

Morphological, Functional, and Molecular Characterization of the Selected Fungal Endophytes from *Neolamarckia cadamba* (Roxb.) Bosser

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Abstract

Background

Higher plants serve as a valuable source of endophytic fungi, which have been shown to produce a wide range of secondary metabolites with therapeutic potential. Despite extensive studies on endophytes from various plant species, there have been no prior reports on the isolation and characterization of endophytic fungi from *Neolamarckia cadamba* (Roxb.) Bosser is a medicinally important tree native to tropical Asia. This study aimed to isolate and identify endophytic fungi from the leaves and stems of *N. cadamba* and assess their phytochemical constituents and biological activities, particularly antioxidant and antimicrobial potential.

Results

A total of eight endophytic fungi were isolated from *N. cadamba*, with seven from leaves (NKL1 to NKL7) and one from the stem (NKS1). NKL1, NKL3, and NKL4 exhibited rapid growth and distinct morphological features, and were therefore selected for further analysis. Phytochemical screening of fungal extracts revealed the presence of alkaloids, flavonoids, and carbohydrates. The antioxidant activity, assessed via the DPPH assay, showed a concentration-dependent increase in free radical scavenging ability, with NKL1 and NKL3 exhibiting the highest antioxidant potential, comparable to that of the standard ascorbic acid. The crude extracts of endophytic fungi NKL1, NKL3, and NKL4 exhibited concentration-dependent antibacterial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*, with NKL1 and NKL3 demonstrating the highest inhibitory effects, thereby confirming their antimicrobial potential. Morphological characterization and 18S rRNA gene sequence analysis identified NKL1 and NKL3 as *Curvularia lunata* and *Curvularia tropicalis* (NCBI GenBank accession numbers PX205205 and PX218454, respectively), both of which belong to the phylum Ascomycota.

Conclusion

This study provides the first report of *Curvularia lunata* and *Curvularia tropicalis* as endophytic fungi associated with *Neolamarckia cadamba*. The isolates NKL1 and NKL3 exhibited strong antioxidant and antibacterial properties, suggesting their potential as promising sources of bioactive compounds. These findings support further exploration of *N. cadamba*-associated endophytes for their therapeutic and industrial applications.

Background

Endophytes are microorganisms, predominantly bacteria and fungi, that colonize the inter- or intracellular spaces of plant tissues without causing apparent disease symptoms (Pimentel et al. 2011; Singh and Dubey, 2015). These symbionts complete part or all of their life cycle within host plants, forming intricate

mutualistic relationships that significantly influence plant physiology. Within the host environment endophytes, particularly fungi and bacteria, produce a wide array of secondary metabolites and signaling molecules that contribute to plant growth, development, and defense (Eid et al. 2021; Saxena, 2014). The unique environmental conditions inside plant tissues can induce endophytes to synthesize metabolites not typically produced in free-living states, making them valuable in drug discovery and agriculture (Strobel and Daisy, 2003).

Fungal endophytes also synthesize phytohormones that regulate various stages of plant development (Al-Kahtani et al. 2020). Prolonged plant-endophyte interactions facilitate metabolite exchange and may enhance the biosynthesis of unique compounds through symbiotic co-evolution. As Schulz et al. (2002) noted, these intimate interactions can drive the production of novel metabolites that are rarely found in other microorganisms, offering a promising platform for the discovery of biologically active compounds. Remarkably, some fungal endophytes can produce the same metabolites as their host plants compounds such as digoxin (*Digitalis lanata*), ginkgolides (*Ginkgo biloba*), hypericin (*Hypericum perforatum*), podophyllotoxin (*Juniperus communis*), and paclitaxel (*Taxus baccata*), suggesting either horizontal gene transfer or metabolic mimicry (Kusari et al. 2009; 2012; 2014; Kaul et al. 2013; Cui et al. 2012; Vigneshwari et al. 2019). In some cases, endophytes utilize alternative or induced biosynthetic pathways to produce related or entirely different bioactive compounds (Ludwig-Muller, 2015). These findings underscore the significant biotechnological potential of fungal endophytes as a source of novel chemical scaffolds (Sadrati et al. 2013; Gupta et al. 2020).

Endophytic fungi isolated from diverse plant species have been shown to produce a wide range of bioactive secondary metabolites with antimicrobial, antioxidant, anticancer, and plant growth-promoting properties (Aly et al. 2011; Kusari et al. 2012; Verma et al. 2021). Some endophytes can even synthesize metabolites structurally similar to those of their host plants, contributing to both plant health and potential pharmaceutical applications. Strobel et al. (2004) reported the isolation of endophytic fungi from medicinal plants, notably *Taxomyces andreanae* from *Taxus brevifolia*, which was capable of synthesizing taxol, an anticancer compound originally attributed to the host plant. This finding highlights the potential of endophytes as alternative sources of pharmaceutically important metabolites. Proper identification and characterization of these endophytes are essential for harnessing their metabolic capabilities in pharmaceutical and agricultural applications (Kharwar et al. 2011). In this context, the present study aims to isolate and characterize fungal endophytes from *Neolamarckia cadamba*, an ecologically and medicinally important yet endangered tree species. Investigating its endophytic microbiota not only enhances our understanding of plant-microbe interactions but also holds great promise for the discovery of novel bioactive compounds.

Materials And Methods

Plant Material

The plant material *Neolamarckia cadamba* (Roxb.) Bosser was collected from the Botanical Garden of SNGS College, Pattambi. Fresh, healthy, and mature stems and leaves of *N. cadamba* were meticulously selected for the isolation of endophytes to minimize any risk of contamination.

Isolation of Endophytic Fungi

Surface-sterilized stem and leaf segments were carefully placed on PDA media containing antibiotics. The plates were then sealed with cling film and incubated at 25–28°C for 5 to 14 days. The Petri plates were monitored frequently to check the growth of endophytic fungal colonies. Hyphal tips that appeared were transferred to fresh PDA plates and slants of PDA for subculture to develop a pure culture (Piska et al. 2015).

Morphological Identification of Endophytic Fungi

The identification of endophytic fungi was based on morphological features of fungal colonies, fruiting bodies, and spores (Alurappa and Chowdappa, 2018). For microscopic observation of fungal mycelium, a drop of Lactophenol cotton blue was placed on a clean microscopic slide, and the hyphal thread was placed on it and carefully dissected with needles. The stained slides were then observed under a microscope to study the microscopic characteristics of the fungi. Non-sporulating cultures were differentiated from one another by their cultural characteristics, including colony morphology, hyphal mat characteristics, and pigmentation of the colony in the medium.

