to various abiotic stresses, using high salt concentration, drought, and high temperature, with slight modifications based on the study by Farhana *et al*. (2019).

4.3 Materials and methods

4.3.1 Isolation of fungal endophytes

The fungal endophytes isolated from *L. camara and L. indica* were used for the stress studies. Based on their abundance across all five sites, fungal endophytes were chosen for the study. The fungal endophytes were grown in potato dextrose agar (PDA) and potato dextrose broth (PDB) amended with streptomycin.

4.3.2 Salinity stress tolerance

Ten selected fungal endophytes were evaluated for salt tolerance under *in vitro* conditions. A seven-day-old culture was used as an inoculum. A 5 mm diameter mycelial disc from the peripheral region of the fungal culture was removed and placed aseptically on PDA plates amended with streptomycin and varied NaCl concentrations (ranging from 1.00, 1.5, 2.00, and 2.50 M). The PDA plates without added NaCl are taken as control. All the treatment was carried out in triplicate. The plates were incubated at 28°C - 30°C for 7-9 days. The results were expressed as mycelial growth in diameter (mm) (Moghaddam *et al*., 2021).

4.3.3 Drought stress tolerance

Ten selected fungal endophytes were evaluated for salt tolerance under *in vitro* conditions. A seven-day-old culture was used as an inoculum. A 5 mm diameter mycelial disc from the peripheral region of the fungal culture was removed and placed aseptically into sterile glass bottles containing potato dextrose broth (PDB) amended with streptomycin and varied Polyethylene glycol (6000 Da) concentrations (ranging from 5 to 10 to 15% in w/v). The glass bottles containing PDB without added PEG (6000 Da) are taken as control. All the treatment was carried out in triplicate. The bottles were incubated at 28°C - 30°C for 7-9 days. The results expressed the weight of the mycelia (gm) (Lü *et al*., 2023).

4.3.4 Temperature stress tolerance

Ten selected fungal endophytes were evaluated for salt tolerance under *in vitro* conditions. A seven-day-old culture was used as an inoculum. A 5 mm diameter mycelial disc from the peripheral region of the fungal culture was removed and placed aseptically on PDA plates amended with streptomycin. The inoculated plates were kept at different temperatures ranging from 35 to 50°C, and control plates were incubated at 28°C - 30°C. All the treatment was carried out in triplicate and incubated for 7-9 days. The results were expressed as mycelial growth in diameter (mm) (Sangamesh et al., 2018).

4.4 Results

A total of 12 fungal isolates were selected for the study, with 5 isolates from *Lantana camara* and 7 isolates from *Lantana indica*. The isolates were chosen based on prevalence, being present in at least three locations. However, due to the inability of EF to grow *in vitro* after multiple subcultures, we had to proceed with only 3 isolates from *Lantana indica and 5 isolates from L. camara* (Table

4.1). This limitation emphasizes the difficulties associated with preserving the viable EF *in vitro*, which frequently fails to replicate the natural environments required for the endophytes (Sarkar et al., 2021; Alam et al., 2021; Bhardwaj et al., 2023). The growth of the EF grown in PDA growth media was measured in mm, and the endophytic fungi grown in PDB was measured in mg.

4.4.1 Drought Tolerance

Isolates, namely *Fusarium oxysporum* and *Diaporthe sojae* (from *Lantana camara*), and *Diaporthe discoidispora* and *Diaporthe melonis* (from *Lantana indica*), failed to grow after successive subcultures and were excluded from further screening.

The growth of fungal endophytes under PEG-induced drought stress was assessed by measuring mycelial weight (g) in potato dextrose broth supplemented with polyethylene glycol (PEG) at concentrations of 5%, 10%, and 15% (w/v). Among the isolates from the invasive plant, *Aspergillus* sp. and *Fusarium nirenbergiae* demonstrated strong tolerance to osmotic stress, exhibiting increased mycelial biomass with rising PEG concentrations compared to the control. In contrast, *Diaporthe* sp. displayed enhanced growth at 10% PEG but declined at 15%, indicating sensitivity to higher PEG-induced stress (Figure 4.1).

