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Isolation, Molecular Identification and Bioactivity of *Aspergillus nomiae* Against Three *Fusarium spp.*

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Abstract

The world's population relies mostly on traditional medicinal herbs, utilizing their extracts or active ingredients. The goal of this work was to isolate and identify a fungal isolate using ITS sequencing, optimize the nutritional factors, and evaluate for phytochemical, antioxidant, and antifungal activity against three *Fusarium spp*. viz., *Fusarium proliferatum, Fusarium fujikuroi,* and *Fusarium oxysporum*. Nutritional changes at the carbon and nitrogen source levels, as well as culture conditions such as specific incubation periods, resulted in the production of a novel and modified medium in which our fungus demonstrated increased levels of various bioactivity. Phytochemical test of selected fungal endophyte showed the presence of various secondary metabolites. Antioxidant properties were shown in the fungal culture though differed as per culture condition and nutritional factors. The highest antifungal activity was seen in the 18-day-old *Aspergillus* nomiae culture and maltose-potassium nitrate is the chosen C and N sources. Achievement of this changed medium composition may be effective in eliciting the synthesis of secondary metabolites, useful for pharmaceutical research and antagonistic principle against plant pathogens.

Keywords: Endophytic fungi, *Terminalia spp.*, Carbon source, Nitrogen source, Antifungal, *Aspergillus nomiae*

Introduction

Endophytes are microorganisms present inside the host tissue and mimic similar metabolism without causing any disease or damage to the host (Stone et al., 2000; Soltani, 2017). Plant endophytes are a "gold mine" of bioactive chemicals with potential applications in agriculture, medicine, and the food business. These microbial chemicals have antibacterial, insecticidal, cytotoxic, and anticancer activities (Samantaray and Gupta, 2024). Metabolites isolated from endophytic fungi have been potentially used for human medicine in pharmaceutical research and one recent report has exhibited that 51% of bioactive secondary metabolites and substances isolated from endophytes (Schulz et al., 2002, Strobel and Daisy, 2003). Terpenoids, alkaloids, flavonoids, glycosides, and phenolics which act as an

important source of bioactive ingredients in modern medicine and pharmaceuticals are also obtained from fungal endophytes and these metabolites are not only used in agriculture but also used for coloring agents, flavouring agents, or texturizing agent, and in food industries (Seca and Pinto, 2019). Endophytic fungi like *Aspergillus spp., Penicillium spp., Nectria spp.*, are active against pathogens and produce various compounds having various bioactivity. Deoxypodophyllotoxin showing antimicrobial activity against bacterial pathogens obtained from *Aspergillus spp.* (Kusari et al., 2009). The above endophytic fungal strain known as a versatile producer of new bioactive metabolites like Asperfumoid (1) and Asperfumoid (2) (Liu et al., 2004), two new compounds, fumitremorgin 12-methoxy-13-[5'-hydroxy-2'-(1"- hydroxy-3"-methoxy-5"-methylbenzoyl)-3'-methoxy]benzoic acid methyl ester (fumitremorgin D, 1) and 4,8,10,14-tetramer thyl-6-acetoxy-14-[16-acetoxy-19-(20,21-dimethyl)-18-ene]-phenanthrene-1-ene-3,7-dione possess cytotoxic activity (Liang et al., 2015). Anticancer potential against breast cancer cell lines was shown in *Aspergillus nomiae* (Naser and Thoppil, 2023) and produced secondary metabolites having antifungal properties (Hatmaker et al., 2022). *A. nomiae* has the potential to be utilized as a biocontrol since it can kill a wide range of insect pests directly while also generating resistance to phytopathogens (Zhang et al., 2024). As antibiotic resistance is the most essential topic to work on, these secondary metabolites can substitute antibiotics to reduce the consumption of medications and be employed for infection treatment (Gangadevi and Muthumary, 2008).

The importance of ethnopharmacology is being acknowledged since the quest for possible therapeutic plants has been fruitful. Plants are always a good source of food, cosmetics, and especially drugs used by humans. Plants belonging to the Combretaceae family are very well known for their therapeutic potential (Mandloi et al., 2013). Members of the genus Terminalia are used to treat a variety of conditions, including cardiovascular diseases, wound healing, abdominal disorders, bacterial infections, colds, sore throats, conjunctivitis, ulcers, headaches, heart diseases, hypertension, jaundice, leprosy, pneumonia, skin diseases and other microbial diseases (Dwivedi, 2007; Li et al., 2011; McGaw et al., 2001; Eloff et al., 2008). *Terminalia arjuna* is known for its rich bark which possess phytochemicals like flavonoids, phenols, tannins, and glycoside-like metabolites used for the treatment of cardiovascular problems and showed antimicrobial, antioxidant, and anti-inflammatory activities. These characteristics make it an appealing option for developing innovative therapeutic treatments for cardiovascular disorders (Kumar et al., 2023).