Mass Cultivation of Endophytic Fungi

Endophytic fungal isolates exhibiting distinct morphological characteristics and rapid growth were selected for mass cultivation. Actively growing mycelial plugs (3 mm in diameter) from 7-day-old pure cultures were aseptically transferred into 250 ml Erlenmeyer flasks containing 100 ml of sterile Potato Dextrose Broth (PDB). Cultures were incubated at room temperature ($25 \pm 2^\circ\text{C}$) under shaking conditions at 120 rpm in a rotary shaker. After incubation, cultures were filtered through sterile cheesecloth to separate mycelial mats, retaining the culture filtrate for further analysis (Selvi and Balagengatharathilagam, 2014).

Extraction of Secondary Metabolites from Endophytic Fungi

After mass cultivation of endophytic fungi, the fungal metabolites from different endophytic mycelial mats were extracted using ethyl acetate. An equal volume of the filtrate and solvent was taken in a conical flask and shaken vigorously for one hour. The solution was then allowed to stand, the cell mass was separated, and the solvent so obtained was collected. Then the solvent was evaporated to yield the crude extracts (Selvi and Balagengatharathilagam, 2014).

Phytochemical Analysis of Crude Extract of Endophytic Fungi

Test for Alkaloids

Dragendroff's Test: Dragendroff's reagent was added to a little of the extract dissolved in its solvent. Alkaloid gives an orange-red precipitate (Alurappa and Chowdappa, 2018).

Test for Flavonoids

NaOH Test: To 1 ml sample, add 3 ml of dilute NaOH; the sample turns yellow color which disappears upon adding dilute HCl, indicating the presence of flavonoids (Alurappa and Chowdappa, 2018).

Test for Terpenoids

Salkowski Test: Take 1 ml of the sample in a test tube and add a few drops of Chloroform along the sides of the test tube. A few drops of concentrated Sulphuric acid were added carefully. Reddish brown coloration at the interface indicates the presence of terpenoids (Alurappa and Chowdappa, 2018).

Test for Saponin

Froth Test: To a 1 mL sample in a test tube, add 2 mL of distilled water. The froth appearance on shaking of the mixture shows the presence of saponin (Alurappa and Chowdappa, 2018).

Test for Phenol

FeCl₃ Test: To 5 drops of the sample solution taken in a test tube, add 3 drops of FeCl₃ (5%W/V). A dark green color indicates the presence of phenol (Alurappa and Chowdappa, 2018).

Test for Coumarins

NaOH Test: 10% NaOH was added to the extract, and Chloroform was added. The formation of a yellow color shows the presence of coumarins (Vimalkumar et al. 2014).

Test for Cardiac Glycosides

Keller Killani Test: To a 5 ml extract taken in a test tube, add 2 ml of acetic acid, along with one drop of FeCl₃ solution and 1 ml of concentrated sulfuric acid. The appearance of a brown ring indicates the presence of cardiac glycosides.

DPPH free radical scavenging assay

The free radical scavenging activity of fungal organic extracts was assessed by the DPPH assay. 200 µL of 0.1 mM of DPPH solution (prepared by dissolving DPPH in ethanol) was combined with varying concentrations of fungal extracts (20–100 µg/ml), vortexed vigorously, and incubated for 30 minutes in

the dark at room temperature. The absorbance was subsequently measured at 517 nm using a microplate reader with ethanol as blank (Dhayanithy et al. 2019). Ascorbic acid was used as a positive control

The percentage of radical scavenging potential was calculated using the formula

% scavenging = $(1 - (\text{Abs}_{(517 \text{ nm})} \text{ of the sample} / \text{Abs}_{(517 \text{ nm})} \text{ of the control})) \times 100$.

Antibacterial Assay

Disc Diffusion Method

20 mg of the fungal crude extract dissolved in 1 ml of DMSO was used to treat the bacteria. Sterilized paper discs of diameter 6 mm were taken and impregnated with 10, 15, 20, and 25 µl endophytic fungal crude extracts (20mg/ml) and allowed to evaporate in air, and then placed on the lawn of bacteria. 25 µL of DMSO was used as a negative control. The plates were incubated at 37°C for 24 hours, and the zone of inhibition was measured (Ramesha and Srinivas, 2014).

Molecular identification and phylogenetic analysis of endophytic fungi

Molecular identification was conducted using the strains on PDA and incubated for 5–7 days at 29°C. The fungal DNA was extracted using a DNA isolation kit, NucleoSpin Plant II Kit (Macherey-Nagel), according to the manufacturer's protocol. DNA quality was assessed via 1% agarose gel electrophoresis. PCR amplified the ITS1 region of the fungus with the primers ITS1F (5'TCCGTAGGTGAACCTGCGG3') and ITS4R (5' TCCTCCGCTTATTGATATGC 3'). PCR reaction conditions were 30 sec at 98°C; 40 cycles of 98°C for 5 s, 58°C for 10 s, and 72°C for 15 s; and 72°C for 15 sec. The PCR products were checked in a 1.2% agarose gel. The PCR products were sequenced by using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following the manufacturer's protocol. Sequence alignment and editing were performed using Geneious Pro v5.1 (Kearse et al. 2012). The resulting DNA sequences were analyzed and compared with those obtained from GenBank via a BLAST search. The obtained nucleotide sequence was submitted to GenBank. Each sequence was refined by removing noise and ambiguous peaks, followed by the assembly of forward (5'–3') and reverse (3'–5') sequences to produce a high-quality consensus sequence. These consensus sequences were subjected to BLAST analysis to determine the taxonomic identity of the endophytic isolates by comparison with sequences available in the International Nucleotide Sequence Database Collaboration. The most similar INSDC sequences were retrieved and aligned with the query sequences using ClustalW for multiple sequence alignment. Phylogenetic circumscription and analysis have been conducted to determine the evolutionary relationships among the fungal endophytes. Sequence alignments and phylogenetic tree construction were performed using MEGA v6 software (Tamura et al. 2013). The neighbor-joining method was employed to reconstruct phylogenetic trees, providing a robust framework for evolutionary inference. The reliability and statistical support for the inferred phylogenies were assessed using bootstrap analysis

with 1,000 replicates, a standard test for evaluating the confidence of branching patterns within the tree (Ramesh et al. 2017).

