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In vitro Antagonistic Assessment of Endophytic Fungi Associated with *Prunus napaulensis* (Ser.) Steud. against Fungal Phytopathogens

Asha Kiran Lakra* and Highland Kayang

Microbial Ecology Laboratory, Department of Botany, North-Eastern Hill University, Shillong, Meghalaya, India.

Abstract

Endophytic microorganisms reside within the plant tissues in a symbiotic association and generally refer to fungi and bacteria. Endophytes benefit the host plants in several ways. They are known to protect the plants from harsh environmental conditions, produce variety of compounds which boost the growth of the plants, render protection against herbivores and also serve as potent biocontrol agents. Screening of fungal species for implementation on such fields has been the area of interest. The current study aims to isolate fungal endophytes from *P. napaulensis* and screen for its antagonistic efficiency against common wide host range fungal pathogens via extracellular enzymatic activities and dual culture methods. Thirteen fungal endophytes were isolated which showed varying range of extracellular enzymes production and growth inhibition of the fungal pathogens. The study highlights potent fungal isolates, which can be implemented in bio-agriculture (*Trichoderma atroviride*, *T. koningiopsis* and *Xylaria feejeensis*) and also for mass and sustainable production of hydrolytic enzymes such as cellulase and amylase and which have now gained interest in industrial sectors (*Chaetomium globosum*, *Colletotrichum gloeosporioides* and *Fusarium graminearum*).

Keywords: *Prunus napaulensis*, Fungal Endophytes, Antagonists, Extracellular Enzymatic Activities

***Correspondence:** asha.lakra93@gmail.com

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INTRODUCTION

Endophytes, generally referred to fungi and bacteria growing within the plant tissues in symbiotic association, have been characterized as defenders of the host plants against the attack of other microorganisms, insects and herbivores.[1] Besides, they also produce phytohormones, enzymes and other bioactive compounds which are of advantages to the host plant.[2] Endophytic fungi have pulled great attention in the recent past due to its efficiency in producing novel secondary metabolites that can be exploited in various sectors such as agriculture, varied industries and medicines.[1] The implementation of biocontrols in agriculture have also gained parallel interest. Unlike application of synthetic chemicals, biocontrol agents offer a more sustainable approach by reducing reliance on chemicals which not only deteriorates the environment but also pose ill impact on humans and animals.[3,4] Antagonists significantly combat the population of fungal pathogen residing on the aerial parts of the host plant and decrease the intensity of diseases.[5,6] Recent researches have been focused on isolation of potent endophytic antagonists. Antagonists exhibits strong colonization ability and have superior environmental adaptability.[7] Fungal biocontrol agents have gained popularity alongside bacteria (particularly *Bacillus thuringiensis*), primarily because of its effectiveness as disease control agent against broad spectrum of pathogens.[8,9] Besides, extracellular hydrolytic enzymes are also known to be secreted by these fungi for instance, amylase, cellulase, protease, catalase, etc, which assist the antagonists in combating growth and effect of pathogens.

Prunus napaulensis (Ser.) Steud. (syn. *Prunus nepalensis* L.), a wild indigenous fruit tree growing in the temperate Himalayan regions of India at altitudes between 1200-3000 m, is popular for its high nutritional and medicinal attributes. It is also considered to have potent antidiabetic and anticancer properties.[10-12] In Meghalaya the plant is distributed in the Khasi and Jaintia Hills districts, and is locally known as Sohiong. This study provides input on fungal endophytes isolated from *P. napaulensis,* their potency to produce various extracellular enzymes and antifungal efficacy against wide host range pathogens.

MATERIALS AND METHODS

Study area and collection of samples

Fresh and healthy leaves, twigs, roots and barks of *P. napaulensis* were collected from Shangpung (Lat. 25.47816°, Long. 92.35328°) West Jaintia Hills, Meghalaya, India. The samples were transferred in sterilized plastic zip bags and brought to laboratory to carry out further studies.