Plants are susceptible to illnesses caused by both biotic and abiotic factors. The biotic causes are mostly pathogenic microorganisms like bacteria and fungi (Sabat and Gupta, 2009). Developing biocontrol agents is one way to combat these

infections. Microbes in nature can hinder or stop the growth of other organisms. Antibiotics are a significant and accurate form of antagonism. As far as antimicrobial activity is concerned, there are several reports available which are otherwise called antibiotics producing capacity of microbes (Debnath et al., 2013; Fyhrquist et al., 2004). Several studies have been carried out regarding extracellular metabolite production by considering the cultural and nutritional conditions (Kim et al., 2010; Kanari et al., 2002). Carbon and Nitrogen sources are very important components of media used for different bioactivity. Basal Sabauraud dextrose medium was modified to obtain better antifungal activity against *Fusarium spp*. by using different C and N sources and laboratory culture conditions. An attempt was also made to partially describe the bioactive metabolite(s) present in the fungal culture. *Fusarium spp.* are ubiquitous in the environment, and several strains that are harmful to plants or animals and produce mycotoxins have been described. *F. oxysporum* is typical of soilborne pathogens and lives in the soil for a long time in the form of chlamydospores, penetrates the roots, spreads throughout the tissues, colonizes and metastasizes in xylem vessels, and causes systemic yellowing, wilting, 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 spp.* 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 these above pathogens, we can develop various antifungal metabolites which is used in pharmaceutical research in the future. In the current study, the existence of endophytes was studied in several regions of *Terminalia spp.,* including the leaf and bark. The isolated fungal endophytes were characterized both morphologically and by ITS gene sequencing. Furthermore, the extract's biological activities were investigated.

Materials and Methods

1. Isolation & Identification of Fungal Endophytes

1.1.Isolation of Fungal Endophytes

The endophytic fungal strain was isolated from *Terminalia arjuna* grown on the campus of Regional Plant Resource Centre, Nayapalli, Bhubaneswar, Odisha. Leaf and bark samples were chopped into with sterile scalpels. Sample fragments 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 (Tejasvi et al. 2007). The fungi tested for antifungal activity against *Fusarium proliferatum, Fusarium fujikuroi*, and *Fusarium oxysporum* were obtained from culture

collection of Microbiology Laboratory, Regional Plant Resource Centre, Bhubaneswar.

1.2. Molecular Identification of selected fungi

The selected fungal cultures were identified through molecular identification method in collaboration with MTCC, CSIR-IMTECH, Chandigarh. Phylogenetic analyses were performed using the closely related ITS-type sequences of Aspergillus species downloaded from GenBank. At the MAFFT server (http://mafft.cbrc.jp/alignment/server/), multiple sequence alignments for the ITS gene were performed online (Katoh et al., 2019). Alignments were then manually corrected using BioEdit (Hall, 1999). The phylogenetic analyses include the AiTS region dataset, composed of 47 taxa belonging to Aspergillus with *Penicillium* chrysogenum*as* an outgroup taxon. Maximum parsimony analysis of the ITS dataset showed that our taxon *Aspergillus nomiae* T1F5 grouped within the *Aspergillus nomiae* clade The evolutionary history was inferred using the Maximum Parsimony method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei and Kumar, 2000) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates).

2. Effect of Nutritional factors like different Carbon sources of selected fungal isolates

During the screening program, the sabouraud dextrose medium was found to be the best medium for higher antimicrobial activity. The best period for optimum growth of *Aspergillus nomiae* is 18 days of the incubation period. Hence, the sabouraud dextrose agar medium and preferred culture condition were taken as the basal medium for the modification of nutritional factors. 12 different carbon sources (2%) taken were fructose, arabinose, raffinose, galactose, maltose, xylose, sucrose, lactose, mannitol, inositol, aesculin, and starch. In each case, the carbon source was taken in 2% w/v and added to the basal SD medium in place of dextrose (Sabat and Gupta, 2009: Behera and Gupta., 2019). The fungal culture was grown in sabouraud dextrose broth medium to determine the best carbon source for optimum growth. The culture filtrate was filtered, and concentrated by the Soxhlet apparatus and ethyl acetate was added to the concentrated filtrate for 72 hours. The upper layer was separated and evaporated by Soxhlet. Evaporated samples were dissolved in methanol and preparation of methanolic extract was completed. Methanolic extract of the selected fungi was screened for antimicrobial, phytochemical, and antioxidant activity to know the best carbon source for eliciting production of secondary metabolite (Lahouar et al., 2016; Patro and Gupta, 2022).