Result

In this study, a total of eight endophytic fungi were isolated from various parts of *Neolamarckia cadamba* (Roxb.) Bosser. Among these, seven were sourced from the leaves and designated as NKL1 (*Neolamarckia cadamba* leaf strain 1), NKL2, NKL3, NKL4, NKL5, NKL6, and NKL7 (Fig. 1). Only one isolate was obtained from the stem, named as NKS1 (*Neolamarckia cadamba* stem strain 1). The majority of the fungal endophytes isolated from *N. cadamba* were extremely slow-growing, with a very slow vegetative growth rate on PDA medium. Consequently, from the total of eight fungal endophytes isolated, only three were selected for further studies, including characterization, antioxidant activity, and antimicrobial screening. The chosen fungal isolates were NKL1, NKL3, and NKL4, which demonstrated better growth rates on both PDA and PDB media.

Morphological Identification of Endophytic Fungi

The identification of the fungi was done by macroscopic and microscopic observations (Fig. 1 and Fig. 2). Table 1 provides a summary of the key characteristics of the fungal isolates from the leaf and stem of *Neolamarckia cadamba*. Colony colour varied among the isolates, ranging from dark green (NKL1, NKL4, NKL7) to light green (NKL2, NKL5), brown (NKL6), a mixed light brown and light green (NKL3), and light yellow (NKS1). Mycelial morphology showed diversity in branching patterns, septation, and cell shape. Heavily branched, anastomosing hyphae were observed in NKL1, NKL3, and NKL7, whereas NKL4 exhibited pointed terminal ends, and NKL5 possessed irregularly sized barrel-shaped cells with rounded ends. Smooth-walled barrel-shaped cells were recorded in NKL6 and NKL7.

Table 1
The characteristics of endophytic fungal isolates from *Neolamarckia cadamba*.

Fungal isolates	Colony characters	Mycelial characters	Spore characters
NKL1	Dark green colored	Heavily branched, anastomosing, septate mycelium with barrel-shaped cells and the tips of terminal cells are somewhat rounded.	Spore like-structures produce inside the hyphae.
NKL2	Light green colored	Branched, septate mycelium having barrel-shaped cells and are anastomosing.	Spore like structures produce outside the hyphae.
NKL3	A mixture of light brown and light green colored	Heavily branched, septate and anastomosing hyphae.	Spores are produced at the end of branches which are two and three celled.
NKL4	Dark green colored	Branched and septate mycelium with barrel shaped cells and the ends are pointed.	Spore producing structures were not found.
NKL5	Light green colored	Branched and septate mycelium and the cells are barrel shaped which are irregular in size with rounded ends.	Spore producing structures were not found.
NKL6	Brown colored	Branched, septate mycelium which are anastomosing and the cells are smooth walled and barrel shaped.	Spore like structures produce inside the hyphae.
NKL7	Dark green colored	Heavily branched and septate mycelium that is anastomosing and the cells with smooth walls	Spores are produced which are oval in shape.
NKS1	Light yellow colored	Branched and septate hyphae.	Spore producing structures were not found.

Spore production was detected in five isolates, with variations in location and morphology. NKL1 and NKL6 produced spore-like structures within hyphae, NKL2 produced spores externally, NKL3 formed terminal 2 to 3-celled spores, and NKL7 produced oval-shaped spores. NKL4, NKL5, and NKS1 did not exhibit spore-producing structures under the observed conditions. Overall, leaf isolates demonstrated greater morphological complexity and sporulation diversity compared to the single stem isolate.

The microscopic investigation of the spores of NKL1 and NKL3 revealed their similarity to *Curvularia* spp. At the same time, the absence of identifiable spore structures precluded the definitive identification of NKL4. But its morphology and mycelial characters show its resemblance to *Aspergillus* spp. There was no published record of any endophytic fungi isolated from *Neolamarckia cadamba* (Roxb.) Bosser. However, several endophytic bacteria have been isolated from this evergreen tree.

Phytochemical screening of crude extracts of isolated endophytic fungi

Phytochemical screening of crude extracts from three selected endophytic fungal isolates (NKL1, NKL3, NKL4) revealed the presence of various secondary metabolites (Table 2). Alkaloids and flavonoids were consistently detected in all three isolates, indicating their widespread occurrence among the tested fungi. Terpenoids were observed only in NKL3, while saponins, phenolics, coumarins, glycosides, and carbohydrates were absent in all isolates. Cardiac glycosides were present in all three isolates, whereas proteins were not detected (Table 2).

Table 2
Phytochemical screening of fungal extracts

	Alkaloid	Flavonoid	Terpenoid	Saponin	Phenolics	Coumarin	Glycosides	Carbohydrate	Protein
							Cardiac		
NKL1	+	+	-	-	-	-	-	+	-
NKL3	+	+	+	-	-	-	-	+	-
NKL4	+	+	-	-	-	-	-	+	-

Antioxidant Activity of Crude Extract of Endophytic Fungi

The antioxidant potential of the crude extracts of three endophytic fungal isolates NKL1, NKL3, and NKL4, was evaluated using the DPPH free radical scavenging assay (Table 3). The antioxidant activity was calculated by plotting the concentration of the extract in µg/ml on the x-axis and the corresponding percentage of inhibition on the y-axis, using ascorbic acid as the standard. It was found that there is a gradual increase in the inhibition percentage when the concentration of the extract is high.

Table 3
DPPH free radical scavenging of the fungal extracts

Concentration of the extract ($\mu\text{g/ml}$)	Inhibition (%) of Ascorbic Acid	Inhibition (%) of NKL 4	Inhibition (%) of NKL1	Inhibition (%) of NKL3
20	56.79 ± 1.754	52.01 ± 1.965	67.59 ± 1.757	65.04 ± 1.954
40	78.64 ± 1.904	57.66 ± 1.986	37.98 ± 1.896	75.20 ± 1.895
60	83.23 ± 1.568	58.87 ± 1.688	70.67 ± 1.709	80.48 ± 1.954
80	90.15 ± 1.734	60.48 ± 1.953	76.25 ± 1.998	86.58 ± 1.925
100	95.32 ± 1.756	73.79 ± 1.762	91.89 ± 1.962	89.43 ± 1.627

At the lowest concentration tested (20 $\mu\text{g/ml}$), NKL1 and NKL3 exhibited high radical scavenging activity (67.59% and 65.04%, respectively), approaching the activity of ascorbic acid (56.79%), whereas NKL4 displayed comparatively lower inhibition (52.01%). The high inhibition values recorded for NKL1 and NKL3 suggest a richer presence or higher activity of antioxidant compounds such as alkaloids, flavonoids, and terpenoids, as supported by the phytochemical screening results. These compounds are known to act as hydrogen donors and free radical quenchers, thus stabilizing reactive oxygen species.