Endophytes from the native plant showed variable responses. *Lasiodiplodia pseudotheobromae* displayed consistent growth improvements at 15% PEG, suggesting enhanced drought tolerance. *Corynespora cassiicola* showed a steady increase in biomass across all PEG levels relative to the control. *Macrophomina phaseolina* exhibited variable growth patterns across PEG concentrations, indicating intermediate or strain-specific responses to drought stress.

4.4.2 Salt Tolerance

Salt stress tolerance was evaluated by measuring the mycelial diameter (mm) of fungal endophytes grown on PDA supplemented with NaCl at increasing concentrations (1.0 M, 1.5 M, 2.0 M, and 2.5 M). Except for *Aspergillus* sp., all isolates showed reduced growth with increasing salt concentrations compared to their respective controls.

Among the endophytes isolated from the invasive plant, *Aspergillus* sp. exhibited consistent mycelial growth across all NaCl concentrations, indicating high salt tolerance up to 2.5 M. *Fusarium nirenbergiae* grew up to 2.0 M NaCl, while *Fusarium oxysporum* and *Diaporthe phaseolorum* grew only up to 1.5 M, with noticeably reduced growth relative to the control.

Among the native plant isolates, *Macrophomina phaseolina* and *Lasiodiplodia pseudotheobromae* showed moderate tolerance up to 2.0 M NaCl, despite a reduction in mycelial growth. In contrast, *Diaporthe discoidispora* and *Diaporthe melonis* exhibited sharp declines in growth with increasing salt concentration, indicating high sensitivity to salt stress (Figure 4.2).

4.4.3 Temperature Tolerance

The thermal tolerance of fungal endophytes was assessed by measuring mycelial diameter (mm) at 30°C (control), 40°C, and 45°C. Overall, increasing temperatures negatively affected fungal growth (Figure 4.4 & Figure 4.5).

Among the isolates from the native plant *Lantana indica*, *Macrophomina phaseolina* showed the highest mycelial growth at 40°C and retained minimal growth at 45°C. *Lasiodiplodia pseudotheobromae* exhibited slight growth at 40°C, while *Corynespora cassiicola* and *Diaporthe* sp. showed no growth at either 40°C or 45°C (Figure 4.3).

In the case of invasive plant isolates, *Fusarium oxysporum* and *Aspergillus* sp. displayed limited growth at 40°C but not at 45°C. *Diaporthe sojae*, *Diaporthe phaseolorum*, and *Fusarium nirenbergiae* failed to grow beyond the control condition, indicating poor thermal tolerance.

4.5 Discussion

This study aimed at evaluating the abiotic stress tolerance of EF isolated from *Lantana camara* and *Lantana indica*, focusing on their responses to high temperature, drought, and salinity. These stress responses provide valuable insights into the adaptive resilience of invasive plants conferred by endophytic fungal symbionts.

4.5.1 Drought Stress Tolerance

The drought stress induced by polyethylene glycol (PEG) showed that *Aspergillus sp.* maintained consistent mycelial biomass across increasing PEG concentrations, indicating its osmotic adjustment capabilities and strong desiccation tolerance. This makes *Aspergillus sp.* a potential candidate for applications in drought-prone environments. Conversely, isolates such as *Corynespora cassiicola*, *Fusarium nirenbergiae*, and *Diaporthe sp.* exhibited reduced growth under increasing PEG concentrations, highlighting their sensitivity to water deficit conditions.

Lasiodiplodia pseudotheobromae displayed intermediate tolerance, suggesting that while it is affected by drought stress, it retains some capacity for adaptation. These differences suggest that the fungal endophytes have variations in cellular osmolyte production, membrane stability, and antioxidant mechanisms among the same or different fungal taxa, all of which are critical for coping with water stress (Lü et al., 2023).

4.5.2 Salt Stress Tolerance

Salt stress is one of the most pervasive abiotic stresses affecting plant health, particularly in arid and semi-arid regions. Here, *Aspergillus sp.* showed consistent growth across increasing NaCl concentrations, implying robust salt tolerance. This is consistent with the studies highlighting

Aspergillus sp., showing halotolerance is due to their ability to maintain ionic balance and express Na^+ efflux transporters under saline stress (Siddiqui *et al*., 2022).

