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Assoc. Prof. Dr. Ahmet KARDAŞLAR

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ASSESSMENT OF ENDOPHYTIC FUNGAL DIVERSITY AND COLONIZATION PATTERNS IN CROTON SPARSIFLORUS COLLECTED ACROSS VARIOUS REGIONS

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ABSTRACT

Background: Endophytic fungi inhabit internal plant tissues without causing disease and contribute significantly to plant growth, stress tolerance, and defense. Their diversity and colonization patterns vary with host species, tissue type, and environmental factors. *Croton sparsiflorus*, a medicinal shrub native to southern India, remains largely unexplored for its endophytic community. The present study aimed to isolate and evaluate the diversity and colonization frequency of endophytic fungi associated with the leaves, stems, and roots of *C. sparsiflorus* collected from three different locations in Tamil Nadu—Villupuram, Kanchipuram, and Thiruvallur.

Methods: Healthy and asymptomatic plant samples were collected from each location. Surface sterilization was performed using 70% ethanol and 2% sodium hypochlorite to remove epiphytes. Fifty tissue segments each from leaf, stem, and root were plated on Potato Dextrose Agar (PDA) amended with streptomycin and incubated at 28 ± 2 °C for three weeks. Emerging fungal colonies were purified and identified based on morphological and microscopic features using standard keys. Colonization frequency (CF%) were calculated

Results: A total of eight fungal taxa were isolated: *Alternaria* sp., *Curvularia* sp., *Aspergillus niger*, *Colletotrichum* sp., *Aspergillus flavus*, *Aspergillus tamarii*, *white sterile forms*, and *Rhizophagous* sp. Among the locations, Kanchipuram exhibited the highest fungal diversity and colonization frequency, followed by Thiruvallur and Villupuram. *Curvularia* sp. dominated the Villupuram samples (CF = 100% in leaves), while *Aspergillus niger* showed the highest CF in Kanchipuram (100%) and Thiruvallur (48%). Leaves harbored the maximum fungal colonization across all locations, followed by stems and roots. The EIR ranged between 18% and 46% depending on tissue type and site. These findings indicate that both tissue specificity and local environmental conditions influence endophytic distribution in *C. sparsiflorus*.

Conclusion: The present study revealed a diverse assemblage of endophytic fungi associated with *Croton sparsiflorus*, varying across different tissues and geographical locations. Kanchipuram samples exhibited the highest colonization frequency and species richness, while *Curvularia* and *Aspergillus* spp. were dominant across all sites. These results highlight the influence of both tissue type and locality on endophytic fungal diversity, emphasizing their ecological and potential biotechnological significance.

Keywords: *Croton sparsiflorus*; Endophytic fungi; Colonization frequency; Tissue specificity; Fungal diversity, Interaction.

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INTRODUCTION

Endophytic microorganisms, encompassing a wide range of bacteria, fungi, and actinomycetes, are known to colonize the internal tissues of plants without causing any apparent harm. Unlike pathogenic microbes, these endophytes often establish mutualistic relationships with their hosts, contributing to enhanced growth, improved stress tolerance, and defense against various pathogens (Alvarez-Loayza et al., 2011; Kumar & Verma, 2018). With the growing emphasis on sustainable agricultural practices and the reduction of chemical fertilizers and pesticides, endophytes have gained immense importance as potential natural allies for crop improvement.

A key functional role of endophytes is their ability to enhance plant defense mechanisms through Induced Systemic Resistance (ISR). This process involves the activation of plant defense pathways, particularly the jasmonic acid (JA) and salicylic acid (SA) signaling systems, which play vital roles in plant immunity (Pieterse et al., 2014). Fungal endophytes such as *Trichoderma* spp. are well-documented for their ability to trigger ISR, secrete bioactive metabolites, and inhibit phytopathogens, thereby improving plant resilience (Contreras-Cornejo et al., 2016).