Antimicrobial activity

A methanolic extract of *Aspergillus nomiae* cultured on distinct carbon sources was tested for antifungal efficacy against *Fusarium proliferatum*, *Fusarium fujikuroi*, and *Fusarium oxysporum*. The percentage (%) of growth decrease was estimated using morphological growth from the solid plate culture method.

Phytochemical profiling

Secondary metabolites, alkaloids, flavonoids, phenols, tannins, saponins, glycosides, and steroids were detected in the methanolic extracts of fungal endophyte cultured in distinct C-sources (Shivaputrappa and Vidyasagar, 2018).

Antioxidant activity test

The radical scavenging action of the stable DPPH free radical was measured in the methanolic extract of fungal endophyte cultured under different incubation conditions using the methodology (Brand-Williams, 1995). DPPH solution (0.006% w/v) was produced in 95% methanol. Methanol extracts (1 ml) were combined with 2 ml of DPPH solution to make a final volume of 3 ml, and discoloration was detected at 517 nm with a UV-Vis Spectrophotometer after 30 minutes of incubation in the dark. In the case of control, methanol was used instead of the sample. The percentage (%) of inhibition was estimated (Rout and Basak, 2012; Nayak and Basak, 2015).

Percentage (%) of inhibition= [(Blank absorbance-Sample absorbance)/ Blank absorbance]×100

3. Effect of Nutritional factors like different Nitrogen sources of selected fungal isolate

10 different nitrogen sources taken 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, the nitrogen source was taken in 2% w/v and added to the basal SD medium in place of peptone (Sabat and Gupta, 2010). The cultures were grown in sabouraud dextrose broth medium to determine the best nitrogen source for optimum growth. Solvent extraction was done and antimicrobial, phytochemical, and antioxidant activity of the methanolic extract was completed to know the best nitrogen source for eliciting production of secondary metabolite as described earlier.

Results

The current study was carried out to isolate and identify the endophytic fungi from different plant parts of *Terminalia arjuna*. The effect of nutritional factors on antifungal activity, phytochemical test, and antioxidant test has given some nice results to explicate its preference for production of secondary metabolites.

Molecular identification of fungi

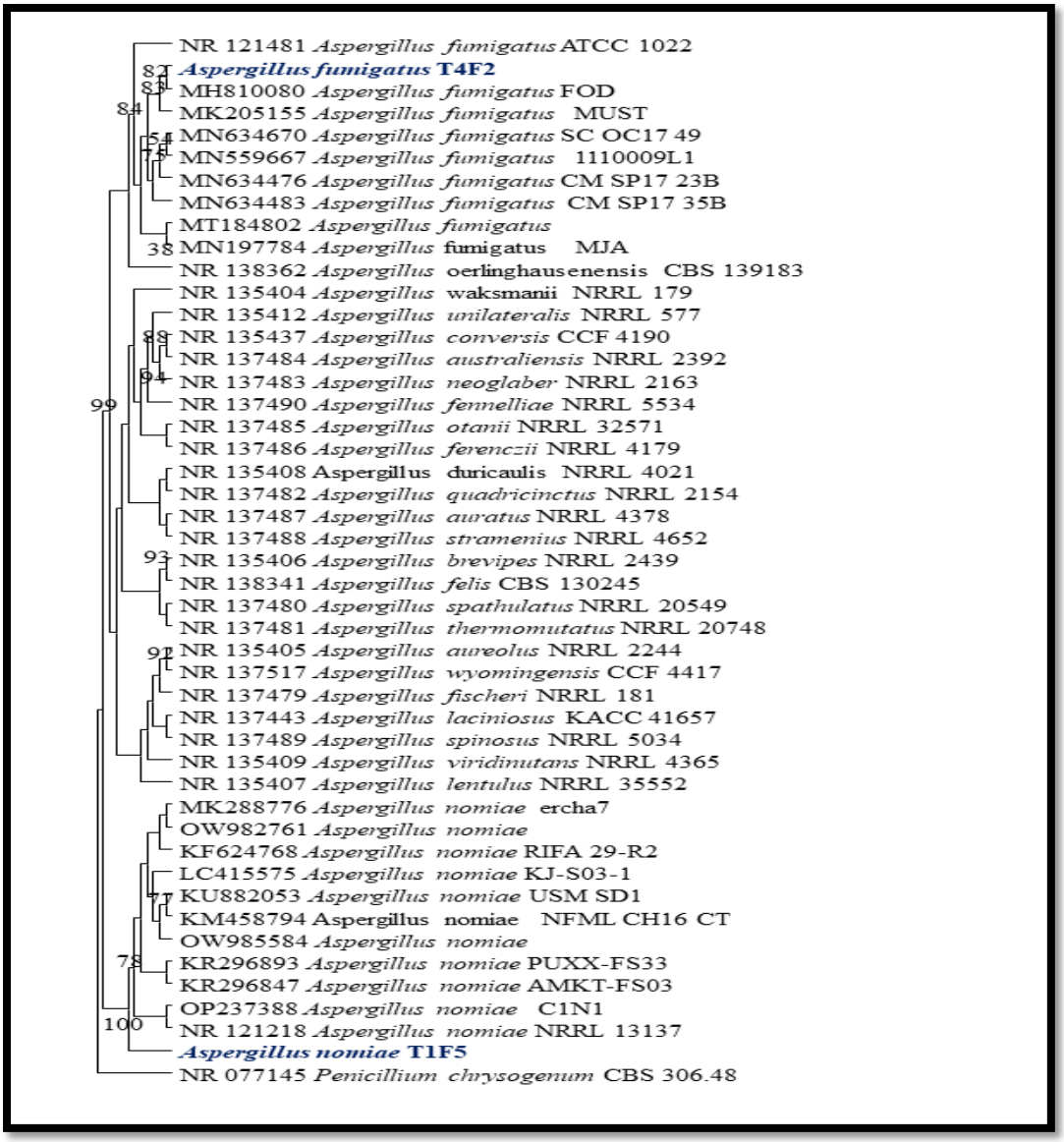
This analysis involved 47 nucleotide sequences for *Aspergillus nomiae*. There were 662 positions in the final dataset for *Aspergillus nomiae*. Evolutionary analyses were conducted in MEGA11(Tamura et al., 2021).

