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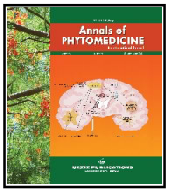

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Evaluation of antimicrobial efficacy of mycoendophytic isolates from rice (*Oryza sativa* L.) crop

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Abstract

Antibiotic-resistant microbes are really a rising threat, necessitating innovative approaches to drug discovery. Possible therapeutic potential lies in the inhibition of such harmful microorganisms. To combat the growing problem of drug-resistant strains of human and plant pathogens, screening antimicrobial substances from endophytes is an exciting new direction. In addition to learning more about the determinants that influence plant growth, this study aims to shed light on the endophytic fungi that can be found in healthy rice seedlings and explore the endophytes' potential for biocontrol as well as their antibacterial properties against phytopathogens and clinically significant ailments. From several parts of rice plants, a total of 66 endophytic fungi were isolated. Their dominance and colonization frequency percentages were calculated. The antibacterial and antagonistic activity was evaluated using an agar well diffusion and dual culture experiment with eight pathogenic bacteria and four phytopathogenic fungi. All extracts exhibited significant levels of inhibitory activity on at least one pathogenic microorganism. Crude ethyl acetate extracts of LS 13 and LL 14 showed inhibition zones ranging from 16-29 mm and 13-28 mm, respectively. Isolate LS 13 showed the highest zone of inhibition (29.70 ± 0.60 F) against *Klebsiella pneumoniae*, whereas isolate LL 14 showed the highest zone of inhibition (28.00 ± 1.00 D) against *Salmonella typhi*. Growth of all studied pathogens was potentially inhibited by *Aspergillus* sp. (GS 01), mycelia sterilia sp. 1 (HS 08), and mycelia sterilia sp. 2 (KL 10), such a zone of inhibition ranging from 44.43 ± 0.99 mm to 67.06 ± 0.81 mm for GS 01, 42.25 ± 2.17 mm to 63.41 ± 1.40 mm for HS 08, and 42.53 ± 1.47 to 53.03 ± 1.50 mm for KL 10, respectively. 50.0% of the isolates showed the protease production. Based on these findings, it implies that some endophytic fungi found in *Oryza sativa* L. plants may serve as a source of antimicrobial compounds and may even have the capacity to support crop growth improvement.

1. Introduction

Cancer and diseases ailments are a growing global threat that continues to gain momentum. Antibiotics with a more antiquated nature might lose just about all of their effectiveness over a period of time. The emergence of "drug" resistance is an issue for both human pathogens and fungal phytopathogens. The increasing world population is demanding innovative therapeutics that can improve their lives in different circumstances. Efforts have been made, and continue to be made, to remove a number of synthetic agricultural compounds from the market. Reasons for this include concerns over security and longevity. Finding adequate alternatives for these components will be essential to maintain current food production rates while reducing negative impacts on the environment, human health and preventing the spread of disease. Now more than ever, drugs and safer antifungal agents derived from biological sources are good candidates for lead compounds. The focus of drug development has shifted from plants to microbes owing to the progress of some therapeutic drugs of microbial origin, including the antibiotic penicillin from *Penicillium* sp., the immunosuppressant cyclosporine from *Tolypocladium inflatum*, *Cylindrocarpon lucidum*, the antifungal agent griseofulvin from *Penicillium griseofulvum*, and the cholesterol biosynthesis inhibitor lovastatin from *Aspergillus terreus* (Balagurunathan and Radhakrishnan, 2007).

Endophytes are perfect for addressing the aforementioned issue. It has been revealed that mycoendophytes, a type of microbe, can exist in both the intercellular and the intracellular spaces of plant tissue and that they cause no harm to the plants they inhabit. It is worth noting that every single one of the world's approximately 300,000 plant species harbors one or more endophytes (Strobel and Daisy, 2003). Some hosts may have facilitated the colonization of a million different endophyte species. On the other hand, only a small fraction of them has been documented (Andrew and Hirano, 1991). Thus, there is a great chance of uncovering unique and selective natural compounds from intriguing endophytic microorganisms amid the vast diversity of plants found in various niches and habitats.

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It has been envisioned that research into endophytic fungi focuses on plants growing in unusual surroundings, notably those with exceptional biology and having effective approaches for endurance or on plants used for therapeutic purposes since they are more likely to hold unique endophytes, some of which may generate distinctive compounds possessing extensive implementation (Strobel and Daisy, 2003).

Most apparently, fungal endophytes mediate induced systemic resistance (ISR) in plants, a crucial mechanism for crop protection and prevention of disease (Arnold and Herre, 2003). In addition, endophytic fungi are exceptionally efficacious at thwarting plants counter to a wide variety of diseases caused by pathogens and mitigating yield losses by secreting a broad range of biologically active metabolites (Murali *et al*., 2017).

Despite their special adaptations for survival, endophytic fungi in crops have received little research. Allelochemicals, some of which may have inhibitory effects and help the competitiveness of the crop plants that host the associative endophytes, may be produced by these plants (Sadrati *et al*., 2013). The presence of endophytic fungus on these plants indicates that they have adapted special survival mechanisms and may represent untapped resources for secondary metabolites with potential biotechnological applications.

In light of this, we set out to investigate more about the antimicrobial potential of an endophytic fungal strains isolated from healthy rice plants in combating a variety of pathogens, including some that are particularly problematic from a clinical standpoint as well as some that have become particularly ingrained as plant pathogens.