Recent advances in *omics* technologies, including genomics, transcriptomics, and metabolomics, have greatly expanded our understanding of endophyte–plant interactions (Wehner et al., 2019). Genome analyses of beneficial species like *Bacillus subtilis* have revealed genes encoding lipopeptides and polyketides that are crucial for pathogen suppression and plant growth promotion (Chen et al., 2018). Additionally, metagenomic studies have revealed the remarkable diversity of endophytic communities across plant species, opening new avenues for discovering novel microbial strains with unique biotechnological properties (Compant et al., 2019). The introduction of beneficial endophytes into crops has been shown to improve resistance to pathogens, enhance yield, and even enrich nutritional quality (Hardoim et al., 2015).

However, the successful application of endophytes in agriculture depends on several factors, including plant genotype, environmental conditions, and microbial interactions within the host. Understanding these dynamics is essential for utilizing endophytes effectively in sustainable agriculture.

In the present study, the plant selected for investigation is *Croton sparsiflorus*, collected from three distinct locations in Tamil Nadu — Villupuram, Kanchipuram, and Thiruvallur. This approach aims to explore the diversity of endophytic fungi associated with different plant parts (leaf, stem, and root) of the same species across varying environmental conditions. Such comparative analysis helps in understanding how geographical and ecological factors influence the diversity and distribution of endophytes.

MATERIALS AND METHODS

1. Collection of Plant Samples

Healthy and symptom-free plants of *Croton sparsiflorus* were collected from three different locations (Villupuram, Kanchipuram, and Thiruvallur). From each site, fresh leaves, stems, and roots were sampled separately using sterile scissors and placed in sterile polyethylene bags. The samples were immediately transported to the laboratory for further processing (Figure 1).

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Figure 1 : Collection of plant samples from various locations

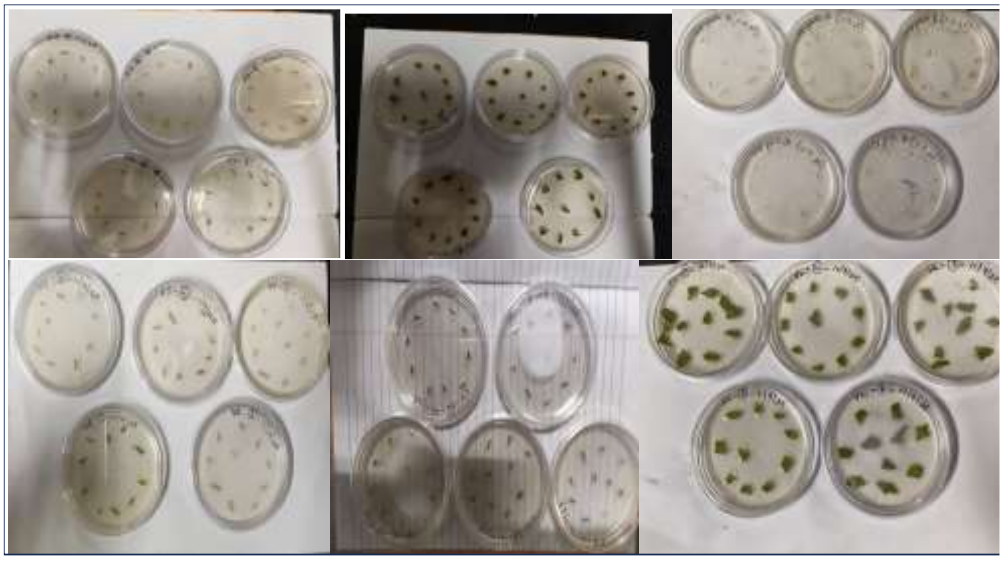
2. Surface Sterilization of Plant Tissues

To eliminate epiphytic microorganisms, plant tissues were subjected to a standardized surface sterilization protocol: Plant materials (leaf, stem, and root) were washed thoroughly under running tap water to remove dust and debris. Tissues were cut into small segments (approximately 0.5–1 cm in size) using sterile scissors. The segments were sequentially treated as follows. The segment was first rinsed in 70% ethanol for 1 minute followed by 2% sodium hypochlorite (NaOCl) for 2–3 minutes. It was then Rinsed three times with sterile distilled water to remove traces of sterilizing agents.