>*Aspergillus nomiae*T1F5

CCGTAGGCGAAACATCATGTGCTTAAATTCAGCGGGTATCCCTTCTTGGTCCGAG
GTCCACCTCCACCNGAGTTTTTGTTNCCTTTCTGCTTGGGCGGGCCGGCCGCAC
GGCGGCCGGGGGGCATCCGCCCCCGGGCCCGCGCCCGCCGGAGACACCAC
GAACTCTGAACGATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACT
TTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGAT
AACTAGTGTGAATTGCAGAATTCCGTGAATCATCGAGTCTTTGAACGCACATTGC
GCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCA
AGCACGGCTTGTGTGTTGGGTCGTCGTCCCCTCCTCCGGGGGGGGACGGGCCC
TAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCAC
CCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAAACAACCATTCTTTCC
CAGGTTGACCTCGGATCAGGAGG

(Fig 1- Phylogenetic analysis of fungal taxa by using ITS sequencing)

Tree #1 out of 2 most parsimonious trees (length = 262) is shown. The consistency index is 0.797710 (0.708791), the retention index is 0.939359 (0.939359), and the composite index is 0.749336 (0.665810)and for Nectria Tree #1 out of 10 most parsimonious trees (length = 666) is shown. 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).



(Fig 2- Phylogenetic tree generated from a maximum parsimony analysis based on ITS, sequences of species of Aspergillus. The tree was rooted to *Penicillium chrysogenum*CBS 306.48. Values above the branches represent parsimony bootstrap support values (> 50%).

Extraction of Secondary Metabolites & evaluation for Antifungal Activity using different carbon sources

The ethyl acetate extracts of fungal metabolites grown in different carbon sources were evaluated for antifungal properties. Observations recorded for a percentage of growth reduction of test *Fusarium spp.* by *Aspergillus nomiae* are presented in Figures 3.1, 3.2, and 3.3. Growth of *F.proliferatum* and *F. fujikuroi* were inhibited by extracts of maltose of *Aspergillus nomiae* (20.13% & 31.76%). The growth inhibition potential of *Aspergillus nomiae* was less (0.93%) in maltose against *F.oxysporom.* The

growth inhibition potential of *Aspergillus nomiae* was higher in maltose, xylose, and raffinose against *F. proliferatum*, higher in maltose and aesculin in the case of *F. fujikuroi* whereas it was gradually lesser in the case of *F.oxysporom.*

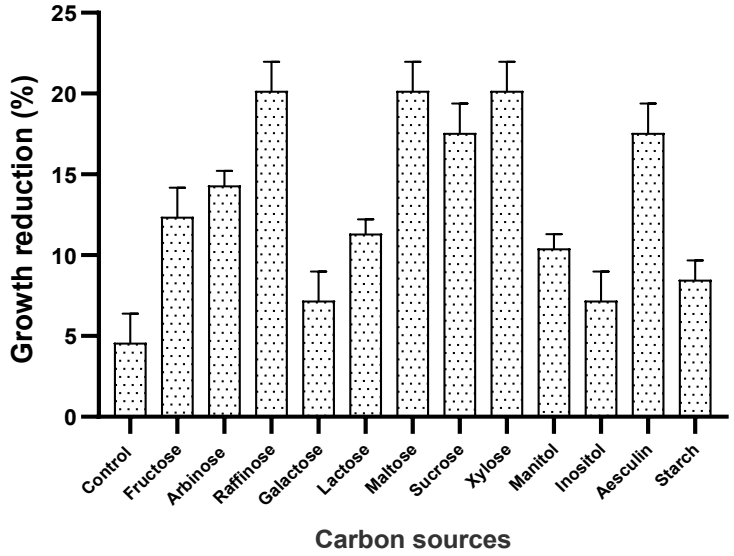


Fig 3.1- Effect of carbon sources on antifungal activity of *A.nomiae* against *Fusarium proliferatum*