In contrast, *Corynespora cassiicola* and *Fusarium nirenbergiae* showed reductions in mycelial diameter with increasing salt concentration, indicating hypersensitivity to salt stress. *Diaporthe sp.* and *Macrophomina phaseolina* showed moderate growth, suggesting partial tolerance.

4.5.3 Temperature Stress Tolerance

Among all the tested fungal endophytes, *Macrophomina phaseolina* showed increased growth at 40°C compared to the control plates at 30°C and remained viable at 45°C, demonstrating higher thermotolerance. This aligns with the study (Srinivas et al., 2017), indicating that certain strains of *Macrophomina sp.* show adaptations to higher temperatures due to the presence of heat shock proteins and membrane fluidity regulation, which allow them to survive under heat stress. Similarly, *Fusarium nirenbergiae* and *Rhizoctonia bataticola* showed relatively higher growth across all the temperature gradients, suggesting their potential to tolerate higher temperatures. In contrast, *Aspergillus sp.*, *Fusarium oxysporum*, and *Lasiodiplodia pseudotheobromae* exhibited reduced growth with increasing temperature, indicating resistance to heat. These results suggest that while some endophytes can thrive under moderate thermal stress, extreme conditions that limit their functional roles within the host. Different *Fusarium* species exhibited varying levels of stress tolerance, indicating significant intraspecific variation and strain-specific responses, even among closely related taxa.

Finally, the differential tolerance patterns observed among fungal isolates emphasize the complexity and specificity of endophytic responses to abiotic stress. Notably, the *Aspergillus sp.* from the invasive plant displayed higher tolerance across multiple stressors compared to the other isolates from *Lantana indica*. These findings suggest that salt tolerance and drought tolerance among EF depends on intrinsic physiological and biochemical traits, such as Na^+ exclusion, compatible solute synthesis, and vacuolar compartmentalization (Xiao & Zhou, 2023) however with respect to high temperature the fungi are limited to grow at higher temperature, unless specialized heat-shock systems are present in the host (Hosseyni et al., 2002).This observation does not provide enough ecological insight into the success of *L. camara.* However, the study demonstrates that specific fungal endophytes possess significant potential to enhance plant tolerance to abiotic stress. Harnessing such endophytes as bioinoculants could offer eco-friendly solutions for improving crop performance under stress conditions.

Plant Species	S.No	Operational Taxonomic Unit	Molecular Identification	Accession Number
Lantana indica	1.	LI_1	*Macrophomina phaseolina*	PV875640
	2.	LI_2	*Lasiodiplodia pseudotheobromae*	PV875641
	3.	LI_3	*Corynespora cassiicola*	PV875642
Lantana camara	4.	LC_4	*Fusarium oxysporum*	PV888603
	5.	LC_5	*Aspergillus sp*	PV888604
	6.	LC_6	*Diaporthe sojae*	PV888605
	7.	LC_10	*Diaporthe phaseolorum*	PV888609
	8.	LC_19	*Fusarium nirenbergiae*	PV888615