3. Isolation of Endophytic Fungi

A total of 50 segments from each plant tissue (leaf, stem, and root) of *Croton sparsiflorus* were used for isolation. From each geographical location (Villupuram, Kanchipuram, and Thiruvallur), 10 segments of each plant tissue type were placed on Potato Dextrose Agar (PDA) medium amended with streptomycin (50 µg/mL) to inhibit bacterial contamination (Figure 2).

Figure 2: Surface Sterilization and Inoculation of plant samples



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The Petri dishes were incubated for a period of three weeks at 28 ± 2 °C, as described by Suryanarayanan (1992). To prevent the rapidly growing fungi from inhibiting slow-growing species, the former were carefully removed following isolation, as suggested by Bills (1996). Emerging fungal colonies from the tissue segments were sub-cultured onto fresh PDA plates to obtain pure isolates. Sporulating isolates were identified to the species level based on their microscopic and macroscopic morphology using standard manuals (Ellis, 1971; Ellis, 1976; Sutton, 1980; Udayaprakash, 2004). Sterile isolates, which did not produce identifiable reproductive structures and could not be assigned to any known taxonomic group, were categorized as 'sterile forms' following the criteria of Fröhlich et al. (2000) and Suryanarayanan et al. (2000).

4. Statistical Analysis

The number of fungal colonies emerging from each tissue type was recorded. The colonization frequency (CF %) and endophytic infection rate (EIR %) were calculated following Fisher and Petrini (1987):

1. Colonization Frequency (CF%)

This indicates how frequently a particular fungal species colonizes plant tissue segments.

$$\text{CF } (\%) = \frac{\text{Number of segments colonized by a particular fungus}}{\text{Total number of segments examined}} \times 100$$

RESULTS

A total of eight distinct fungal taxa were isolated from the leaves, stems, and roots of *Croton sparsiflorus* collected from three geographical locations — Villupuram, Kanchipuram, and Thiruvallur (Figure 3).

Figure 3: Isolation of fungal endophytes form the plant samples

The isolated taxa included *Alternaria sp.*, *Curvularia sp.*, *Aspergillus niger*, *Colletotrichum sp.*, *Aspergillus flavus*, *Aspergillus tamarii*, white sterile forms, and *Rhizophagous sp.* (Table 1).

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Table 1. Distribution of fungal endophytes isolated from leaves, stems, and roots of *Croton sparsiflorus* collected from three geographical locations

Fungal Taxa	Villupuram			Kanchipuram			Thiruvallur		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Alternaria sp.	–	2	–	7	4	6	9	4	1
Curvularia sp.	52	4	–	15	8	7	10	3	2
Aspergillus niger	–	–	17	24	12	15	9	8	7
Colletotrichum sp.	–	1	–	5	2	1	1	–	–
Aspergillus flavus	–	–	–	3	5	3	1	1	1
Aspergillus tamarii	–	–	–	–	2	–	–	–	–
White sterile forms	–	–	–	3	3	2	2	1	–
Rhizophagous sp.	–	–	–	2	–	–	–	–	–
Total isolates (Ni)	**52**	**9**	**7**	**51**	**28**	**33**	**32**	**17**	**11**
Total species (Ns)	**1**	**3**	**1**	**7**	**7**	**6**	**6**	**5**	

A total of 240 fungal isolates were obtained from 450 tissue segments, with the number and diversity of isolates varying among locations and plant parts. The maximum number of isolates was recorded in leaf tissues from Villupuram (n = 52) and Kanchipuram (n = 51), while the lowest number was observed in root tissues from Villupuram (n = 7). Species richness was highest in Kanchipuram, where up to seven species were identified from both leaf and stem tissues, whereas Villupuram leaf and root samples yielded only one dominant species (*Curvularia sp.*).

Colonization Frequency

The colonization frequency (CF%) of endophytic fungi isolated from *Croton sparsiflorus* exhibited distinct variations across plant parts and sampling locations (Table 2).