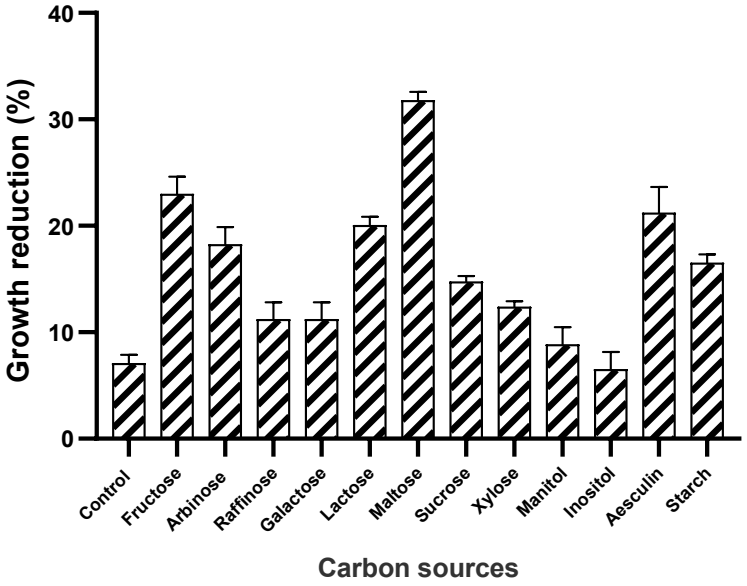


Fig 3.2- Effect of carbon sources on antifungal activity of *A.nomiae* against *Fusarium fujikuroi*

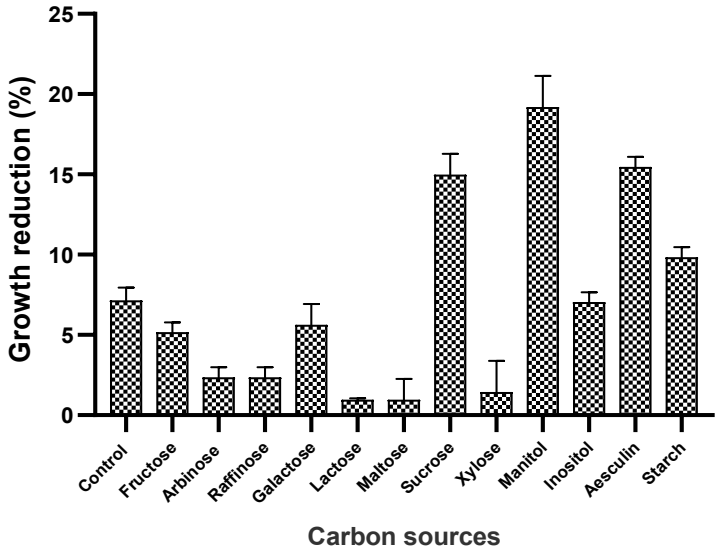


Fig 3.3- Effect of carbon sources on antifungal activity of *A.nomiae* against *Fusarium oxysporom*

The antioxidant test results revealed that practically all extracts of *Aspergillus nomiae* displayed DPPH activity at varied percentages (-10.7- 87.45) (Figure 4). In the case of *Aspergillus nomiae* highest percentage of DPPH activity was observed in lactose followed by maltose and aesculin,i.e., 87.45,80.26 & 79.03 (Figure 3). A very weak percentage of DPPH activity was observed in fructose (-10.7%).