Table 4.1: List of endophytes selected for screening for stress tolerance

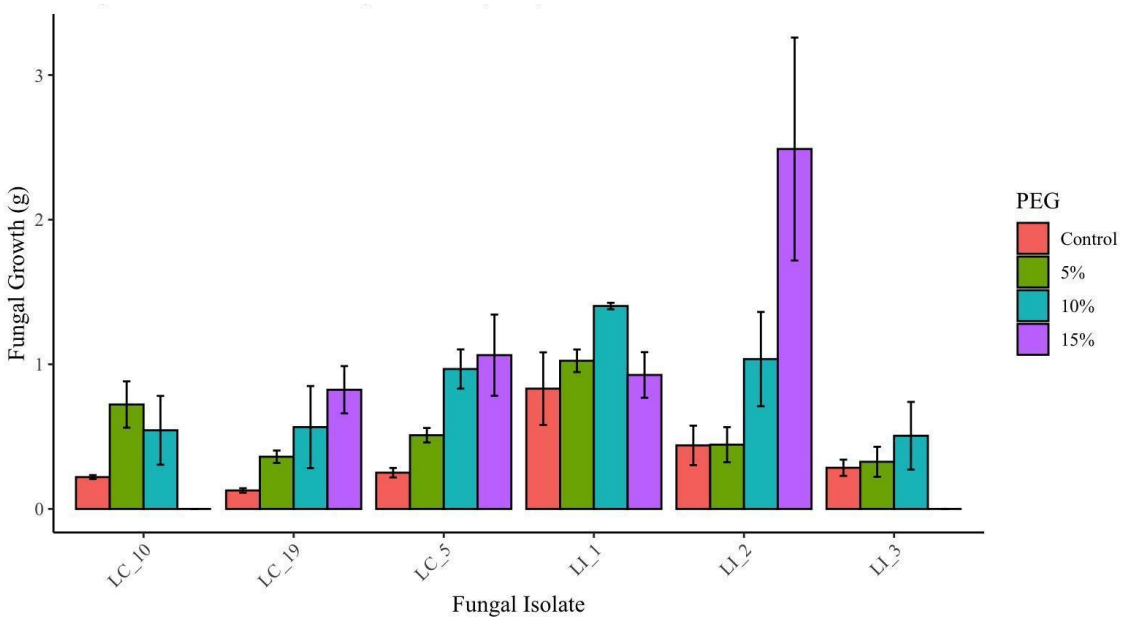


Figure 4.1: Drought stress tolerance of EF isolated from *L.camara & L.indica*

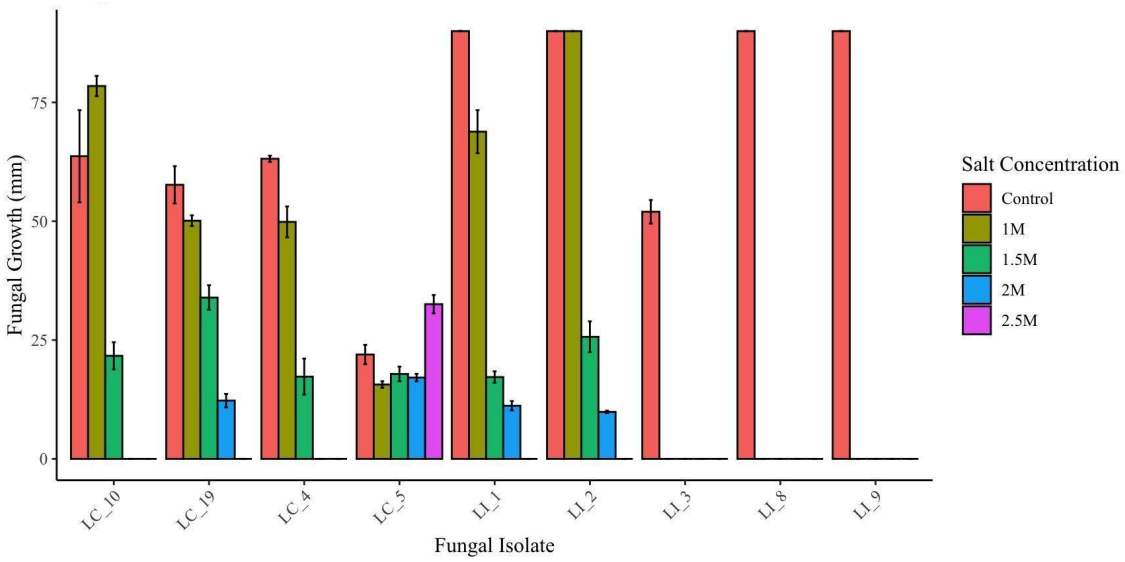