Antibacterial Activity of Crude Extract of Endophytic Fungi

The antibacterial activity of crude extracts from NKL1, NKL3, and NKL4 was evaluated against *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli* using the disc diffusion method at concentrations ranging from 10–25 $\mu\text{l/disc}$ (stock: 20 mg/ml) (Table 4; Fig. 3). Tetracycline was used as a positive control. *All three fungal crude* extracts remarkably inhibited *Staphylococcus aureus*. NKL3 demonstrated broad-spectrum antibacterial activity with consistently larger zones of inhibition against all three bacterial strains, particularly *K. pneumoniae* and *E. coli*. NKL1 showed moderate, dose-dependent activity against *K. pneumoniae* and *S. aureus*. NKL4 was active only against *S. aureus* and exhibited no effect on the Gram-negative strains tested.

Table 4
Antibacterial activity of endophytic fungi NKL1, NKL3, NKL4

Sl. No	Dosage (µl/ disc) (Stock: 20 mg/ ml)	Zone of Inhibition (mm)								
Bacterial strains		<i>Klebsiella pneumoniae</i>			<i>Staphylococcus aureus</i>			<i>Escherichia coli</i>		
		NKL1	NKL3	NKL4	NKL1	NKL3	NKL4	NKL1	NKL3	NKL4
1.	10µl	-	14 ± 1.76	-	11 ± 1.64	14 ± 1.86	6 ± 1.66	-	11 ± 0.97	-
2.	15 µl	7 ± 1.75	16 ± 0.65	-	13 ± 1.53	15 ± 1.89	12 ± 0.63	-	15 ± 1.38	-
3.	20 µl	8 ± 1.90	16 ± 1.81	-	15 ± 1.00	15 ± 1.08	14 ± 0.64	-	15 ± 1.76	-
4.	25 µl	10 ± 1.20	17 ± 1.99	-	16 ± 1.96	15 ± 1.17	14 ± 0.57	-	16 ± 1.06	-

Molecular Characterization and Phylogenetic Analysis

Among the three endophytic fungi (NKL1, NKL3, and NKL4), two were chosen (NKL1 and NKL3) for species identification due to their notable antibacterial and antioxidant properties. Morphological characteristics, including spore and mycelial characteristics, allowed the identification of the endophytic fungal isolate NKL1 as *Curvularia lunata* (NCBI GenBank accession number PX205205). This identification was further corroborated by the sequence analysis of its 18S rRNA gene, which exhibited a more than 99% sequence similarity to those available in the NCBI database for *C. lunata*. In a similar vein, based on ITS sequences along with morphological and molecular characterization, the endophytic fungus NKL3 is classified as *Curvularia tropicalis* (NCBI GenBank accession number PX218454). The NKL1 and NKL3 sequences shared 90.59% identity (Fig. 4). The high degree of conservation suggests close genetic relatedness between NKL1 and NKL3. Two endophytes, NKL1 and NKL3, isolated from *Neolamarckia cadamba* were successfully identified as *Curvularia lunata* and *Curvularia tropicalis*, respectively, based on their similarity with reference sequences available in the NCBI database. These fungi have been documented as endophytic in various host plant species. To confirm their taxonomic placement and evolutionary relationships, a phylogenetic tree was constructed using reference sequences retrieved from GenBank (Fig. 5). The resulting phylogram resolved four major clusters. Cluster II contained NKL1 (*Curvularia lunata*, PX205205), which grouped tightly with other *C. lunata* accessions and *C. petersonii*, indicating a close evolutionary relationship to *C. lunata*. Cluster III contained NKL3

(*Curvularia tropicalis*, PX218454), which clustered with *C. tropicalis* sequences and related species (*C. phaenospora*, *C. chiangmaiensis*), confirming NKL3's identity as a *C. tropicalis* isolate. Other clusters comprised *Cochliobolus*, *Sarocladium*, *Bipolaris*, *Phoma*, *Cercospora*, and *Agaricus*, serving as related taxa and outgroups. The distinct separation of NKL1 and NKL3 within the *Curvularia* clade supports the BLAST results and demonstrates their evolutionary proximity to well-characterized *Curvularia* species. This is the first report of *C. tropicalis* being isolated as an endophyte from *N. cadamba*, suggesting a potentially unique and symbiotic association.

Discussion

The present study highlights the diverse bioactivities exhibited by endophytic fungal isolates NKL1, NKL3, and NKL4 derived from *Neolamarckia cadamba*. The phytochemical screening (Table 2) revealed a consistent presence of alkaloids, flavonoids, and cardiac glycosides across all isolates, with terpenoids uniquely detected in NKL3. Alkaloids and flavonoids are well-known for their potent antioxidant and antimicrobial properties (e.g., flavonoids acting as hydrogen donors and metal chelators (Rahim et al. 2022)). This finding aligns with previous studies demonstrating that endophytic fungi often mirror the medicinal plant's metabolite profile and can synthesize analogous bioactive compounds (Venieraki et al. 2017)

Previous phytochemical and bioactivity studies on *Neolamarckia cadamba* provide a valuable framework for interpreting the present findings. Shikha and Kumar (2024) quantified flavonoid and tannin contents in different parts—leaves, bark, unripe fruits, and ripe fruits—and found that the bark possessed the highest concentrations of both flavonoids and tannins, highlighting its potential contribution to the plant's medicinal properties. Similarly, Konatham et al. (2025) demonstrated that aqueous extracts of *N. cadamba* leaves exhibited notable antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The phytochemical screening revealed the presence of alkaloids, saponins, phenolic compounds, proteins, and amino acids. In addition, Haritha et al. (2025) evaluated aqueous, ethanolic, and cyclohexane extracts of *N. cadamba* leaves and detected alkaloids, tannins, saponins, steroids, and glycosides, with the aqueous extract showing significant antifungal activity against *Aspergillus niger*. Collectively, these studies highlight the rich phytochemical diversity and broad-spectrum antimicrobial potential of *N. cadamba*, underscoring the need for further exploration of its endophytic fungal community for novel bioactive metabolites.