Isolation of fungal endophytes and maintenance of pure culture

The plant samples (leaves, twigs, roots and barks) were washed clean with running water in order to remove any unwanted entities which might have adhered to the surface of the samples. Surface sterilization was done by subjecting the plant samples to 70% ethanol (Analytical AR Grade Ethanol Absolute) for 2-3 minutes, followed by 1% sodium hypochlorite (HiMedia) for 1-2 minutes and 2-3 times washing with sterile distilled water.[13] About 1 cm of the explants were cut from the sterilized samples, air dried and inoculated in potato dextrose agar (HiMedia) plates containing 100 mg/L of streptomycin sulphate (HiMedia). The plates were incubated for 10 days at 28 ± 2 °C and the pure cultures for the obtained endophytic fungal isolates were maintained following the same procedure.[14]

Identification of fungal endophytes

The isolated fungal endophytes were identified morphologically and microscopically based on their vegetative and reproductive structures using standard manuals.[15,16] For molecular characterization the fungal isolates were cultured in potato dextrose broth (HiMedia) for 10 days and were then used for identification by adapting the procedures given by National Bureau of Agriculturally Important Microorganisms (NBAIM) by analysing the Internal transcribed Spacer (ITS) region. The amplification of locus analysed (ITS) was performed using universal primers for the ITS region, ITS 1 and ITS 4.[17] The obtained PCR products were purified and

sequenced at Eurofins Genomics India Pvt. Ltd., Bengaluru, Karnataka, India. Analysis of the sequences were carried out using BLAST program from GenBank database (NCBI), sequence with highest similarity was downloaded. Sequences were aligned using MEGA 11 software and using Clustal W alignment program. The resulting sequences were deposited to the GenBank database and the accession numbers were acquired.

Analysis of enzymatic assay

Amylase test

Qualitative analysis for amylase was done by inoculating isolates in starch agar (HiMedia) plates and incubated at 28 ± 2 °C for 10 days. Following incubation, the plates were flooded with 0.5% iodine solution for a minute. Occurrence of clear zones around the colonies depicted positivity for amylase production.[18] Quantification of amylase was done by using the filtrates of 10 days old fungal broth grown in potato dextrose broth (PDB) containing 1% starch. A reaction mixture comprising of 1 ml of filtrate (enzyme broth) and 0.5 ml of soluble starch (1%) was incubated for 30 minutes at 37 °C and then 0.5 ml Dinitrosalicylic acid (DNS) was added. The solution was diluted to 5 ml by adding distilled water. The optical density (OD) of the developed reddish brown colour was recorded at 540 nm using UV/Vis Spectrophotometer (Lambda 35, Perkin Elmer, USA). Readings were taken in triplicate and quantity of sugar produced was read off by considering the standard curve of maltose. The result was expressed in unit per ml (Uml^{-1}), where one unit of amylase is the amount of enzyme releasing a µmol of reducing sugar, expressed as maltose, per min, per ml, under given condition.[19]

Cellulase test

Qualitative analysis for cellulase was determined by inoculating fungal isolates in PDA plates amended with 1% carboxymethylcellulose (CMC) and incubating for 10 days at 28 ± 2 °C. Following incubation, plates were flooded with congo red solution (0.1%) for 15 minutes, the excess stain was washed with 1M NaCl solution for 10 minutes.[20] Occurrence of clear zones around the colonies indicated positive activity for cellulase. Quantification of Endo-β-1,4-glucanase (Carboxymethyl cellulase) was done by centrifuging 10 days grown PDB cultures of endophytic fungi at 10,000 rpm (Eppendorf, Centrifuge 5430 R) for 15 minutes. The reaction mixture comprised of 1 ml supernatant and 1 ml of 1% CMC in 0.05 M sodium citrate buffer with pH 4.8. The mixture was incubated at 50 °C for an hour and the 3 ml of alkaline DNS reagent was added to halt the reaction. Absorbance was measured at 540 nm and readings were taken in triplicates. The result was expressed in Unit per ml (Uml^{-1}), where one unit of enzyme (CMC-ase) is the amount of enzyme releasing a µmol of reducing sugars, expressed as glucose, per min, per ml, under given condition.[19]

