RESEARCH ARTICLE

Molecular Identification and Bioactive Potential of Endophytic *Nectria pseudotrichia* from *Terminalia catappa*

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Abstract

Endophytic fungi associated with *Terminalia spp.* (Combretaceae) are reported to possess significant therapeutic potential. The present study aimed to isolate, identify, and evaluate the bioactive properties of *Nectria pseudotrichia* obtained from *Terminalia catappa*. The isolated fungus was screened for antifungal activity against three *Fusarium spp.* and was molecularly identified as *N. pseudotrichia* through ITS sequencing. Further investigations focused on optimizing culture media with different carbon and nitrogen sources to assess their effects on Fusarium growth suppression, phytochemical production, and antioxidant activity. Phytochemical analysis confirmed the presence of diverse bioactive metabolites, while antioxidant activity was detected but varied with growth conditions and nutrient composition. The sturdiest antifungal activity was observed in 12-day-old cultures, with arabinose and sodium nitrate identified as the most effective carbon and nitrogen sources, respectively. These optimized conditions boosted secondary metabolite production, highlighting the potential of *N. pseudotrichia* as a promising source of compounds for pharmaceutical applications and as an antagonist against plant pathogenic fungi.

Keywords: Endophytic fungi, Secondary metabolite, *Fusarium spp.,* Carbon source, Nitrogen source.

Highlights

- *Terminalia catappa* is considered an effective medicinal plant
- Endophytic fungus isolated from *Terminalia catappa* and molecular identification through ITS sequencing
- *Nectria pseudotrichia* was isolated from *T. catappa,* and its methanolic extract has various bioactivities like Antimicrobial, Antioxidant properties, and is also involved in the production of secondary metabolites.
- Effect of various nutritional amendments like different Carbon and Nitrogen source on the bioactive potential of *N. pseudotrichia.*

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Introduction

Plants are an excellent source of food, beauty, and, most importantly, medications for humans. Since the search for potential medicinal plants has shown positive results, the relevance of ethnopharmacology is being recognized. Most of the population in developing countries is dependent upon herbal medicine as a main source. Additionally, the herbal one is used as the main alternative due to its fewer complexities, safety, and efficiency (Mwangi *et al.,* 2024). Medicinal plants belonging to the family Combretaceae are well addressed for their therapeutic potential (Mandloi *et al.,* 2013). Among these, one species, i.e., *Terminalia catappa,* is known as a tropical almond base in both tropical and subtropical regions and has high nutritional value with various bioactive compounds, which are of benefit to health (de Oliveira *et al.,* 2024). Bioactive compounds include anthocyanins, hesperidin, rutins, and tannins like metabolites with macronutrients and minerals (Adefegha *et al.,* 2017; Hussain *et al.,* 2021). It is considered a useful herbal plant in Asia (Kaneria *et al.,* 2018), and in Africa, it is used in the development of drugs for antifungal, antibacterial, antiinflammatory, antioxidant, antidepressant, and chemoprotective activities (Abiodun *et al.,* 2016; Silalahi, 2022; Punniyakotti *et al.,* 2019). Despite rising scientific curiosity in *Terminalia catappa*, understanding of its bioactive content and characteristics remains limited (Pham *et al.,* 2023). Nowadays, the main public health concern is the spread of multidrug-resistant microbes. This occurs due to the lack of antimicrobials that treat an infection effectively. Therefore, to overcome the issue, the investigation for new antimicrobial agents is urgent. According to fossil evidence, plants and fungi have shown mutualism for millions of years (Tripura *et al.,* 2024). Endophytic fungi are prevalent in the plant world and also serve an important role in stabilizing the microenvironments within the host tissue. Fungal endophytes isolated from ethnobotanically significant plants produced several bioactive metabolites with putative bioactivity. Endophytes live within host tissue and do not cause harm; instead, they generate or imitate the same metabolites as the host plant, making them more significant. Fungal endophytes can produce secondary metabolites enriched with antimicrobial, insecticidal, cytotoxic, and anticancer properties (Samantaray and Gupta., 2024), and a study revealed that 51% of bioactive secondary metabolites and materials were segregated from endophytes (Strobel and Daisy, 2003; Schulz *et al.,* 2002). They create bioactive chemicals that boost plant development and increase host resistance to plant diseases