Table 2: Colonization frequency (CF%) of endophytic fungi isolated from leaves, stems, and roots of *Croton sparsiflorus* collected from three different geographical locations

Fungal Taxa	Villupuram	Kanchipuram	Thiruvallur
Alternaria sp.	4.00	34.00	28.00
Curvularia sp.	100.00	60.00	30.00
Aspergillus niger	34.00	100.00	48.00
Colletotrichum sp.	2.00	16.00	2.00
Aspergillus flavus	-	22.00	6.00

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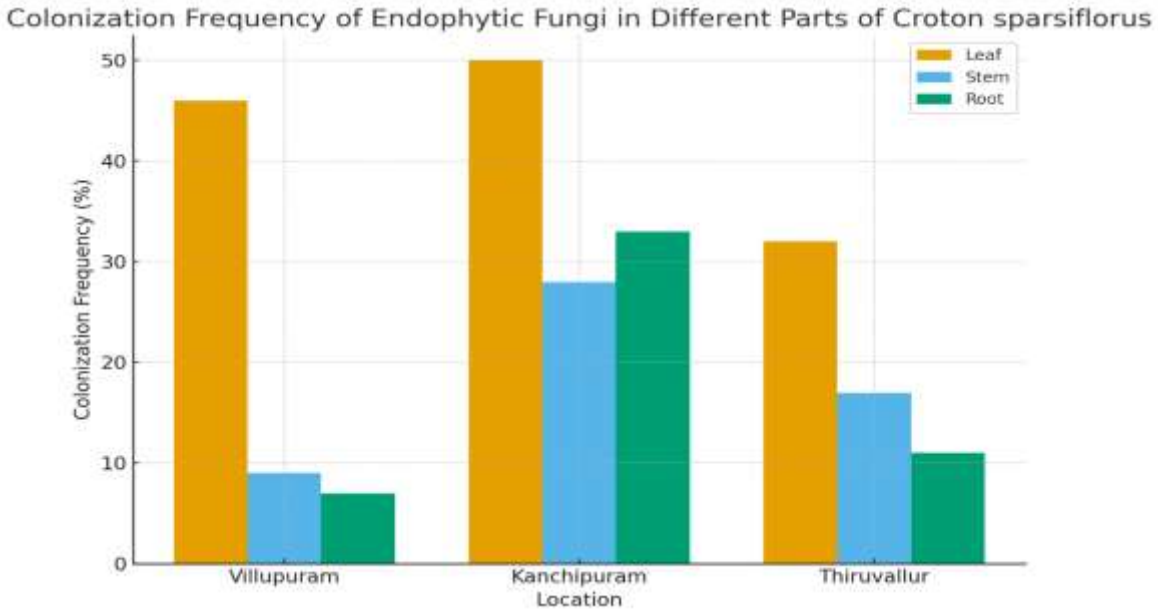
Aspergillus tamarii	-	4.00	-
White sterile forms	-	16.00	6.00
Rhizopus sp.	-	4.00	-

Among the three geographical sites, the overall fungal colonization was highest in Kanchipuram, followed by Thiruvallur and Villupuram, indicating regional differences in endophytic diversity and abundance (Figure 4).

Figure 4: Colonization frequency of endophytic fungi from various plant parts



Villupuram:

Endophytic fungi were isolated from the leaves, stems, and roots of *Croton sparsiflorus* collected from Villupuram (Figure 5). The colonization frequency (CF%) varied among plant parts and fungal taxa. *Curvularia* sp. exhibited the highest colonization frequency in leaf tissues (100%), followed by *Aspergillus niger* in roots (34%). Moderate colonization was observed for *Alternaria* sp. and *Colletotrichum* sp. in stem tissues, with CF values of 4% and 2%, respectively. Other taxa such as *Aspergillus flavus*, *Aspergillus tamarii*, white sterile forms, and *Rhizopus* sp. were not detected in this location. Overall, leaves showed the highest fungal colonization, while roots harbored fewer endophytes.