Protease test

Protease activity was screened by inoculating fungal isolates in skim milk agar plates followed by incubating for 10 days at 28 ± 2 °C.[21] Occurrence of clear zones around the colonies specified positive activity of protease. Protease quantification was done by centrifuging the fungal cultures grown in PDB amended with 1% casein (10 days) at 10,000 rpm (using Eppendorf, Centrifuge 5430R) for 15 minutes. The reaction mixture comprised of 1 ml of the supernatant and 5 ml of 0.5 N NaOH. The mixture was mixed vortex and 1.5 ml of Folin-Ciocalteu reagent (diluted to 1:1 v/v) was added and incubated for 30 minutes in the dark at room temperature. Development of blue colour indicated protease activity and absorbance was measured at 660 nm using tyrosine standard. The result was expressed in unit per ml (Uml^{-1}), where one unit of protease is the amount of enzyme releasing µmol of tyrosine per ml per min under the given condition.[22]

Catalase test

Catalase activity was determined by mixing small inoculum of the isolates with 3% hydrogen peroxide solution. Intensity of bubble evolution indicated the catalase producing potential of the fungi: (-) no activity, (+) low activity, i.e. few bubbles, (++) moderate activity, (+++) high activity, i.e. abundant bubbles.[23]

Hydrogen cyanide (HCN) production test

Production of HCN was detected by inoculating isolates onto PDA plates. The media used for the test contained 0.4% L-Glycine. Thin

Whatman filter paper strips were soaked in a solution of 2% Na_2CO_3 and 0.5% picrate. Single strips were adhered to the lids of petri dish and secured using parafilm. The plates were incubated for 10 days at 28 ± 2 °C. Any alteration in the strips colour after the incubation period indicated the evolution of HCN.[24]

Urease test

Urease test was done by inoculating fungal isolates in plates containing Christensen's urea agar (HiMedia) and incubating for 10 days at 28 ± 2 °C. Change in the colour medium denoted the urease activity: no change in colour implies negative (-), orange colour means weak activity (+), pink colour means moderate activity (++) and red colour means strong activity (+++).[23]

Isolation, identification and culture of fungal phytopathogens

Fungal phytopathogens were isolated from diseased parts of *Sechium edule* (leaf), *Solanum tuberosum* (leaf) and *Mangifera indica* (fruit pericarp and leaf), and identified following the methods as described above for the endophytic fungi.

Assessment of antagonistic potential of endophytic fungi in in vitro condition

The in vitro assessment of antagonistic potential of the isolated endophytic fungi was carried out by dual culture technique. A 6 mm mycelial disc of fungal pathogens and fungal endophytes obtained from 4 days old cultures were placed opposite to each other on PDA plates, secured with parafilm and incubated for 10 days at 28 ± 2 °C.[25] Percentage of growth inhibition was determined using the formula:

$$\text{Growth Inhibition \%} = R1\text{-}R2 / R1 \times 100$$

Where, R1 indicates the radial growth of the pathogen's control colony and R2 indicates the radial growth of the pathogen in a treated colony.

Determination of the zone of interaction between the fungal endophytes and pathogens' colonies

The interaction zone between the fungal endophytes and pathogens in dual culture were studied using Scanning Electron Microscopy (SEM) in Sophisticated Analytical Instrument Facility, North-Eastern Hill University, Shillong, India. Samples were prepared based on the method given by NEHU-SAIF. Mycelial cubes of 0.5 cm were cut using sterilized blade at the interaction