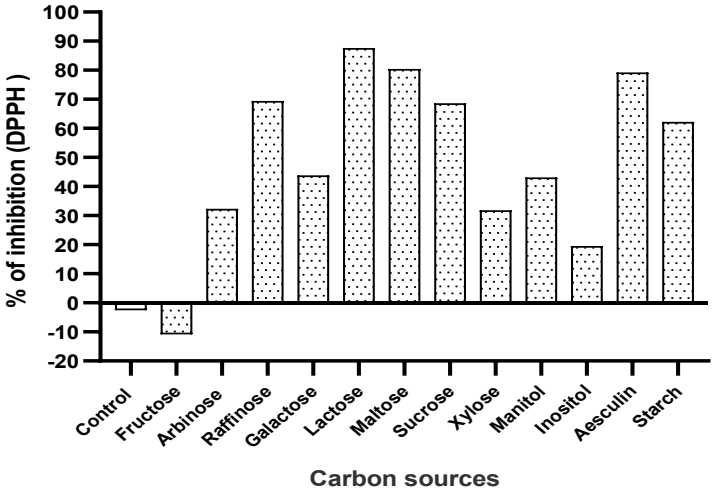


Fig. 4- Antioxidant profile of extracts of *Aspergillus nomiae* grown in different carbon sources

The data revealed that almost all methanolic extracts of different carbon sources were rich in alkaloids, phenols, flavonoids, tannins, and saponins, in contrast, the extracts are poor in the presence of glycosides and steroids (Table 1). Methanolic extract of *A. nomiae* showed a good presence of alkaloids, phenols, and tannins and very little availability in saponins, steroids & glycosides. Phenols, flavonoids, and tannins are very effectively present in the case of *A.nomiae*.

Table -1- Phytochemical profile extracts of *A. nomiae* 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

From above three bioactivity test, i.e., antifungal, antioxidant and Phytochemical test, the data showed the *Aspergillus nomiae* preferred maltose as Carbon source for better production of secondary metabolite.

Extraction of Secondary Metabolites & evaluation for Antifungal Activity using different nitrogen sources

The ethyl acetate extracts of fungal metabolites grown in different nitrogen sources were evaluated for antifungal properties. Observations recorded for % growth inhibition of test *Fusarium spp.* by *Aspergillus nomiae* are presented below. The data revealed from the result (Figure 5.1, 5.2 & 5.3) that among the 10 tested nitrogen sources of selected endophytic fungi *Aspergillus nomiae*, the best growth decrease was observed in potassium nitrate. The highest percentage of growth reduction was observed in Potassium nitrate (14.37%, 13.11%, 12.44%) against *F.proliferatum*, *F.*

fujikuroi, and *F.oxysporom* followed by sodium nitrate, calcium nitrate. Data on the percentage growth reduction of *Fusarium spp*. by *A.nomiae* extracts revealed that these fungi exhibit antifungal activity against three Fusarium species examined. The presence of bioactive metabolites differed depending on the nutritional conditions.

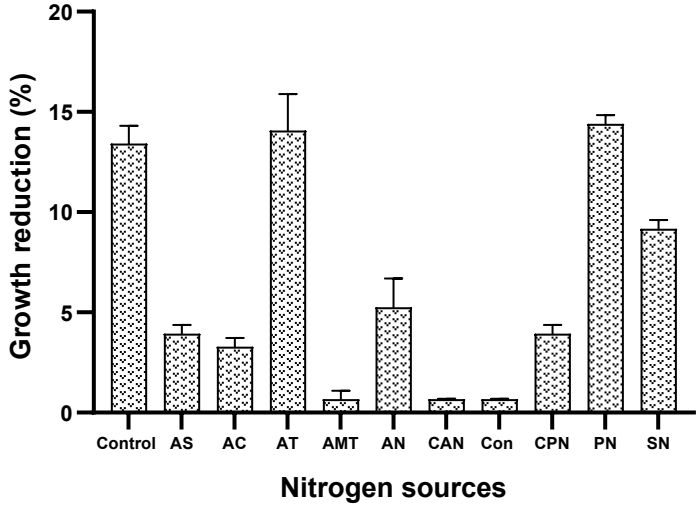


Fig. 5.1- Effect of Nitrogen sources on antifungal activity of *A.nomiae* against *F.proliferatum*

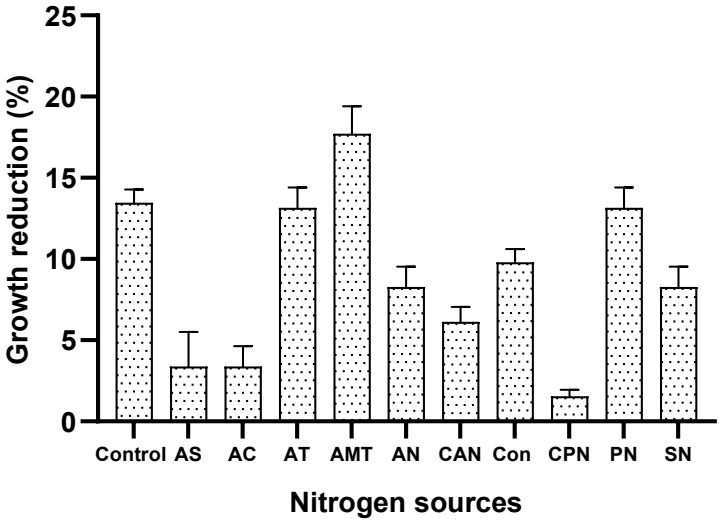


Fig. 5.2- Effect of Nitrogen sources on antifungal activity of *A.nomiae* against *F.fujikuroi*