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(Nguyen *et al.,* 2020). Important sources of bioactive ingredients are mainly Terpenoids, alkaloids, flavonoids, glycosides, and phenolics used in modern medicine, and pharmaceuticals are also obtained from fungal endophytes (Seca and Pinto., 2019). From *Terminalia spp*., more than 47 fungal genera have been described, and the metabolites generated by some of these fungi show different biological functions (Kouipou and Boyom., 2019). Endophytic fungi, including *Aspergillus spp*., *Penicillium spp., and Nectria spp.,* are active against infections and create a variety of chemicals with varying bioactivity. *The Nectria* genus is a rich source of biologically active secondary metabolites with various structures and bioactivity. Two sesquiterpene-epoxy cyclohexanone conjugates, nectrianolins A (1) and B (2), as well as a sesquiterpene, nectrianolin C (3), were isolated from the brown rice culture of *Nectria pseudotrichia* and had different biological activities (Ariefta *et al.,* 2017). As we know, the plant and its endophytic fungus have a wide range of bioactive potential. A current study is needed to generate antifungal metabolites, which will boost medical and pharmaceutical research and make it valuable for future generations. Plants are vulnerable to diseases induced by both biotic and abiotic agents. Pathogenic microorganisms like bacteria and fungi are the main biotic factors (Sabat and Gupta., 2009). In terms of antimicrobial activity, there are numerous studies accessible, also known as antibiotics, on the antibiotic-generating capability of bacteria (Debnath *et al.,* 2013; Fyhrquist *et al.,* 2004; Rubini *et al.,*2013). Several studies have been conducted on extracellular metabolite synthesis while considering cultural and dietary circumstances. The basal medium was modified using different carbon and nitrogen sources to obtain better antifungal potential. Experiments were constructed to partially describe the bioactive metabolite(s) found in the fungal culture.

Fusarium spp. are omnipresent in the environment, and several strains of *Fusarium spp.* Showing infection in both plants or animals and producing mycotoxins are reported. One of the typical soilborne pathogens, *F. oxysporum* lives in the soil for an extended period in the form of chlamydospores, penetrates throughout the roots and tissues, and colonizes in xylem tissue, and causes wilting, yellowing, and death in plants (Arie, 2019). *F. fujikuroi*, a phytopathogen, was later shown to cause severe illness in man's economically significant plants, including rice, maize, sugarcane, wheat, asparagus, etc (Qiu *et al.,* 2020). *F. proliferatum*, a ubiquitous and diversified fungal disease, infects numerous plants, including maize, wheat, and pine (Proctor *et al.,*2010). *Fusarium sp.* causes various diseases like dry rot in various plants (Galvez and Palmero, 2022), bakanae disease in rice (Raghu *et al.,*2018), tracheomycosis (Fravel *et al.,* 2003), vascular wilt disease (Flood, 2006), and so on. By suppressing the growth of the above pathogens, we can develop various antifungal metabolites that can be used in pharmaceutical research in the future. In the present study, various parts of *Terminalia catappa.* They were studied for the presence of endophytes. The selected fungal endophyte was identified morphologically as well as by ITS gene sequencing. Further, various biological activities like antifungal potential, phytochemical, and antioxidant properties of the extract were evaluated. Endophytic fungi are recognized as promising sources of bioactive compounds, yet many species, including *Nectria pseudotrichia*, remain underexplored for their molecular characteristics and therapeutic potential. Although *Terminalia catappa* is known for its medicinal properties, there is limited information on its associated endophytic fungi and their bioactive metabolites. This study addresses this gap by combining molecular identification with bioactivity screening, providing new insights into the diversity, metabolite potential, and biotechnological relevance of endophytic *N. pseudotrichia*. The work not only enhances understanding of endophyte–host interactions but also identifies novel candidates for antifungal and pharmaceutical applications, highlighting its significance in natural product discovery.

Methodology

Isolation of Endophytic Fungus

Fungal endophyte was segregated from the various parts of *Terminalia catappa* grown on the campus of the Regional Plant Resource Center, Bhubaneswar, Odisha. Plant components were securely stored in sterile polyethylene pouches. Parts of plant samples were cut with sterile scalpels. Fragments of plant sample were successively surface sterilized in 70% ethanol for 1 minute, 2.5% sodium hypochlorite for 2 minutes, and sterile distilled water 2 times for 1 minute each (Srivastava *et al.,* 2024). The isolated fungus was screened against three *Fusarium* species—*Fusarium proliferatum*, *Fusarium fujikuroi*, and *Fusarium oxysporum*—obtained from the culture collection of the Microbiology Laboratory, Regional Plant Resource Centre, Bhubaneswar.