Figure 4.2: High-salt stress tolerance of EF isolated from *L.camara & L.indica*

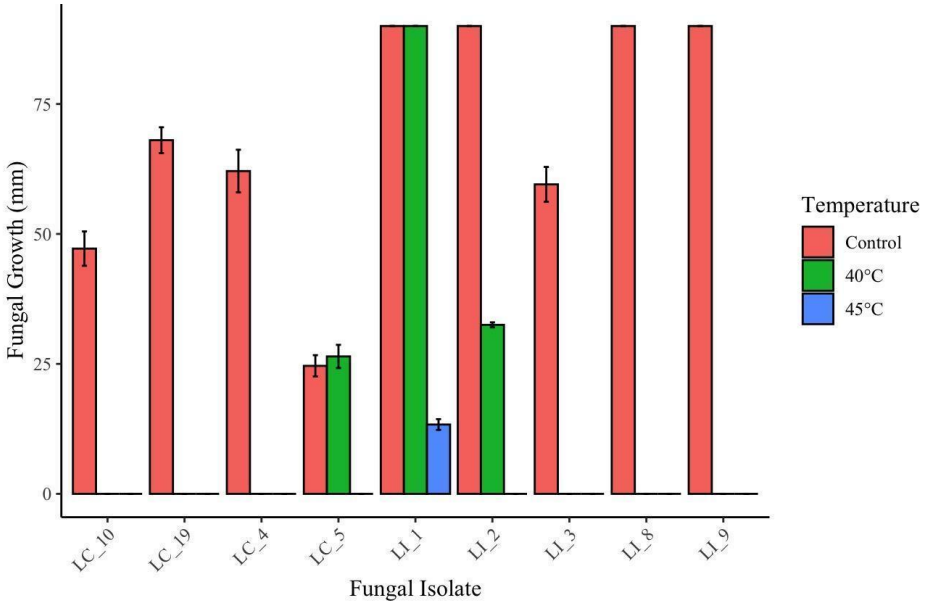


Figure 4.3: High-temperature stress tolerance of EF isolated from *Lantana camara & Lantana indica* illustrates the mycelial growth of the isolates at varying temperatures (30°C, 40°C, and 45°C).