The antioxidant assays (Table 4) underscored a concentration-dependent increase in DPPH radical scavenging activity, with NKL3 and NKL1 achieving inhibition values comparable to those of ascorbic acid (89–92% at 100 µg/ml). NKL4, however, showed significantly lower activity (74% at 100 µg/ml). Such elevated antioxidant capacities are characteristic of endophytic fungi rich in phenolics and flavonoids, as evidenced in prior research where certain ethyl acetate extracts displayed radical scavenging activities on par with vitamin C (Gupta et al. 2020). In the assessment of the antioxidant potential of endophytes isolated from *Catharanthus roseus* and *Calotropis procera*, it was noted that the total antioxidant activity in the case of the culture filtrate of all the endophytic crude samples was higher than that of the mycelium, of which the highest antioxidant activity in the culture filtrate was noted in

Curvularia geniculata (Chowdhury et al. 2018). The free radical percentage activity was reported maximum in the aqueous extract of the stem of *N. cadamba* at 81% with 1.5 ml and the minimum percentage of scavenging activity was reported in its aqueous extract of leaf part 3.33% with 0.5ml, whereas the methanol extract of stem and leaf showed intermediate activity free radical scavenging activity of 60% and 30% with 1.5ml respectively (Kumar et al. 2020). The high scavenging potential of NKL1 and NKL3 likely reflects their richer repertoire of phenolic and flavonoid compounds, as observed in other *Curvularia* endophytes (Pinheiro et al. 2013).

The antibacterial assay demonstrated that NKL3 exhibited broad-spectrum activity, with the highest inhibition zones across *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli*. NKL1 showed moderate activity against Gram-positive *S. aureus* and *K. pneumoniae*, whereas NKL4 was effective only against *S. aureus*. These results resonate with numerous reports describing endophytic fungi as prolific sources of antibacterial compounds, particularly alkaloids and terpenoids as part of their ecological role in plant protection (Strobel 2003; Wiyakrutta et al. 2004). Mensah et al. (2016) carried out the antibacterial activity of *Curvularia lunata*, *Aspergillus parasiticus*, and *Mucor* spp. against two gram-negative bacteria (*Escherichia coli*, *Salmonella typhi*), two gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), and one fungus (*Candida albicans*) by the broth dilution method. For all fungi, the hexane extracts exhibited a more potent microbial inhibitory activity as demonstrated by the low recorded MIC values: 0.10 mg/ml for *Curvularia lunata*, 0.068 mg/ml for *Aspergillus parasiticus*, and 0.018mg/ml for *Mucor* spp. Rani et al. (2017) studied the endophytic fungal extracts of *Aspergillus nidulans*, *Curvularia tropicalis*, *Chaetomium aruenum*, and *Chaetomium atrobrunneum* and demonstrated significant microbial controlling capacity.

Curvularia is a member of Ascomycota that exhibits a facultative pathogenic and endophytic lifestyle (Mehta et al. 2022). Kharwar et al. (2011) investigated the diversity and antibacterial activity of endophytic fungi isolated from the medicinal plant *Adenocalymma alliaceum*. Among the isolated fungi, *Curvularia lunata* was identified and noted for its significant antibacterial properties. Similarly, Al-Mahi et al. (2013) isolated the endophytic fungi from *Kigelia africana* and evaluated their antibacterial activity. *Curvularia lunata* demonstrated notable antibacterial effects against several pathogenic bacteria. *Curvularia* and *Alternaria* exhibit similarities in conidial formation and share somewhat similar morphologies. *Curvularia* also showed similarity with *Bipolaris* in the sequence structure and conidial features (Manamgoda et al. 2012).

Identification based on ITS sequence analyses indicated that all the endophytic isolates obtained from *N. cadamba* belonged to the phylum Ascomycota. The clustering of NKL1 with *Curvularia lunata* and NKL3 with *C. tropicalis* corroborates their BLAST-based identification. This finding is consistent with earlier studies that demonstrate the reliability of ITS phylogenies in resolving *Curvularia* species (Huang et al. 2009). Similar phylogenetic approaches have been successfully used to clarify the taxonomic placement of plant-associated endophytic fungi, highlighting ITS sequencing as a robust tool for evolutionary and diversity assessments (Bongiorno et al. 2016; Oliveira et al. 2020). Diversity of leaf fungal endophytes from two *Coffea arabica* varieties and antagonism towards coffee leaf rust. Previous studies have also

shown that Ascomycota is the most dominant fungal lineage associated with plants as endophytes (Ramos-Garza et al. 2016; Gonzalez-Teuber et al. 2017). The predominance of this group is often attributed to its adaptive potential, high sporulation capacity, and ability to colonize diverse plant tissues under varying environmental conditions (Arnold and Lutzoni, 2007). The identification of two *Curvularia* species with strong bioactive potential underscores the ecological and pharmaceutical significance of *N. cadamba* endophytes. *Curvularia* spp. are known to produce a range of secondary metabolites, including polyketides, alkaloids, and terpenoids, with documented antimicrobial, antioxidant, and cytotoxic properties (Kharwar et al. 2011). For instance, *Curvularia lunata* isolated from medicinal plants has been shown to possess strong antibacterial properties, even against drug-resistant strains like MRSA, along with notable anti-inflammatory potential (Kaur et al. 2024). Similarly, *Curvularia tsudae* from *Cynodon dactylon* has been reported to produce coumarin derivatives with potent antioxidant and antimicrobial activities (Nischitha et al. 2020). The bioactive potential of *Curvularia* isolates aligns with the increasing recognition of endophytes as an alternative source for novel natural products (Kusari et al. 2012). The discovery of *C. lunata* and *C. tropicalis* in this host plant opens new avenues for exploring species–host specificity and the bioprospecting of novel compounds.