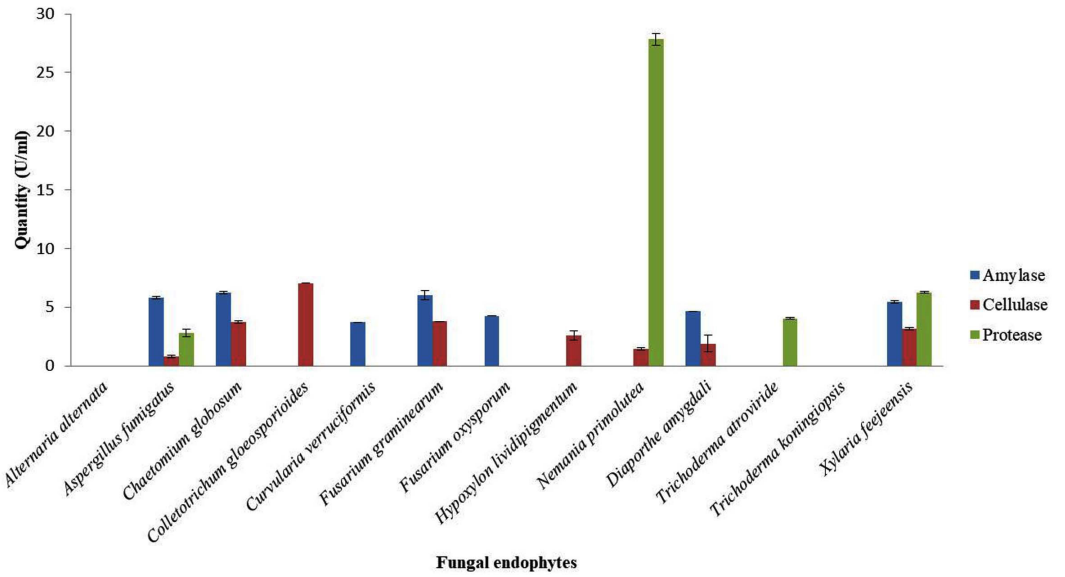

Figure 1. Graphical presentation of quantitative analysis of extracellular enzymes produced by the isolated fungal endophytes

Table 1. Species identification of fungal endophytes isolated from *P. napaulensis*

No.	Fungal endophytes	Accession No.
1	*Alternaria alternata*	PP855860
2	*Aspergillus fumigatus*	PP856661
3	*Chaetomium globosum*	PP855861
4	*Colletotrichum gloeosporioides*	PP856664
5	*Curvularia verruciformis*	PP855862
6	*Fusarium graminearum*	PP856667
7	*Fusarium oxysporum*	PP856665
8	*Hypoxylon lividipigmentum*	PP856668
9	*Nemania primolutea*	PP855863
10	*Diaporthe amygdali*	PP856669
11	*Trichoderma atroviride*	PP856670
12	*Trichoderma koningiopsis*	PP855865
13	*Xylaria feejeensis*	PP855866

zone in a dual culture plate. The samples were fixed with 3% Glutaraldehyde for 2 hours and then immersed in 0.1 M Sodium Cacodylate buffer for 15 minutes at 4 °C to wash off the excess fixative (3 changes for 15 minutes each). The samples were then subjected to gradient dehydration using acetone (30%-100%) and followed by immersion in Tetramethylsilane for 10-15 minutes at 4 °C. The samples were oven dried at 26 °C to remove the moisture. The dried samples were mounted on SEM stubs, gold sputter coated and observed under SEM (JEOL JSM-6360 SEM) at 20 kV.

Data analysis

The data analysis (analysis of variance and Tukey's HSD test at $P \leq 0.05$) were done using SPSS, v.25 (Chicago, IL, USA). Evaluation of various extracellular enzymatic activities and antagonistic potential of the isolated endophytic fungi were done in triplicates.

RESULTS

Identification of endophytic fungi isolated from *P. napaulensis*

Following the isolation of endophytic fungi, 13 isolates were identified; their names and accession number of which are listed in Table 1.

Analysis of enzymatic assay

The isolated fungal endophytes showed varying range of extracellular enzymatic activities (Table 2 and Figure 1). For amylase test 7 out of 13 endophytes tested positive. *Chaetomium globosum* (6.25 Uml^{-1}) and *Fusarium graminearum* (6.02 Uml^{-1}) were found to produce higher amount of amylase in comparison to the other isolates. In cellulase test 8 isolates showed positive response, *Colletotrichum gloeosporioides* was found to produce significantly higher cellulase (7.06 Uml^{-1}) followed by *Chaetomium globosum* (3.75 Uml^{-1}) and *Fusarium graminearum* (3.78 Uml^{-1}). For the protease test only 4 isolates showed positive activity. *Nemania primolutea* was found to produce maximum (27.84 ± 0.5 Uml^{-1}) of the enzyme. *Aspergillus fumigatus, Trichoderma atroviride* and *Xylaria feejeensis* produced protease in the range 2.80 ± 0.3 Uml^{-1}, 4.06 ± 0.1 Uml^{-1} and 6.28 ± 0.1 Uml^{-1}, respectively. In the