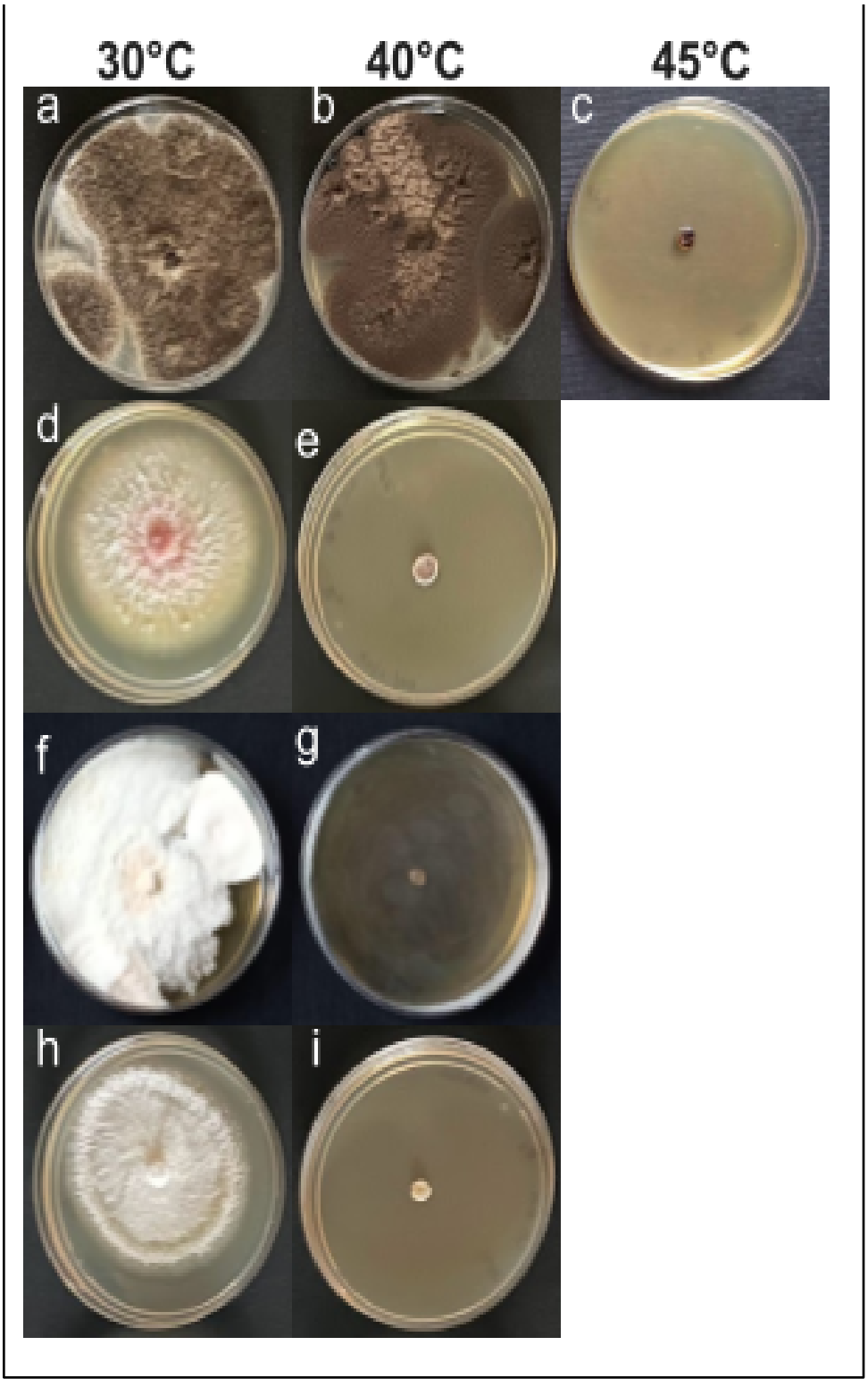


Figure 4.4: High-temperature stress tolerance of EF isolated from *L.camara* showing the mycelial growth at varying temperatures (30°C, 40°C, and 45°C)