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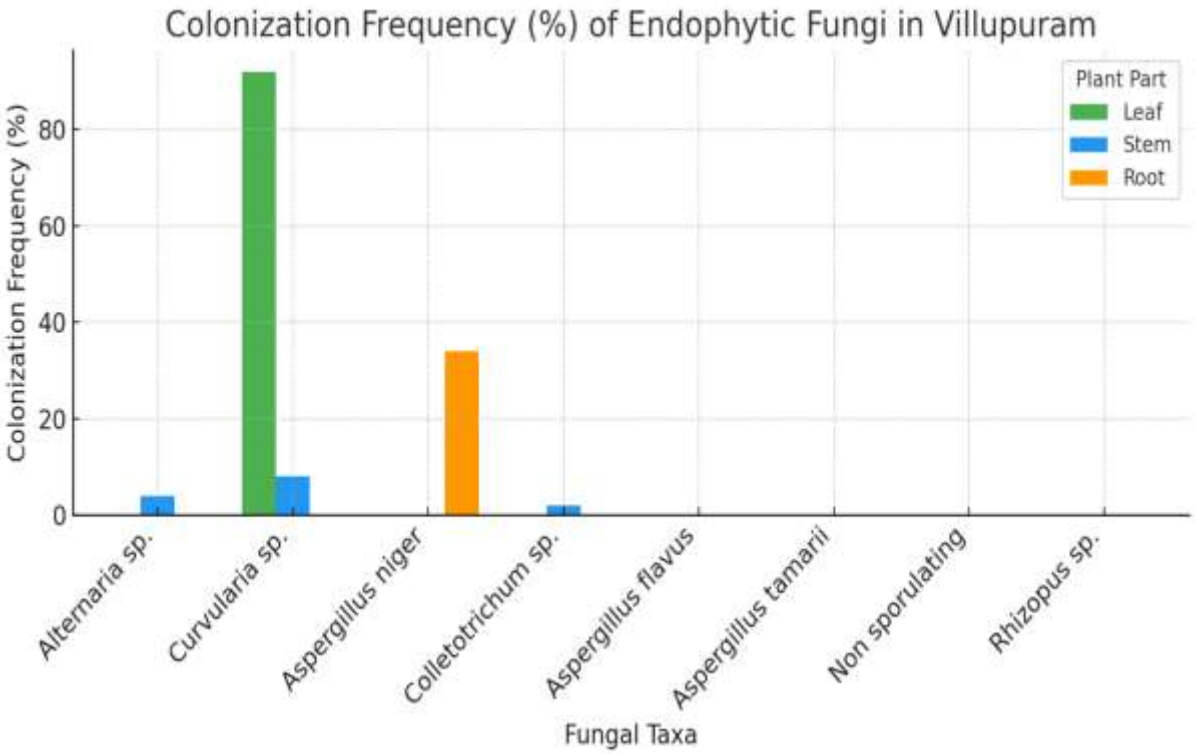
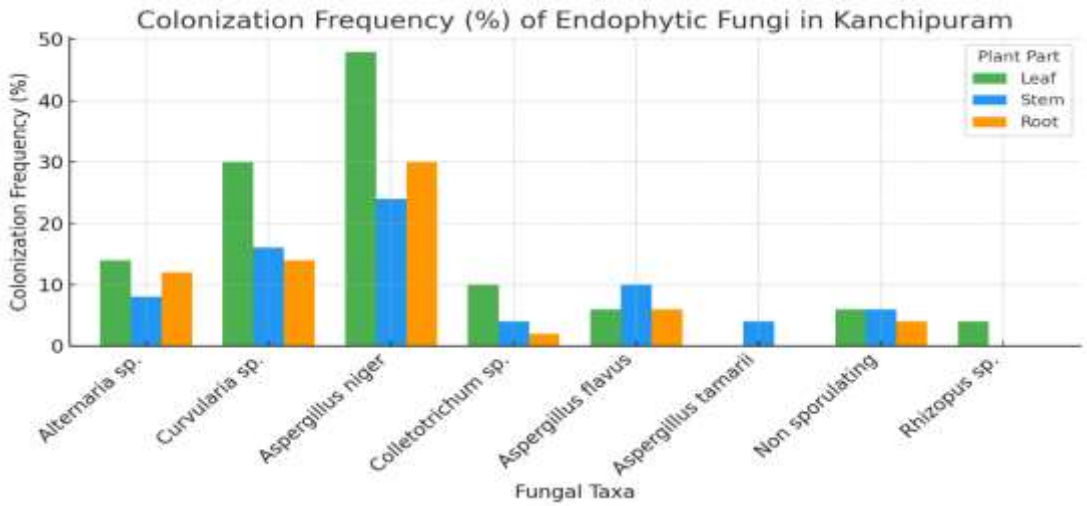
Figure 5: Colonization frequency of endophytic fungi in Villupuram