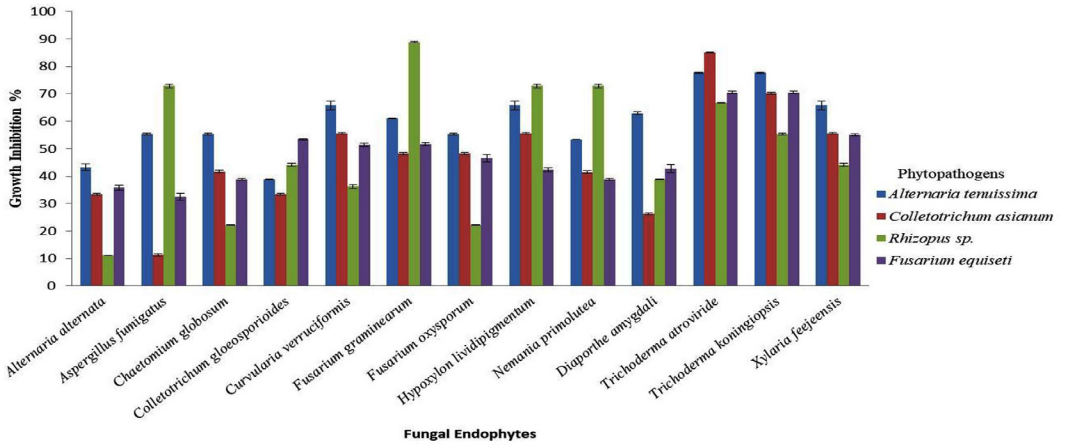

Figure 2. Graphical representation of antagonistic assessment of the isolated fungal endophytes against selected wide host phytopathogens

Table 2. Quantitative assessment of amylase, cellulase and protease produced by the fungal endophytes isolated from *P. napaulensis*

No.	Fungal endophytes	Amylase (Uml^{-1})	Cellulase (Uml^{-1})	Protease (Uml^{-1})
1	*Alternaria alternata*	0	0	0
2	*Aspergillus fumigatus*	5.79 ± 0.1^{bc}	0.8 ± 0.1^{g}	2.80 ± 0.3^{c}
3	*Chaetomium globosum*	6.25 ± 0.1^{a}	3.75 ± 0.1^{b}	0
4	*Colletotrichum gloeosporioides*	0	7.06 ± 0.0^{a}	0
5	*Curvularia verruciformis*	3.73 ± 0.0^{e}	0	0
6	*Fusarium graminearum*	6.02 ± 0.4^{ab}	3.78 ± 0.0^{b}	0
7	*Fusarium oxysporum*	4.27 ± 0.0^{d}	0	0
8	*Hypoxylon lividipigmentum*	0	2.62 ± 0.4^{d}	0
9	*Nemania primolutea*	0	1.47 ± 0.1^{f}	27.84 ± 0.5^{a}
10	*Diaporthe amygdali*	4.64 ± 0.0^{d}	1.91 ± 0.7^{e}	0
11	*Trichoderma atroviride*	0	0	4.06 ± 0.1^{bc}
12	*Trichoderma koningiopsis*	0	0	0
13	*Xylaria feejeensis*	5.48 ± 0.1^{c}	3.14 ± 0.1^{c}	6.28 ± 0.1^{b}

Data shows the mean ± S.D and Tukey's HSD test at $p<0.05$. Values followed by different letters in the same column are significantly different

Table 3. Qualitative assessment of catalase, HCN and urease production by the fungal endophytes isolated from *P. napaulensis*