Molecular identification of fungi through ITS sequencing

The chosen fungal cultures were identified using a molecular identification approach in alliance with MTCC, CSIR-IMTECH, Chandigarh. Phylogenetic studies were carried out using closely related ITS-type sequences of *Nectria* and *Murinectria* species obtained from GenBank. On the MAFFT server, several sequence alignments for the ITS gene were done online (Katoh *et al.,* 2019). By using BioEdit, alignments were then manually corrected (Hall, 1999). The phylogenetic studies contain the AiTS area dataset, comprising 31 species belonging to *Nectria* and *Murinectria*, with *Ophiocordyceps arborescens* NBRC 105891 as the outgroup taxon. A maximum parsimony analysis of the ITS dataset revealed that our taxon, *Nectria pseudotrichia* T4F4, grouped with the *Nectria pseudotrichia* clade. The Maximum Parsimony method was used for the evolutionary history. The proportion of replicate trees in which the connected taxa are grouped in the bootstrap test (1000 repetitions) is displayed next to the branches (Felsenstein, 1985). By using the Subtree-Pruning-Regrafting (SPR) method, the MP tree was created (Nei and Kumar, 2000) at search level 1, with the initial trees generated by random sequence addition (10 replicates).

Optimization of media using different nutrient sources

To investigate the impact of different nutritional sources on metabolite production, the fungal isolate was cultured in media supplemented with various carbon and nitrogen sources. Extracts obtained from each condition were evaluated for three bioactivity test.

Optimization of media using different carbon sources

The Sabouraud dextrose medium was discovered to be the most effective medium for increasing antifungal activity during the screening phase. *Nectria pseudotrichia* grows best throughout the 12-day incubation period. As a result, the Sabouraud dextrose agar medium with the chosen culture condition was used as the base medium for nutritional factor modification. 12 different carbon sources (2%) were taken: fructose, arabinose, raffinose, galactose, maltose, xylose, sucrose, lactose, mannitol, inositol, aesculin, and starch. In each case, the carbon source was obtained at 2% w/v and given to the basal SD medium instead of dextrose. The fungal culture was cultured in a Sabouraud dextrose broth medium to find the optimal carbon source for maximum development. The culture filtrate was filtered and concentrated using the Soxhlet equipment, after which ethyl acetate was added to the concentrated filtrate for 72 hours. The top layer was separated and evaporated using Soxhlet. Evaporated samples were dissolved in methanol, and a methanolic extract was produced. Methanolic extracts of various fungi were evaluated for antifungal, phytochemical, and antioxidant activities to determine the optimal carbon source for secondary metabolite formation (Lahouar *et al.,* 2016; Patro and Gupta, 2022).

Optimization of media using different carbon sources

The ten nitrogen sources used were ammonium sulfate, ammonium chloride, ammonium thiocyanate, ammonium molybdate tetrahydrate, ammonium nitrate, calcium nitrate, cobaltous nitrate, copper nitrate, potassium nitrate, and sodium nitrate. In each case, 2% w/v nitrogen was added to the basal Sabouraud dextrose medium instead of peptone (Sabat and Gupta, 2010). The cultures were cultured in a Sabouraud dextrose broth medium to find the greatest nitrogen source for maximum growth. Solvent extraction was performed, and the antifungal, phytochemical, and antioxidant activities of the methanolic extract were evaluated to determine the optimal nitrogen source for eliciting the synthesis of metabolites as previously reported.

Three different bioactivity tests

Antifungal activity test

Solvent extract of *Nectria pseudotrichia* cultivated on various carbon sources was evaluated for antifungal activity against *Fusarium proliferatum*, *Fusarium fujikuroi*, and *Fusarium oxysporum*. The percentage (%) of growth decline was calculated using morphological growth from the solid plate culture technique.

A= Zone of Diameter of negative control- Zone of Diameter of Test/ Zone of Diameter of negative control×100, B= Zone of Diameter of negative control- Zone of Diameter of Positive control/ Zone of Diameter of negative control × 100
Percentage of growth reduction A-B

Phytochemical test

Secondary metabolites, alkaloids, flavonoids, phenols, tannins, saponins, glycosides, and steroids were detected in the methanolic extracts of endophytic fungi *Nectria pseudotrichia*, cultivated under varied nutritional factors (Shivaputrappa and Vidyasagar, 2018).