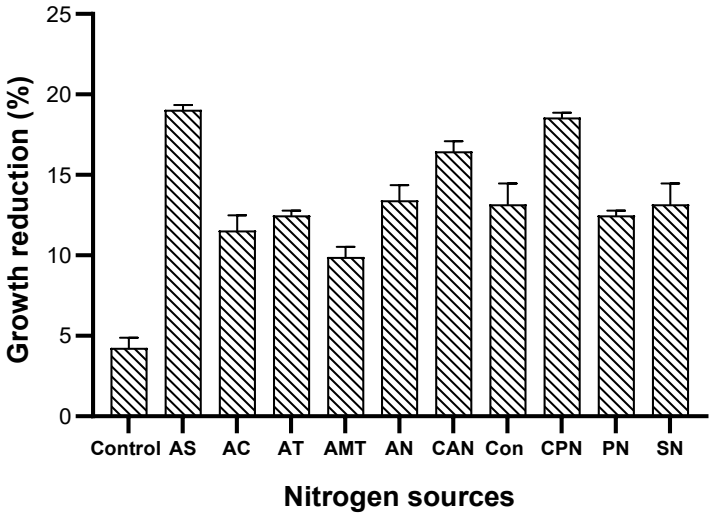


Fig. 5.3- Effect of Nitrogen sources on antifungal activity of *A.nomiae* against *F.oxysporom*

The data exhibited that methanolic extracts of different nitrogen sources of selected fungi named *Aspergillus nomiae* showed DPPH activity at varying percentages (-18.39 to 31.48). In the case of *A.nomiae* highest percentage of DPPH activity was observed in sodium nitrate (31.48) followed by calcium nitrate (28.49) (Figure 6). A very weak percentage of DPPH activity is shown in cobaltous nitrate presented below.

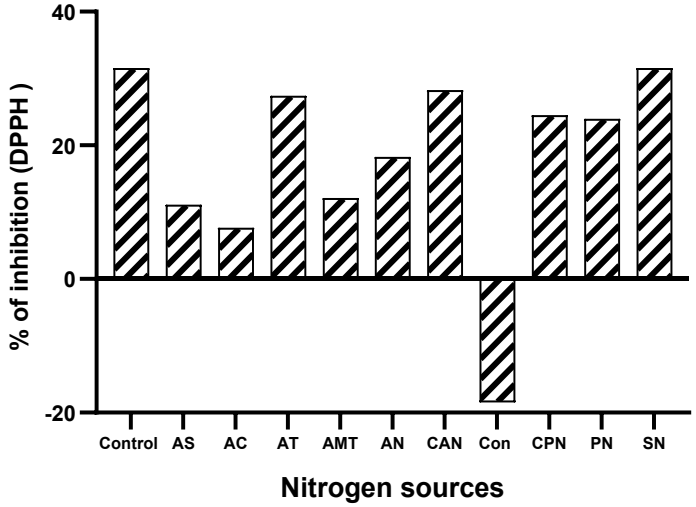


Fig. 6- Antioxidant profile of extracts of *Aspergillus nomiae* grown in different Nitrogen sources

The data revealed that almost all methanolic extracts of different nitrogen sources were rich in alkaloids, phenols, flavonoids, tannins, and saponins, in contrast, the extracts are poor in the presence of glycosides and steroids. The methanolic extract of *A. nomiae* included alkaloids, phenols, tannins, saponins, glycosides, and steroids. *A.nomiae* has a high concentration of phenols and saponins, as well as fewer glycosides and steroids (Table 2).

Table -2 Phytochemical profile extracts of *Aspergillus nomiae* 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 acess; - absent

From the above three bioactivity tests, the data showed that *Aspergillus nomiae* preferred potassium nitrate as a source of N-source for giving the highest percentage of growth and eliciting production of secondary metabolite.