No.	Fungal endophytes	Catalase	HCN	Urease
1	*Alternaria alternata*	-	-	-
2	*Aspergillus fumigatus*	+	-	-
3	*Chaetomium globosum*	-	-	-
4	*Colletotrichum gloeosporioides*	++	-	-
5	*Curvularia verruciformis*	+	-	-
6	*Fusarium graminearum*	++	-	+
7	*Fusarium oxysporum*	-	-	+++
8	*Hypoxylon lividipigmentum*	-	-	-
9	*Nemania primolutea*	-	-	+
10	*Diaporthe amygdali*	++	-	++
11	*Trichoderma atroviride*	+++	+	+
12	*Trichoderma koningiopsis*	-	+	-
13	*Xylaria feejeensis*	-	-	-

Data indicates positive and negative responses of fungal endophytes for the enzymatic tests: '-' for negative response; '+' for positive response; '++' for moderate response; '+++' for high response

qualitative screening, 6 isolates tested positive for catalase, only the *Trichoderma* species reported positive for HCN production and 5 isolates showed positive response for urease production (Table 3). *Alternaria alternata* showed negative response for all the extracellular enzymatic activities and HCN production.

Isolation and identification of fungal phytopathogens

The fungal pathogens isolated from selected crop plants were identified as *Alternaria tenuissima, Colletotrichum asianum, Rhizopus* sp. and *Fusarium equiseti*. The accession numbers obtained for identified pathogens have been specified in Table 4.

Table 4. List of fungal pathogens isolated from crop plants

No.	Fungal pathogen	Host Plant	Plant part	Accession No.
1	*Alternaria tenuissima*	*Solanum tuberosum*	Leaf	PP856662
2	*Colletotrichum asianum*	*Mangifera indica*	Leaf	PP856663
3	*Rhizopus* sp.	*Mangifera indica*	Fruit	PP855864
4	*Fusarium equiseti*	*Sechium edule*	Leaf	PP856666

Table 5. Antagonistic potential of endophytic fungi against fungal phytopathogens

No.	Endophytic fungal isolates	Growth Inhibition % of Fungal Phytopathogens			
		Alternaria tenuissima	*Colletotrichum asianum*	*Rhizopus* sp.	*Fusarium equiseti*
1	*Alternaria alternata*	43.23 ± 1.2^{e}	33.37 ± 0.4^{f}	11.07 ± 0.1^{i}	35.83 ± 0.9^{g}
2	*Aspergillus fumigatus*	55.39 ± 0.3^{d}	11.33 ± 0.4^{h}	72.84 ± 0.6^{b}	32.43 ± 1.3^{h}
3	*Chaetomium globosum*	55.39 ± 0.3^{d}	41.69 ± 0.4^{e}	22.15 ± 0.1^{h}	38.80 ± 0.3^{f}
4	*Colletotrichum gloeosporioides*	38.93 ± 0.1^{f}	33.37 ± 0.4^{f}	44.05 ± 0.6^{e}	53.47 ± 0.2^{bc}
5	*Curvularia verruciformis*	65.79 ± 1.6^{b}	55.67 ± 0.2^{c}	36.31 ± 0.7^{g}	51.37 ± 0.6^{c}
6	*Fusarium graminearum*	61.07 ± 0.1^{c}	48.24 ± 0.3^{d}	88.76 ± 0.2^{a}	51.67 ± 0.6^{c}
7	*Fusarium oxysporum*	55.39 ± 0.3^{d}	48.24 ± 0.3^{d}	22.14 ± 0.1^{h}	46.53 ± 1.3^{d}
8	*Hypoxylon lividipigmentum*	65.79 ± 1.6^{b}	55.67 ± 0.2^{c}	72.84 ± 0.6^{b}	42.40 ± 0.8^{e}
9	*Nemania primolutea*	53.38 ± 0.1^{d}	41.45 ± 0.5^{e}	72.84 ± 0.6^{b}	38.80 ± 0.3^{f}
10	*Diaporthe amygdali*	62.94 ± 0.4^{c}	26.32 ± 0.3^{g}	38.89 ± 0.1^{f}	42.77 ± 1.4^{e}
11	*Trichoderma atroviride*	77.65 ± 0.2^{a}	85.13 ± 0.1^{a}	66.68 ± 0.1^{c}	70.50 ± 0.5^{a}
12	*Trichoderma koningiopsis*	77.65 ± 0.2^{a}	70.26 ± 0.3^{b}	55.39 ± 0.3^{d}	70.50 ± 0.5^{a}
13	*Xylaria feejeensis*	65.79 ± 1.6^{b}	55.67 ± 0.2^{c}	44.05 ± 0.6^{e}	55.13 ± 0.4^{b}