Kanchipuram:

In the Kanchipuram population of *C. sparsiflorus*, colonization frequency was generally higher and more diverse across tissue types (Figure 6). *Aspergillus niger* showed the maximum CF (46% in leaves), followed by *Curvularia* sp. (30% in leaves) and *Alternaria* sp. (14% in leaves). Moderate colonization was also observed in stem and root tissues by *A. niger* (24% and 30%, respectively). Lesser frequencies were recorded for *Colletotrichum* sp. (2–10%), *Aspergillus flavus* (6–10%), *A. tamarii* (4% in stem), and white sterile forms (4–6%). *Rhizopus* sp. was present only in leaves (4%). Among the tissues, leaf segments supported the highest fungal colonization, followed by roots and stems, indicating that the foliar region provided a more favorable microenvironment for endophyte establishment.

Figure 6: Colonization frequency of endophytic fungi in Kancheepuram



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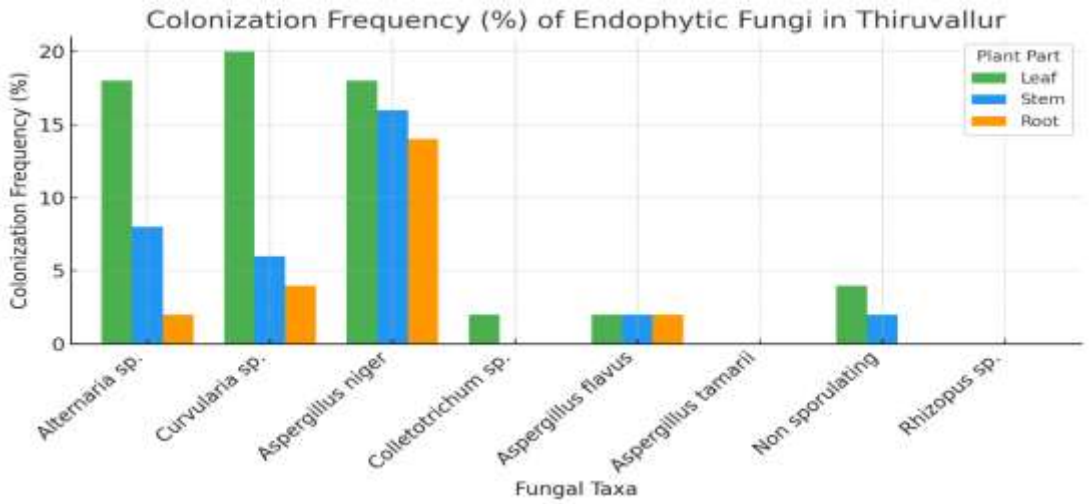
Thiruvallur:

The Thiruvallur population exhibited a moderate range of colonization frequencies (Figure 7). *Aspergillus niger* was the most dominant species, showing CF values of 30% in leaves, 16% in stems, and 12% in roots. *Alternaria* sp. and *Curvularia* sp. also recorded notable colonization, particularly in leaf tissues (18% and 20%, respectively). Minor colonization was observed for *Aspergillus flavus* (2–4%), *Colletotrichum* sp. (2%), and white sterile forms (2–4%). *Aspergillus tamarii* and *Rhizopus* sp. were absent in all tissues. Similar to other locations, leaf tissues recorded the highest overall colonization, while roots had the least.