Discussions

The present study focused on the evaluation of nutritional amendments for *Aspergillus nomiae* isolated from *Terminalia arjuna*. Medicinal plants are important because they offer natural, cost-effective, and often safer alternatives to manufactured medications. The genus Terminalia has roughly 200-250 species that are widely utilized in traditional medicine across the world and also known for anti-parasitic, anti-infectious, anti-diabetic, anti-hypertensive, anti-oxidant, anti-dermatophytic properties (Bognan et al., 2016; Bhattacharyya and Jha, 2011). Terminalia arjuna (Arjuna) is one such plant that has been venerated in traditional

medicine for its multiple health advantages mainly used for cardiovascular diseases (Singh and Kumar, 2024). Its cardioprotective qualities are thought to strengthen the muscles of the heart, control blood pressure, and enhance cardiac function (Hasan, 2023). Many endophytes reside in different parts of the host plant without causing any harm to the host, The majority of the fungal species were discovered from Terminalia species belonging to the Ascomycota (Felber et al., 2016). Various secondary metabolites are present in *Terminalia arjuna* and possess different bioactivity. Triterpenoids showed anti-inflammatory, and cardioprotective activity, Flavonoids showed antioxidant and vasodilatory activity, and Glycosides showed Cardio-tonic properties and regulation of cardiac rhythm (Hasan, 2023). In the case of the present study, we have used the different plant parts of *Terminalia arjuna* and isolated *Aspergillus nomiae*, and the methanolic extract of the fungi showed good bioactivity with different secondary metabolites like alkaloids, flavonoids, saponins, tannins, glycosides, phenols & steroids.

The presence of *Aspergillus nomiae* its antifungal potential and its production of metabolites are corroborated with other scientists. *Aspergillus spp*. is an endophyte and fungi present in one host plant may be a pathogen or an endophyte depending on the balance between endophytisim and pathogenicity of micro-organisms of the different plants (Liu et al., 2004). One report gives an idea about the isolation and identification of alkaloid compounds by Aspergillus from the medicinal plant Bauhinia guianensis showed antibacterial activity and the identified compound like Fumigaclavine C showing, anticolitis, anti-inflammatory, hepatoprotective activity (Pinheiro et al., 2013; Guo et al., 2015; Du et al., 2011). *Aspergillus spp.* from *Juniperus communis*L. Horstmann is a novel source of anticancer compound, deoxypodophyllotoxin (Kusari et al., 2009).

Inhibition of DPPH radicals above 50 % is considered to be significant for the antioxidant properties of any compound (Ramya, 2008). The availability of antifungal activity of different alcoholic extracts of *Terminalia spp.* has been reported in the work of many other scientists (Elizabeth, 2005; Parekh and Chanda, 2006; Shinde et al., 2011). Endophytes reside in the host and mimic the same metabolism as the host does. Present study indicates of such happening as endophytic fungi like *Aspergillus sp.* and *Curvularia sp*. more active against bacteria like *H.influenza* and *E.coli* and produce various antimicrobial compounds (Liu et al.,2016; Mbekou et al., 2021) and Aspergillus genus is known for the excellent producers of cytotoxic metabolites and showed various bioactivity (Wang et al., 2007; Maheswari et al., 2014). The modification needed for the enhanced production of secondary metabolites having antifungal activity against three *Fusarium spp.* Fusarium is a huge genus, several species are into pathogenicity to plants and animals. *F.oxysporom*, *F. fujikuroi*, *F. solani*, *F. proliferatum*, and *F.graminearum* are the plant pathogens reported in one literature (Arie, 2019). *F.oxysporom* is reported as an airborne fungi as a pathogen in

humans and a soilborne pathogen in plants (Y Emoto, 1972; Dignani and Anaissiae, 2004). Vascular wilt is a destructive disease that occurs in oil palm and causes severe loss in some areas by *F.oxysporom* (Flood, 2006). Another disease in plants, i.e., dry rot which is a postharvest disease occurred in garlic crops causing yield losses worldwide by *F. proliferatum* (Galvez and Palmero, 2022). One of the most staple foods in India is Rice. As the world population increases, we need to produce 50% more food grains to fulfill the requirement (Yadav, 2017), but one plant pathogen named *F.fujikuroi* caused rice bakanae disease in different rice species reported mostly in various districts of Odisha mostly in Cuttack, Sambalpur, Bargarh, Ganjam and Jajpur (Raghu et al., 2018) and also affected maize and soybean by producing mycotoxins (Qiu et al., 2020). By understanding the pathogenicity of different *Fusarium spp.,* we focused on the antifungal activity test of methanolic extract of different parameters of isolated endophytic fungi against three destructive pathogens such as *F.proliferatum, F.oxysporom, F.fujikuroi*.

Conclusion

The main issue is antibiotic resistance and to solve the problem it's an urge to discover new substances having different bioactivity. The present research focused on the potential of endophytes from *Terminalia spp*. to produce secondary metabolites that have different bioactivity. Enhancement of new and modified medium elicits the production of secondary metabolites which could be further characterized and it is believed that bioactive compounds from endophytic fungi may be a way to eliminate the issue of resistance and fulfill the demand for less toxic antibiotics effective for pharmaceutical research and secondly, the optimization of culture condition and nutritional amendments is the important factor in essential industrial process to achieve the desired product.

Conflicts of interest: The authors declare that there are no conflicts of interest.

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