Data shows the mean ± S.D and Tukey's HSD test at $p<0.05$. Values followed by different letters in the same column are significantly different

Assessment of antagonistic potential of isolated fungal endophytes

The growth inhibition percentage for fungal endophytes varied from 11.33%-88.76%, depicting that all the isolated endophytes had some inhibitory potential against the test fungal phytopathogens (Figure 2 and Table 5). *Trichoderma atroviride* and *T. koningiopsis* showed the highest growth inhibition against *Alternaria tenuissima* (77.65%), *Colletotrichum asianum* (85.13% and 70.26%, respectively) and *Fusarium equiseti* (77.65%). While highest growth inhibition against *Rhizopus* sp. was observed by *Fusarium graminearum* (88.76%). Scanning electron microscopy between the endophytes and hosts showed morphological alterations in the pathogens' hyphae, viz. distortion of pathogens' hyphae, coiling of antagonist hyphae around the pathogens' hyphae and also perforations in pathogens' conidia (Figure 3).

DISCUSSION

Biocontrol agents render crop protection against wide range of pathogens and unlike chemical pesticides and insecticides, biocontrol agents offer significant result as it ensures environmental safety, are target specific, do not produce hazardous residues and are easy to administer.[26] Fungal endophytes are known to secrete various hydrolytic enzymes which facilitates its initial colonization in the host tissues, nutrient absorption from the host, hydrolyses complex macromolecules, exhibits mycoparasitism

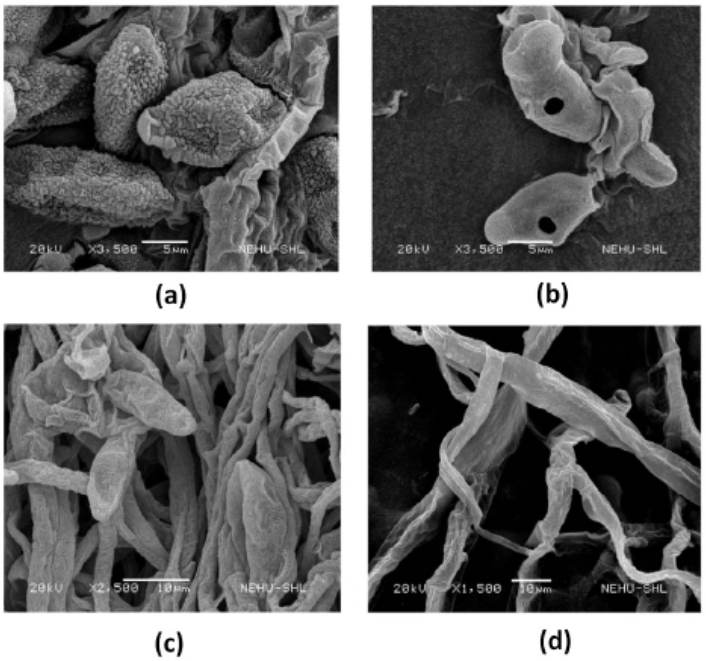

Figure 3. SEM micrographs of: (a) untreated *Alternaria tenuissima* (b) *Alternaria tenuissima* treated with *Xylaria feejeensis* (c) untreated *Fusarium equiseti* (d) *Fusarium equiseti* treated with *Trichoderma koningiopsis*