Antioxidant activity test

The approach was used to assess the DPPH activity of the stable free radical in a methanolic extract of a fungal endophyte cultivated under various nutritional conditions. DPPH solution (0.006% w/v) was prepared in 95% methanol. 2ml of DPPH solution was added with 1ml of the Methanol extracts to form a final volume of 3 ml, and discoloration was measured at 517 nm with a UV-Vis Spectrophotometer after 30 minutes of dark incubation. In the control group, methanol was used instead of the sample. The percentage of inhibition was calculated (Nayak and Basak, 2015).

Results

The current work aimed to segregate and identify endophytic fungi from several plant sections of *Terminalia catappa*. The effect of nutritional amendments on antifungal activity, phytochemical tests, and antioxidant tests has produced some interesting results that explain its predilection for the synthesis of secondary metabolites.

Molecular identification of selected fungi

A total of 31 nucleotide sequences were analyzed for *Nectria pseudotrichia*. There were 646 positions in the final dataset for *Nectria pseudotrichia*. An evolutionary study was conducted in MEGA11(Tamura *et al.,* 2021), and phylogenetic analysis by using ITS sequencing is represented in Fig. 1.

>*Nectria pseudotrichia*T4F4
CCTGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATACCTAT
AATTGTTGCTTCGGCGGGACCGCCCCGGCGCCCACTCGGGCCCGGACCCAGGCGCC
CGCCGGAGGCCCCAAACTCTTTGTTTTTACGTTGTGTATCTTCTGAGTTATACAAGCA
AATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA
GCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACATTGCGCCCGCTAGTATTCTAGCGGGCATGCCTGTTCGAGCGTCATTTCAAC
CCTCAAGCCCCCGTGGCTTGGTGTTGGGGATCGGCCGCAGCCTTCACGGCAGGCGGC
CGGCCCCGAAATCTAGTGGCGGTCTCGCTGTAGTCCCCCTCTGCGTAGTAATACACC
TCGCACTGGAAGCTCGGCCTGACCACGCCGTAAAACCCCCCACTTCTGAAGGTGACC
TCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAT

Fig. 1: Phylogenetic analysis of fungal taxa by using ITS sequencing

For Nectria Tree #1 out of 10 most parsimonious trees (length = 666) is shown in Fig. 2. The consistency index is 0.857357 (0.842975), the retention index is 0.941358 (0.941358), and the composite index is 0.807080 (0.793541) for all sites and parsimony-informative sites (in parentheses).

Extraction of Secondary Metabolites & evaluation for Antifungal and Antioxidant Activity using different nutritional conditions like carbon sources and nitrogen sources

Antifungal activity test

Antifungal activities were assessed in ethyl acetate extracts of fungal metabolites generated over a variety of nutritional factors, such as different carbon sources. Fig. 3.a, 3.b, and 3.c show the observations regarding the percentage growth inhibition of test *Fusarium spp*. by *Nectria pseudotrichia*. Extracts from the culture of arabinose suppressed the growth of *F. proliferatum* and *F. oxysporom* by 25.84% and 12.62% respectively. *Nectria pseudotrichia*'s growth inhibition potential increased significantly against *F.fujikuroi* but decreased gradually against *F.oxysporom*. The antifungal capabilities of *Nectria pseudotrichia* reported below demonstrated the existence of bioactive secondary metabolites in arabinose fungal culture against *Fusarium spp.*

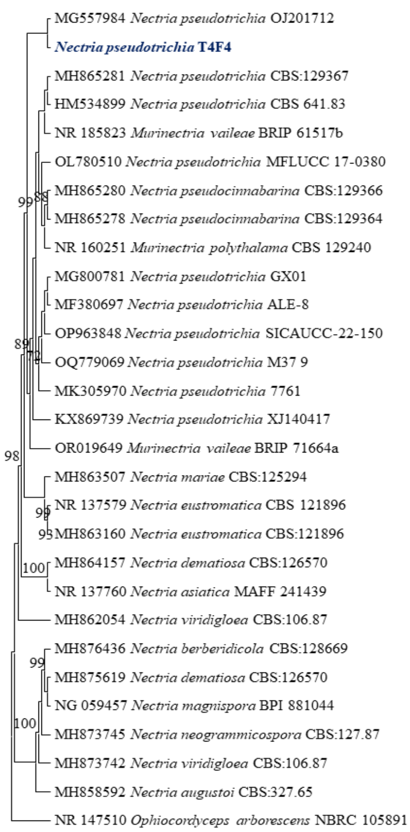

Fig. 2: Phylogenetic placement of *Nectria pseudotrichia* T4F4 by a Maximum Likelihood (ML) analysis of a dataset of the ITS region. Bootstrap support values for MP equal to or greater than 70% (black) are given above the nodes. The tree was rooted to *Ophiocordyceps arborescens* NBRC 105891