Conclusion

Endophytic fungi, a previously underexplored group of microorganisms, have recently garnered attention for their ability to produce a diverse range of bioactive compounds. As a key component of a plant's mycobiome, endophytes contribute significantly to host physiology, resilience, and ecological fitness. The identification of *Curvularia lunata* and *Curvularia tropicalis* as endophytes from *N. cadamba* from the present study represents a novel and significant finding, marking the first report of these fungi inhabiting this plant. The presence of such diverse and potentially beneficial fungal endophytes suggests a complex and possibly co-evolved relationship that may contribute to the plant's stress tolerance, pathogen resistance, or overall ecological fitness. Endophytic fungi, particularly Ascomycetes, are known to influence plant health by enhancing nutrient acquisition, producing secondary metabolites, and offering protection against biotic and abiotic stresses. The conservation of *N. cadamba* should therefore consider not only the plant itself but also its microbial symbionts, which may play crucial roles in its survival and adaptability. The discovery of *C. lunata* and *C. tropicalis* as endophytes opens new avenues for understanding the plant's resilience and supports the broader need for integrated conservation strategies that include microbial biodiversity as a component of endangered plant protection. Further studies are anticipated to carry out the pharmacological profiling of fungal metabolites. This may help in detecting novel pharmacologically active therapeutic molecules that can be used as a lead in the drug discovery process.

Declarations

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Authors contributions

Dr. Sumayya S. S. conceptualized the study, designed the research framework, and contributed to data interpretation and manuscript preparation. Ms. Sheena carried out data collection, performed preliminary analysis, and assisted in drafting the manuscript. Both authors read and approved the final version of the manuscript.

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Data availability and materials

The authors declare that the data supporting the findings of this study are available within the paper. Sequencing data were deposited in the NCBI database.

Conflict of interest

The authors declare that the research was conducted in the absence of any financial relationships that could be construed as a potential conflict of interest.

Ethics approval and consent to participate

This paper does not involve human and animal experiments, so it does not need ethical approval and informed consent.

Consent for publication

All the authors agree to publish the paper.

Competing interests

The authors declare that they have no competing interests.

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Figures

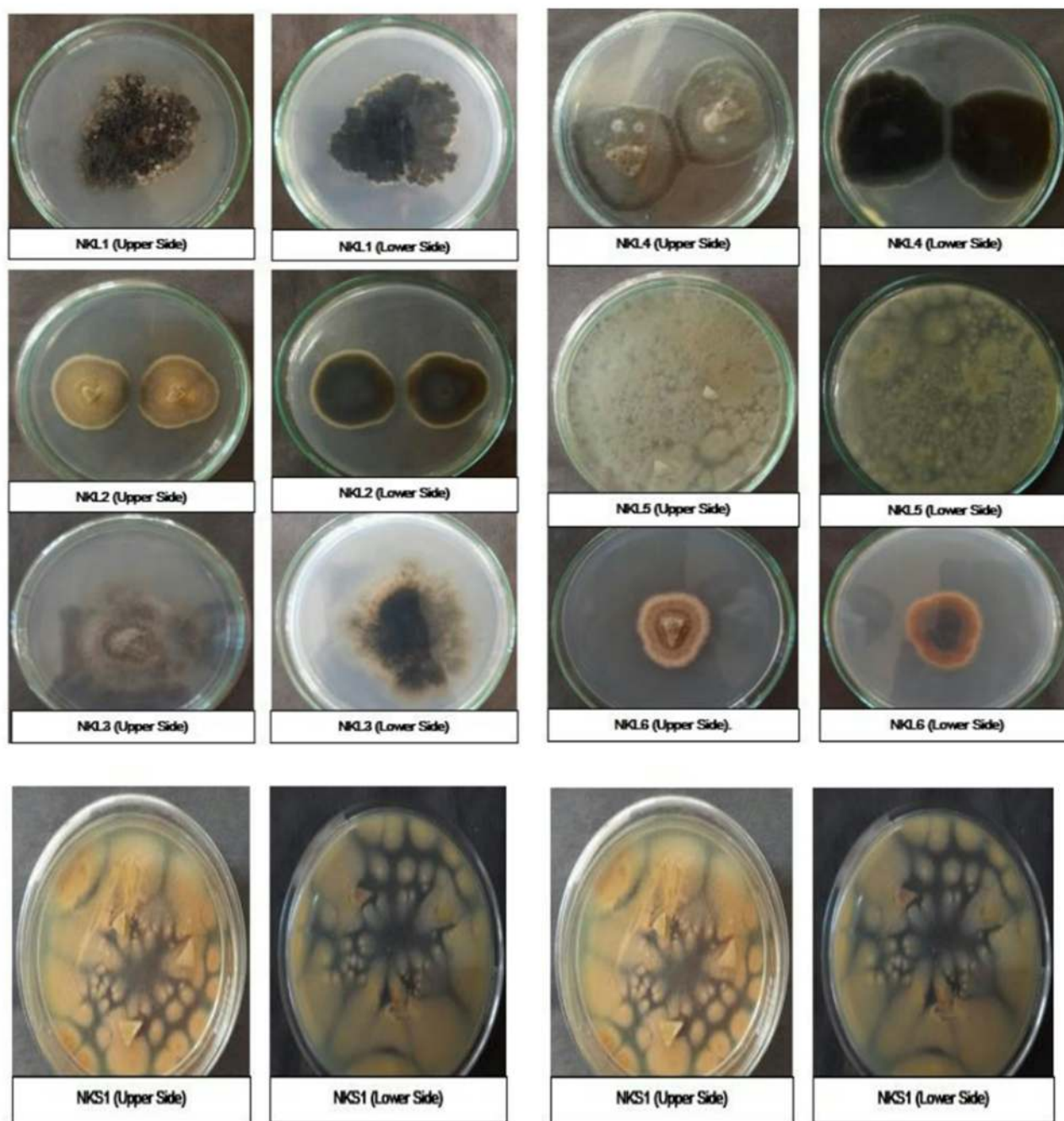


Figure 1

The pure culture of fungal endophytes isolated from the leaves (NKL 1 to NKL 7) and stem (NKS 1) of *Neolamarckia cadamba*.