and also elicits defensive mechanisms against various pathogens or other external stress.[27,28] In the host tissue, endophytes and the pathogens compete under the same micro ecological niche by means of the varied hydrolytic enzymes secreted by the fungal endophytes.[29-31] The commonly produced hydrolytic enzymes by endophytic fungi are amylase, cellulase and protease.[32] In the present study, most of the species were found to produce extracellular enzymes: 8 of 13 fungal endophytes produced cellulase in the range of 0.8-7.06 U/ml, 7 of 13 species produced amylase in the range 3.73-6.25 U/ml and 4 out of 13 species were found to produce protease in the range 2.80-27.84 U/ml. In the current study *Aspergillus fumigatus*, *Chaetomium globosum*, *Fusarium graminearum*, *Diaporthe amygdali* and *Xylaria feejeensis* were found to produce both amylase and cellulase. The qualitative analysis showed 6 of 13 fungal endophytes positive for catalase activity. Microbial catalase renders protection to the host against oxidative stress. In a recent study, *Aspergillus fumigatus* was reported to secrete three types of catalases of which one was found to produce by conidia and the other two by its mycelia.[33] Microbial cyanides in general, are documented to control several plant diseases by increasing plants' resistance during pathogenic attack, and by inhibiting energy metabolism or metalloenzyme activity.[34-36] The present study showed that only the *Trichoderma* species were able to produce HCN. Also in the study, urea hydrolysis was observed only for five isolates- *Fusarium graminearum, Fusarium oxysporum, Nemania primoletea, Diaporthe amygdali,* and *Trichoderma atroviride*. It was thus observed that not all the isolated fungal endophytes produced all the enzymes. Production and regulation of extracellular enzymes by the fungal endophytes is the outcome of its genetic recombination along with the host elicitation and environmental factors.[37] Maccheroni et al., reported in their study that factors such as variation in pH influenced the lytic activity of the endophytes.[38] Similar results were also obtained by Shubha and Srinivas, and Hawar that not all the fungal endophytes could produce all the hydrolytic enzymes.[39,40] Some produce only one of the hydrolytic enzymes while few produced none.

The isolated fungal endophytes all belong to Ascomycetes. Ascomycetes possess high antagonistic activity as they produce bioactive

compounds with antimicrobial properties.[41] As a fungal biocontrol agents *Trichoderma* sp. have been reported to share almost half of the market most preferably as soil or growth enhancers due to its wider antagonism potential against varied plant pathogens.[42,43] *Chaetomium*, *Penicillium*, *Aspergillus* etc., have also been reported as commonly used antagonists.[44] The antagonist activity can be regarded as the outcome of secondary metabolites production, competition, direct parasitism or development of resistance which can sometimes serve as a defensive tool in reducing the enzymatic activities of pathogens.[45,46] In the present study, isolated fungal endophytes from *P. napaulensis* showed varying growth inhibition percentage against the selected fungal phytopathogens irrespective of their efficiency in producing extracellular enzymes. It can also be noted that *Alternaria alternata* showed negative response for all the proposed enzymatic activities and it scored the least per cent for growth inhibition. Studies have reported the involvement of microbial lytic enzymes in controlling infection against fungal pathogens like *Rhizoctonia solani* and *Fusarium oxysporum*.[47,48] Thus, it can be inferred that production of extracellular enzymes enhances the antagonistic efficiency of the fungal endophytes.

Microbial antagonists imparts a significant role in restricting the population of fungal pathogens residing in the aerial parts of the host plants. Colonization by fungal endophytes have been reported in many plants which provides several benefits to the host including enhancing plant growth, increasing resistance against wider pathogens indirectly by some signalling pathways or directly by its antagonistic effect.[49,50] Additionally, several industries like biomaterials, food, cosmetics, textile, fine chemicals, leather, etc., have started employing microbial enzymes such as amylase, cellulase and protease.[51,52] Thus, these potential fungal endophytes can meet the needs of wider sectors.

CONCLUSION

Use of chemical fungicides on crop plants apparently makes the plants unfit for human consumptions as it is reported to have ill impact on human health apart from its adverse effect on the environment. These budding issues have let to the exploration of biocontrol systems which are more natural and environmentally acceptable to combat the problems. Fungi and bacteria are the commonly utilized biocontrol agents. The study provides the insights of fungal endophytes associated with *P. napaulensis*, the extracellular enzymes they produced and their antagonistic potential against various fungal phytopathogens. The tested endophytes showed competitive nature against the pathogens for space, nutrients and also mycoparasitism thereby restricting the growth of the pathogens. The findings add on to the environmentally friendly management strategies to minimise the application of synthetic chemicals for crop plants.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

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