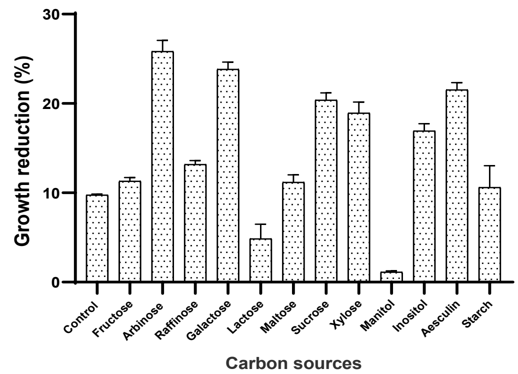

Fig. 3: a. Influence of different carbon sources on the antifungal activity of the isolate against *Fusarium proliferatum*

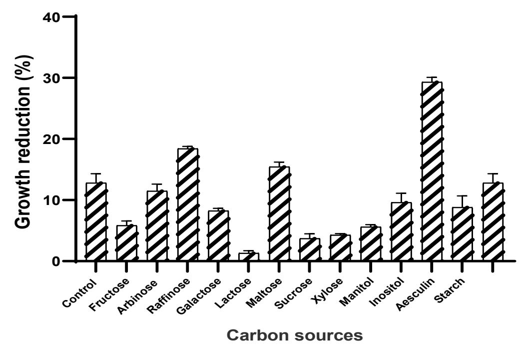

Fig. 3: b. Influence of different carbon sources on the antifungal activity of the isolate against *Fusarium fujikuroi*

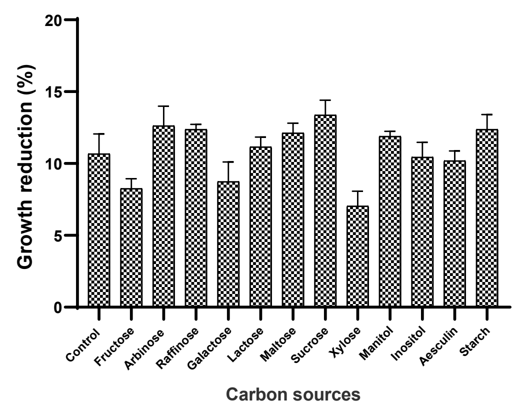

Fig. 3: c. Influence of different carbon sources on the antifungal activity of the isolate against *Fusarium oxysporom*

Antifungal activities were assessed in ethyl acetate extracts of fungal metabolites generated over a variety of nutritional factors, like different nitrogen sources. Fig. 4.a, 4.b, and 4.c show the observations that extracts from the culture of sodium nitrate suppressed the growth of *F. proliferatum*, *F. fujikuroi, and F. oxysporom* by 23.39%, 30.46%, & 8.3% respectively. *Nectria pseudotrichia*'s growth inhibition potential increased significantly against *F.fujikuroi*. The antifungal capabilities of *Nectria pseudotrichia* reported below demonstrated the existence of bioactive secondary metabolites in sodium nitrate,

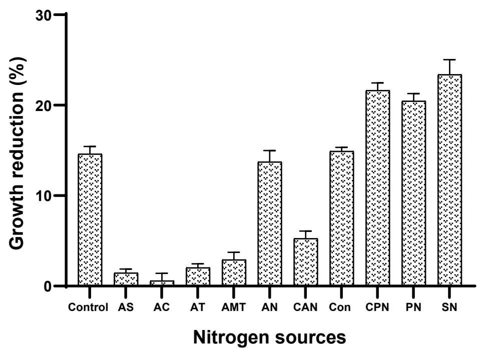

Fig. 4: a. Influence of different nitrogen sources on the antifungal activity of the isolate against *Fusarium proliferatum*