Figure 7: Colonization frequency of endophytic fungi in Kancheepuram



DISCUSSION

A total of eight endophytic fungal taxa were recovered from *Croton sparsiflorus* across three locations, with 240 isolates obtained from 450 plated segments. This level of culturable diversity (8 taxa; many isolates concentrated in leaves) is consistent with culture-based surveys of tropical and subtropical plants, which typically report a modest set of dominant genera (often 5–15 taxa) obtained by standard plating approaches. Modern reviews emphasise that culture-based methods recover only a subset of the true endophyte community but reliably detect the common, fast-growing genera such as *Curvularia*, *Alternaria* and *Aspergillus* (Khan et al., 2023)

Among the taxa identified in this study, *Curvularia* and *Aspergillus niger* emerged as the most abundant and widespread. *Curvularia* dominated leaf samples (highest CF% in Villupuram and substantial CF% in other sites), while *A. niger* was frequent in root samples—patterns that mirror other reports where *Curvularia/Bipolaris* lineages are often dominant foliar endophytes and *Aspergillus* species are commonly recovered from roots and rhizosphere-associated tissues. These genera are frequently reported across diverse hosts and habitats due to their saprotrophic and opportunistic lifestyles and ability to sporulate readily on PDA, which increases their detectability in culture-based surveys (Zakaria L 2024 and Mehta et al., 2022).

The colonization frequency (CF%) results—showing higher CF in leaves than in stems or roots for many taxa—also follow widely observed trends. Leaves provide a larger, more exposed surface area for spore deposition and may present favourable microhabitats (nutrient availability in mesophyll, seasonal humidity) that permit frequent colonization by airborne taxa such as *Curvularia* and *Alternaria*. Several foliar endophyte studies report comparable patterns of leaf > stem ≈ root in terms of isolate numbers and CF% (Khan et al., 2023 and Bernardi-Wenzel et al., 2010).

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Location-specific differences in diversity and CF% were pronounced: Kanchipuram showed the highest overall isolate counts and species richness, followed by Thiruvallur and Villupuram. Such spatial variation is expected because endophyte assemblages are shaped by local abiotic factors (microclimate, humidity, soil properties), host physiology, and surrounding vegetation (inoculum sources). Regional comparisons in other studies have likewise shown site-to-site variation in both isolate abundance and species richness; authors attribute this to microclimatic differences and variation in dispersal of fungal propagules. These findings highlight that even for the same host species, endophyte communities can differ substantially between nearby sites (Liao et al., 2025 and Kumar et al., 2024)

The dominance of culturable, sporulating taxa and the presence of non-sporulating (sterile) morphotypes in the collections is also typical of culture-based surveys. Sterile isolates often represent taxa that require specific conditions to sporulate or slow-growing taxa outcompeted on general media; many studies explicitly record sterile forms and treat them separately in analyses. Use of selective incubation, longer incubation or different media sometimes recovers reproductive structures and permits finer taxonomic placement (Huang et al., 2015).

Comparison with previous numerical metrics: culture surveys of roots in some studies report high colonization by *Aspergillus* spp. (root colonization rates often 20–60% depending on host and method); similarly, foliar dominance by *Curvularia* or *Alternaria* with CF values in the range observed here has been recorded in several tropical plant surveys. Thus, the proportions observed in *C. sparsiflorus* (e.g., *Curvularia* CF up to 100% in a leaf sample, *A. niger* CF up to 100% in Kanchipuram roots) are within the range reported for culture-based field surveys, though absolute values are influenced by plating density, incubation duration, and whether fast growers were removed to allow slow growers to appear (your methods followed the recommended practice of removing rapidly growing colonies to not suppress slow growers).(Surendirakumar et al., 2024)

CONCLUSION

Croton sparsiflorus hosts a modest but recurring assemblage of culturable endophytic fungi dominated by *Curvularia* and *Aspergillus* spp., with leaves showing higher colonization frequencies than stems and roots and Kanchipuram showing the richest endophyte assemblage. The dominance of these genera suggests potential for further functional screening (plant-growth promotion, antifungal metabolite production), but molecular identification and metabolite assays are recommended before any applied use. Future work should combine culture-dependent and culture-independent approaches, increase sampling across seasons, and test endophyte functions in planta.

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