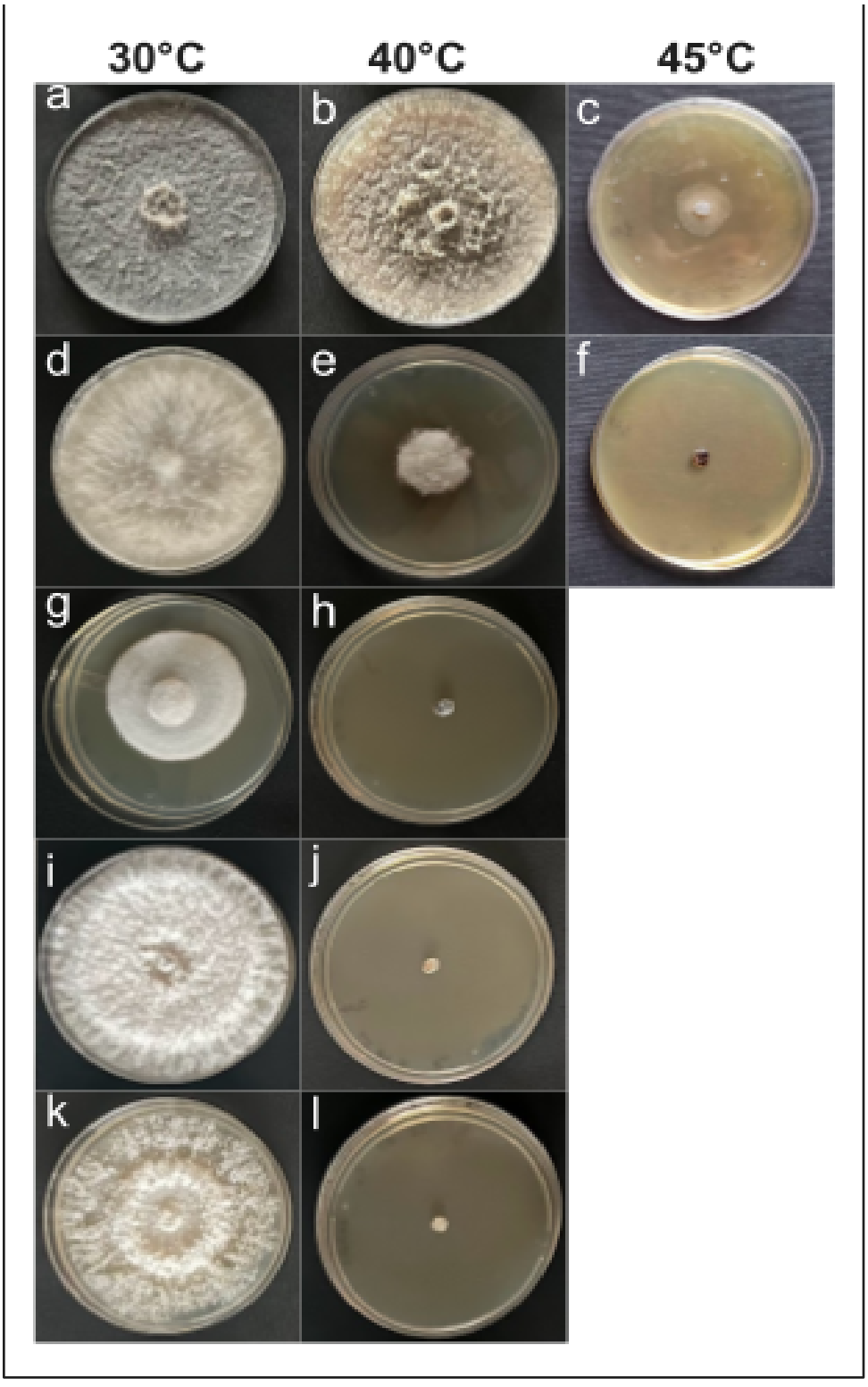


Figure 4.5: High-temperature stress tolerance of EF isolated from *L.indica* showing the mycelial growth at varying temperatures (30°C, 40°C, and 45°C)

Conclusion and Future Perspectives

This study explores microbial communities in invasive and non-invasive plant species, in particular endophytic fungal and bacterial communities in *Lantana camara* (invasive) and *Lantana indica* (native). The study was expected to reveal whether invasive plants harbor distinct microbial assemblages that enhance their adaptability and performance compared to their native congeners, in line with invasion ecology hypotheses. Through both amplicon-based and culture-dependent approaches, the findings revealed that bacterial and fungal communities do differ between invasive and native congeners, either in their richness or the abundance, suggesting that microbes play a role in invasion success. These results align with the initial expectation that invasion may be facilitated through shifts in microbial diversity and function rather than mere taxonomic overlap.

Notably, the invasive *L. camara* hosted distinct and functionally enriched microbial consortia, particularly endophytic bacteria having stress-adaptive pathways predicted using the PICRUSt2 annotation tool. These enriched pathways, including those involved in fatty acid metabolism and pentose phosphate cycles (as highlighted in Chapter 1), suggest microbial support for stress tolerance and energy regulation. In the case of culturable fungi (Chapter 3), the invasive plants harboured isolates that can tolerate salinity, drought, and temperature stress confirming the functional capacity of endophytes to support host persistence under extreme conditions. These findings collectively meet the expected outcome that invasive plants recruit or maintain microbial partners conferring greater resilience to environmental stresses.

These associations align with microbiome-mediated invasion hypotheses like the enhanced mutualism, enemy release, and habitat filtering theories. However, it is important to emphasize that microbial composition alone does not fully explain plant invasiveness. This study mainly underscores the role of host and environmental factors, particularly since both *L. camara* and *L. indica* were sampled from the same geographic location. The shared environment has contributed to the overall similarity in microbial assemblages, especially in fungal communities where no significant differences in diversity were observed. This observation supports the inference that while invasive species exhibit distinct functional microbiota, environmental filtering exerts a dominant influence on fungal community structure.

This suggests that environmental factors in local habitat can override host effects, leading to overlapping microbial communities despite differences in plant identity. Taken together, the outcomes indicate that microbial interactions enhance adaptability rather than directly determine invasiveness echoing patterns from earlier chapters where both species exhibited shared but functionally differentiated endophytes. These findings reinforce the notion that plant-microbe interactions are important but not deterministic in explaining plant invasion success.

Further investigation of microbial dynamics using functional genomics and cross-inoculation trials would disentangle the relative roles of microbes, hosts, and habitats in shaping invasion success. Finally, integrating microbial perspectives into invasion ecology not only fills a critical knowledge

gap but also offers novel perspectives for predicting, managing, and mitigating biological invasions.

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