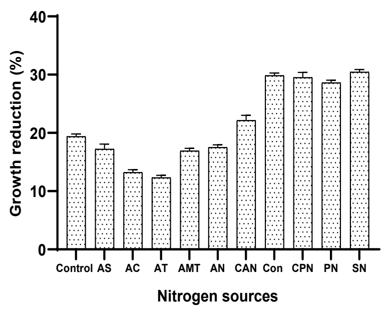

Fig. 4: b. Influence of different nitrogen sources on the antifungal activity of the isolate against *Fusarium fujikuroi*

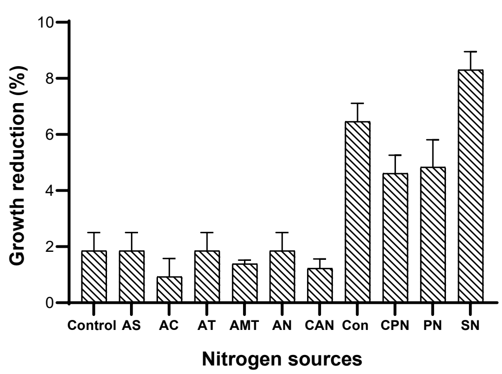

Fig. 4: c. Influence of different nitrogen sources on the antifungal activity of the isolate against *Fusarium oxysporom*

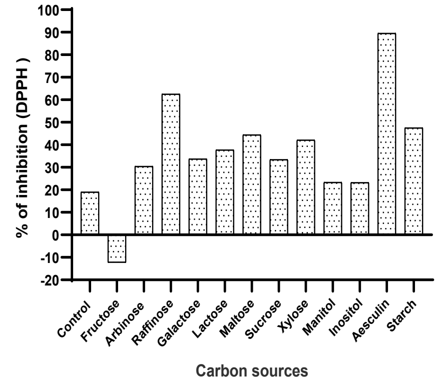

Fig. 5: a. Antioxidant profile of extracts of fungal culture grown in different carbon sources

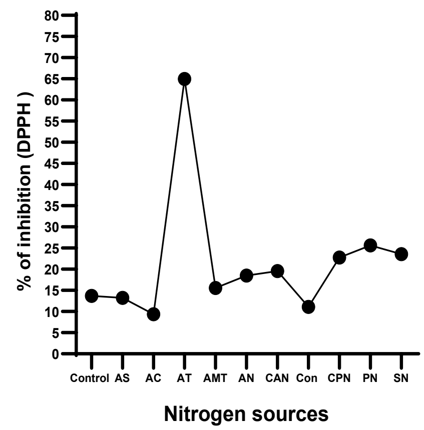

Fig. 5: b. Antioxidant profile of extracts of fungal culture grown in different nitrogen sources

followed by potassium nitrate & copper nitrate of fungal culture against *Fusarium spp*.

The antioxidant test results revealed that practically all extracts of different carbon and nitrogen sources of *Nectria pseudotrichia* displayed DPPH activity at varied percentages (Fig. 5.a and Fig. 5.b). In *Nectria pseudotrichia* a significant proportion of DPPH activity was observed. *Nectria pseudotrichia* showed the greatest percentage (%) of DPPH activity in aesculin (89.53%), raffinose (62.6%), and arabinose (30.44%) in case of carbon source and ammonium thiocyanate (64.95%), potassium nitrate (25.66%), and sodium nitrate (23.54%) in case of nitrogen source.

The data from the phytochemical test revealed that very low availability of secondary metabolites like Phenols, flavonoids, tannins, and saponins was observed in different carbon sources of *N. pseudotrichia*. Tannins and phenols are present in arabinose and aesculin, and flavonoids are present in arabinose, raffinose, galactose, and aesculin of *N. pseudotrichia* (Table 1). Table 2 showed that almost all methanolic extracts of different nitrogen sources were abundant in alkaloids, phenols, tannins, and saponins; in contrast, the extracts were poor in the availability of flavonoids, glycosides, and steroids. Alkaloids, phenols, tannins, and saponins are present in sodium nitrate, which is the best nitrogen source for *N.pseudotrichia*. The findings from the three bioactivity tests, namely antifungal, antioxidant, and phytochemical, revealed that *Nectria pseudotrichia* chose arabinose as a carbon source and sodium nitrate as a nitrogen source for improved secondary metabolite formation.