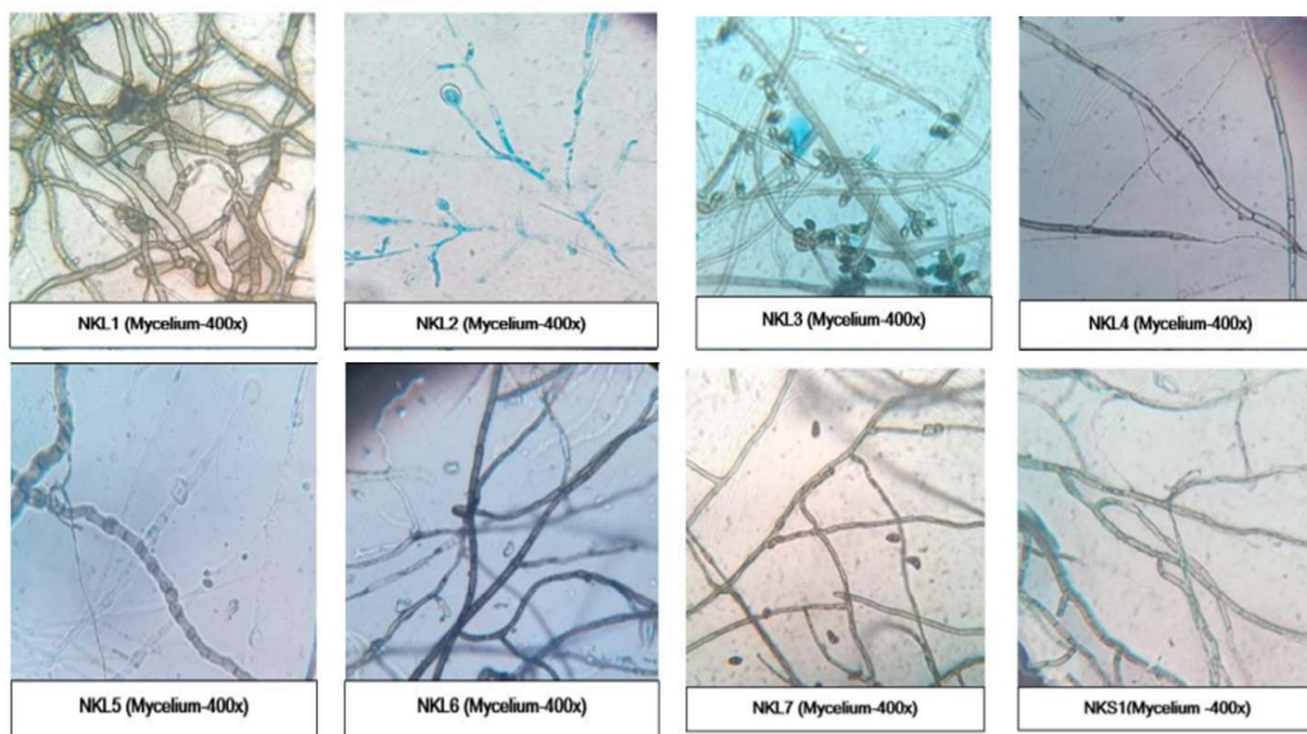


Figure 2

Mycelial characters of endophytic fungi from leaves (NKL1 to NKL7) and stem (NKS1) isolated from *Neolamarckia cadamba*

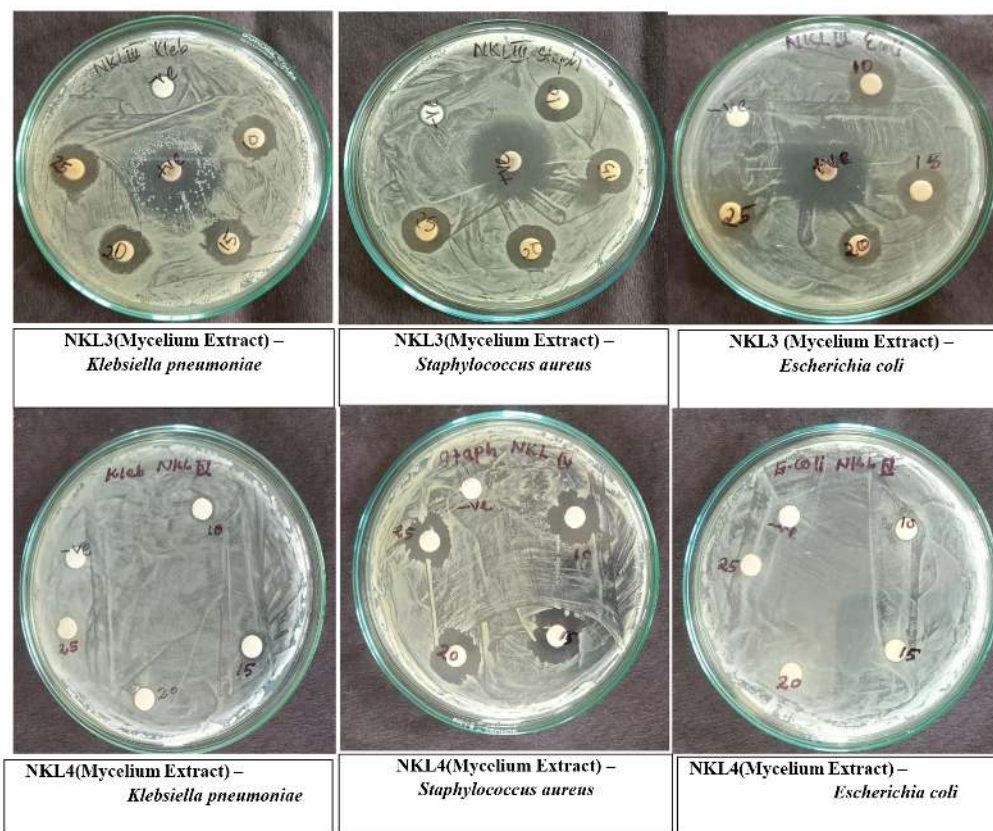


Figure 3

Antibacterial activity of the crude extract of NKL3 and NKL4 against *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*