Results indicated that both the quantity and potency of bioactive metabolites varied depending on the medium composition, suggesting that specific carbon and nitrogen sources selectively influence metabolite synthesis. This differentiation supports that the observed antifungal activity is attributable to fungal metabolites rather than the growth

Table 1: Phytochemical profile of extracts of *N. pseudotrichia* grown with different carbon sources

Carbon sources	*Alkaloid*	*Phenol*	*Flavonoid*	*Tannin*	*Saponin*	*Glycosides*	*Steroids*
Control	-	+	-	++	-	-	-
Fructose	-	-	-	-	-	-	-
Arabinose	-	++	++	+	++	-	-
Raffinose	-	-	+	-	-	-	-
Galactose	-	-	+	-	-	-	-
Maltose	-	-	-	-	-	-	-
Xylose	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-
Mannitol		-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-
Aesculin	-	+++	+	+++	+	-	-
Starch	-	-	-	-	-	-	-

Abbreviations: +, present; ++ present significantly; +++, present in acess; - absent

Table 2: Phytochemical profile extracts of *N. pseudotrichia* grown with different Nitrogen sources

Nitrogen sources	*Alkaloid*	*Phenol*	*Flavonoid*	*Tannin*	*Saponin*	*Glycosides*	*Steroids*
Control	-	-	-	-	+	-	-
Ammonium Chloride	-	-	-	+	+	-	-
Ammonium sulphate	-	-	-	+	+	-	-
Ammonium thiocynate	-	+++	-	++	-	-	-
Ammonium mol Tetrahydrate	-	-	-	+	+	-	-
Ammonium nitrate	-	-	-	+	+	-	-
Calcium nitrate	-	-	-	+	+	-	-
Cobaltous nitrate	-	-	-	+	+	-	-
Copper nitrate	+++	-	-	+	+	-	-
Potassium nitrate	-	-	-	+	+	-	-
Sodium nitrate	++	+	-	+	+	-	-

Abbreviations: +, present; ++ present significantly; +++, present in access; - absent

medium alone. Our results align with the initial hypothesis that different carbon and nitrogen sources can modulate the production of bioactive metabolites. Specifically, extracts obtained from cultures grown on [arabinose-sodium nitrate active C/N sources] exhibited significantly higher antifungal activity compared to other conditions, indicating that nutrient composition directly influences metabolite synthesis. This supports the premise that optimizing growth conditions could enhance the yield of potential bioactive compounds, thereby validating the proposed hypothesis.

Discussions

The goal of the current study was to isolate and identify the fungal endophytes present in *Terminalia catappa* and to assess their bioactivity against *Fusarium spp.* Fungal endophytes produce a considerable range of biologically active metabolites with various bioactivities such as antioxidant, antifungal, and antibacterial (Toghueo, 2020). In the present study, methanolic extracts of isolated and identified endophytic fungi, *Nectria pseudotrichia* from *Terminalia catappa* were evaluated for different bioactive potentials like antifungal, antioxidant, and availability of various secondary metabolites. *Nectria pseudotrichia* showed good antifungal activity against three phytopathogens, such as *Fusarium proliferatum, Fusarium fujikuroi, and Fusarium oxysporom.* Fusarium diseases pose a serious threat to the production of food plants and are extremely challenging to manage (Arie, 2019). The pathogen *F. proliferatum* is polyphagous and can infect a variety of hosts.

A wide variety of toxins, including fumonisins (FB1, FB2, and FB3), beauvericin (BEA), moniliformin (MON), fusaric acid (FA), and fusaproliferin (FUP), can be harmful to both plants and animals and are produced by *F. proliferatum* (Proctor *et al.,* 2010). Oil palm vascular wilt is a destructive disease caused by *F. oxysporum* that can cause severe loss in some areas (Flood et al., 2006). However, it has been reported that a single plant pathogen, *F. fujikuroi*, caused rice bakanae disease in several rice species (Raghu *et al.,* 2018). We concentrated on examining the antifungal activity of methanolic extracts of various parameters of isolated endophytic fungi against three destructive pathogens by studying the pathogenicity of various *Fusarium spp.*