NKL1	TCTCTGTAGGTGAACCTGCGGAGGGATCATTACACAAATACAATATGAAGGCTT----	CG
NKL3	CCTCCGTAGGTGAACCTGCGGAGGGATCATTACACAA-TACAATATGAAGGCTGTCCGCA	
	*** *****	*
NKL1	GCTGGATTATTTATTTACCCCTTGTCTTTTGC	CACTTGTTGTTTCCTGGGCGGGTTCGC
NKL3	GCTGGAGTATTT-TATTACCCCTTGTCTTTTGC	CACTTGTTGTTTCCTGGGCGGGTTCGC
	*****	*****
NKL1	CCGCCACCAGGACCACACCATAAACCTTTTTT---	GTTAATGCAGTCAGCGTCAGTACAA
NKL3	CCGCCACCAGGACCACCCAATAAACCTTTTTTTATGCAGTTGCAATCAGCGTCAGTGTA	
	*****	*****
NKL1	CAAATGTAAATCATTTACAACCTTTCAACAACGGATCTCTTGTTCTGGCATCGATGAAGA	
NKL3	ATAATGTAAATCATTTACAACCTTTCAACAACGGATCTCTTGTTCTGGCATCGATGAAGA	

NKL1	ACGCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG	
NKL3	ACGCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG	

NKL1	AACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCCGAGCGTCATTTGTACC	
NKL3	AACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCCGAGCGTCATTTGTACC	
	*****	*****
NKL1	CTCAAGCTTTGCTTGGTGTGGGCGTTTTGTCTTTGCTCGCC-AAAGACTCGCCTTAAAA	
NKL3	CTCAAGCTTTGCTTGGTGTGGGCGTTTTGTCTTTGGTGCCCAAAGACTCGCCTTAAAA	
	*****	*****
NKL1	CGATTGGCAGCCGACCTCTT--GGTTTCGCAGCGCAGCACAAATTTTGC	GCTTGCAAATCA
NKL3	CGATTGGCAGCCGGCCTTTTCTGGTTTCGCAGCGCAGCACATTTTGC	GCTTGCCA-TCA
	*****	*****
NKL1	GCAAGTTGGCACTCCATCAAGAACATTTTTTTACGTTTGACCTCGGATCAGGTAGGGATA	
NKL3	GCAGACCGGCACTCCATGAAGC-CTCCTTCTCACGTTTGACCTCGGATCAGGTAGGGATA	
	***	*****
NKL1	CCCGCTGAACTTAAGCATATCAT	
NKL3	CCCGCTGAACTTAAGCATAT---	

Figure 4

Comparative Sequence Alignment of Endophytic Fungal Isolates NKL1 and NKL3 (Asterisks (*) indicate identical bases, while gaps (-) represent insertions or deletions)

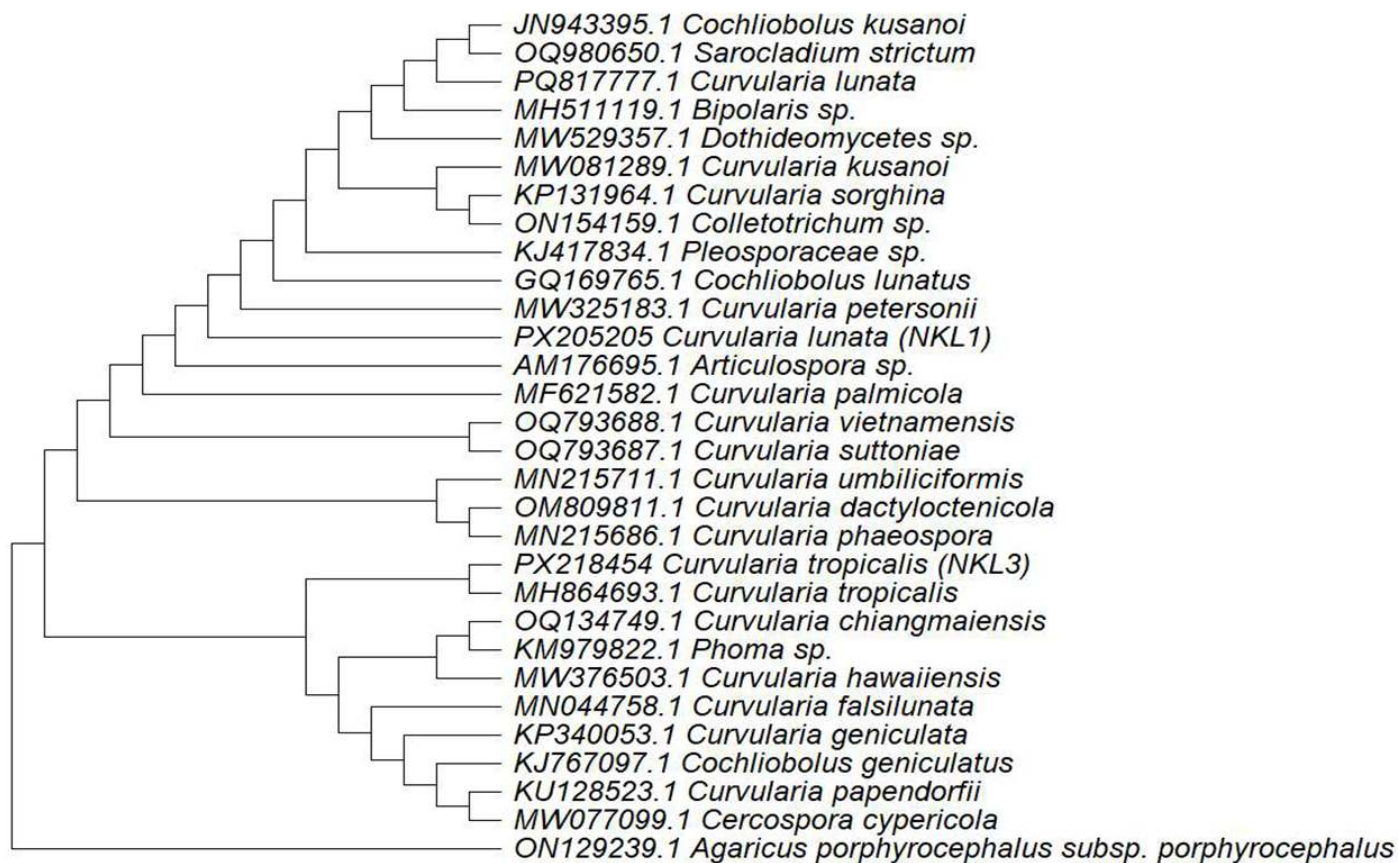


Figure 5

Phylogram depicting the relationship between NKL1 and NKL3 endophytic fungal species inferred from ITS nucleotide sequence data.