The work done on *Nectria spp.* demonstrate the antifungal potential, indicative of good secondary metabolite production. The existence of *Nectria pseudotrichia* and the study of its ability to fight fungal infections and produce secondary metabolites are supported by other scientists. One family, Nectriaceae, whose genus Nectria is well-known for dominant endophytes from various plant sections, may generate a variety of bioactive compounds to combat plant diseases. In addition to producing the cytotoxic Nectrianolins A, B, and C that are active against HeLa and HL60 cells, *Nectria pseudotrichia* from *Gliricidia sepium* and the endophytic fungus *N. pseudotrichia* from *Caesalpinia echinata* also include a variety of leishmanial chemicals (Ariefta *et al.,* 2017; Cota *et al.,* 2018) *Crinipellis perniciosa*is inhibited by the endophytic fungus *Nectria balsamea*, which has an antifungal effect against diseases such as *D. Quercus-Mongolia* and *Nectria haematococca* from cocoa plants (Rubini *et al.,* 2005; Nguyen *et al.,* 2024).

Ackah *et al.,* 2008 reported that Endophytic fungi, including *Aspergillus fumigatus, Candida albicans,* and *Trichophyton mentagrophytes*, were segregated from the bark sample of *T. catappa*, and the ethanolic extract exhibited good activity. For phytochemical, antibacterial, and antifungal activity, fruit, leaf, and bark extracts of *Terminalia spp.* were prepared using various solvents. The results unmistakably show the presence of secondary metabolites (Singh *et al.,* 2018). In the present investigation. we have used the leaf and bark sample of *Terminalia catappa*, isolated and identified *Nectria pseudotrichia*, and the methanolic extract of the fungi showed good antifungal and DPPH activity with different secondary metabolites like alkaloids, flavonoids, saponins, tannins, and phenols.

Free-radical scavenging compounds were also shown by fungal endophytes, and this is investigated through DPPH assay (Rout and Basak., 2012). Methanolic extracts of different carbon and nitrogen sources of *Nectria pseudotrichia* showed DPPH activity at varying percentages. Any compound's antioxidant properties are thought to be significantly affected by DPPH radical inhibition above 50% (Maheshwari *et al.,* 2014). Secondary metabolism may be affected by different growth parameters of the fungi. It may vary and be species-specific, also. Numerous other scientists' works have reported on the antifungal properties of various alcoholic extracts of *Terminalia spp.* (Parekh and Chandra., 2006; Shinde *et al.,* 2011; Javed *et al.,* 2016). *T. catappa* was evaluated for its antifungal activity against four pathogens and allergens. The methanol extracts of the plants revealed remarkable antifungal properties against all four fungi (Liu *et al.,* 2016). The expression of bioactivity in the current study through media optimization with varying C and N sources could help develop a biocontrol agent that fights three pathogenic fungi. N. *pseudotrichia*, endophytic fungi segregated from *T. catappa* are examples of bioactive metabolites that could be further investigated in detail for their extraction, purification, and molecular characterization. In this study, our hypothesis was that the bioactive potential of the fungal extracts would changes depending on growth conditions, particularly carbon and nitrogen sources. Our results demonstrate that different media compositions led to variations in antifungal property, indicating that metabolite production is modulated by nutrient availability. Although we focused on extract-level activity, these findings support the premise that specific metabolites contributing to bioactivity are differentially exhibited under varying growth conditions. Therefore, the observed differences in antifungal activity are consistent with our initial hypothesis regarding the bioactive potential of the fungus

Conclusion

The global challenge of antibiotic resistance underscores the urgent need for novel compounds with distinct bioactivities. Our study highlights the diverse bioactive potential and secondary metabolite production of the endophyte *Nectria pseudotrichia* isolated from *Terminalia spp.* By employing enhanced and modified culture media, we successfully elicited the production of bioactive compounds that warrant further characterization. These findings suggest that natural products derived from endophytic fungi could play a pivotal role in addressing antibiotic resistance while offering less toxic alternatives suitable for pharmaceutical research.

Moreover, the optimization of culture conditions and nutritional amendments emerges as a critical factor in industrial applications to achieve desired metabolite yields. This preliminary investigation provides a promising lead toward the development of antifungal agents targeting fusarial diseases in plants. Importantly, the metabolites identified may also hold potential against a broader spectrum of pathogens, though comprehensive studies are required to validate their efficacy and expand their applications.

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Author's Contribution

Ms. Debajani Samantaray has performed the experiment, organized the data, and prepared the manuscript. Dr. (Mrs.) Nibha Gupta has made a substantial contribution to the concept, designed the experiment, interpretation of data and involved in the supervision of the whole work

Conflicts of Interest

No conflicts of interest.

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