2. Materials and Methods

2.1 Source organism isolation

Endophytic fungi were isolated from asymptomatic, randomly selected leaves of four paddy plant varieties (*Oryza sativa* L. var. Khandagiri, Gitanjali, Hiranyamayee and Lalat) found on the campus of Odisha University of Agriculture and Technology in CBSH, Odisha, India. To isolate these microorganisms, healthy stem and leaf tissues are collected, sliced into numerous short segments (2-3 cm), and surface-sterilized by dipping in sequence in 70% ethanol for 2 min and 0.5% sodium hypochlorite (NaOCl) for 5 min, ensued by a thorough wash with sterilized distilled water, partially adapted from the methods (Tripathy and Rath, 2020; Strobel, 2002 Jabborova *et al*., 2020). After being wiped out with a surface sterilant the stems and leaves were placed on sterile tissue paper and dried in an Air Flow Chamber. Utilizing a sterile scalpel, the leaves were cut into 0.5×0.5 cm^2 segments, and the stems were cut into 0.5 cm segments. The segments were then plated in six mycological media, including Potato dextrose agar (PDA), Water agar (WA), Rose bengal agar (RBA), Sabourd's dextrose agar (SDA), Czapeck'sdox agar (CDA), and Malt extract agar (MEA), which were treated with streptomycin (100 µg/ml) to subdue bacterial growth. The plates were wrapped with sterile parafilm and incubated for 2 weeks at 28 ± 2°C in a BOD incubator. Once a day, the plated pieces were checked for the growth of endophytic fungus. As soon as hyphal tips emerged from the plated segments, they were right away transferred to PDA slants, purified, and kept at 4°C. To make sure, the PDA plate was properly sterilized, the final wash was put on it as a control.

Genus-level identification was achieved by comparing fungal culture morphology, spore production mechanism, and spore characteristic properties to those listed in the classic mycological Manual (Barnett and Hunter 1996). Mycelia sterilia are the nonsporulating fungal isolates, while morphotypes are the fungal isolates with distinctive morphological traits. Isolates that were successfully recovered and identified were each given a special code and maintained by sub-culturing them repeatedly in the appropriate nutritional media. Isolated endophytic fungi were labelled with letters representing the plant parts they were taken from, such as GS-(Gitanjali stem), GL-(Gitanjali leaf), HS-(Hiranyamayee stem), HL-(Hiranyamayee leaf), KS-(Khandagiri stem), KL-(Khandagiri leaf), LS-(Lalat stem) and (Lalat leaf).

2.2 Analyzing data on fungal diversity

This formula, provided by Fisher and Petrini (1987), was used to determine the colonization frequency (CF%) of endophytic fungi:
$CF = (N_{COL}/N_t) \times 100$

where, N_{COL} = The number of stem/leaf segments colonized by a particular fungus.; N_t = Total number of plated stem/leaf segments.

The dominant endophytic fungi obtained were determined using the approach used by Goveas *et al.* (2011):

% Dominance = (Number of isolates collected from the samples/ Total number of leaf/stem samples) × 10

The source organisms were among numerous endophytic fungal isolates that shown antibacterial activity against several clinically relevant pathogens and were thus chosen for further investigation.

2.3 Fungal culture and obtaining of crude extract

To cultivate endophytes, pure culture agar blocks (3 mm in diameter) of the organism of interest were placed in 100 ml of potato dextrose broth (PDB) in a 250 ml Erlenmeyer flask. For three weeks, the flask was kept in a BOD shaking incubator at 28 ± 2°C with intermittent shaking at 150 rpm. Mycelia mats were eliminated by passing the culture through sterile whatman filter paper. After collecting the liquid broths, an equal volume of ethyl acetate was added to a separating funnel and the mixture was agitated continuously for 30 min. The cell mass was separated, and the resultant solvent was collected. To get the concentrated crude extracts, the ethyl acetate was evaporated from the mixture before being dried with $MgSO_4$. DMSO (Dimethyl sulphoxide) was used to dissolve the crude extract for use in antibacterial bioassays (Padhi and Tayung, 2013).

2.4 Antibacterial activity study

The agar well diffusion technique of Hormazabal and Piontelli (2009) was used to evaluate the crude metabolites for antimicrobial activity against eight bacteria that are considered to be clinically relevant human pathogens: *Escherichia coli* (MTCC 406), *Bacillus subtilis* (MTCC 178), *Staphylococcus aureus* (MTCC 737), *Salmonella typhi* (MTCC 98), *Pseudomonas aeruginosa* (MTCC 1688), *Vibrio cholerae* (MTCC 3906), *Klebsiella pneumoniae* (MTCC 109) and *Enterococcus faecalis* (MTCC 109). The Institute of Microbial Technology (IMTECH) in Chandigarh, India, provided all of the reference strains. Overnight cultures of each bacterial suspension were inoculated onto Muller Hinton agar plates. A sterile cotton swab was employed to spread the inoculated organisms uniformly throughout the plates. Agar cups were formed by scooping out the medium with a sterile corkborer (6 mm in diameter). The crude metabolites were then dissolved in DMSO to achieve a concentration

of 1 mg/ml, and 100 µl was added to each cup. For bacterial pathogens, the plates were subsequently incubated at 36 ± 1°C for 24 h. After the specified incubation period, ZOI (zone of inhibition) was measured and compared to controls (*i.e.*, a cup filled with just DMSO solution). Measurements of the inhibitory zone diameters were taken, and the mean of three observations was noted.

2.5 Demonstration of antagonism assessment and disease suppressive mechanism

Antagonistic activity contrary to prevalent crop pathogens were assessed by employing the endophytes., such as including *Fusarium oxysporum* (ITCC No. 4721), *Rhizoctonia solani* (ITCC No. 4503), *Aspergillus niger* (ITCC No. 1624), and *Pythium debaryanum* (ITCC No. 95) procured from the Indian Type Culture Collection (ITCC), New Delhi, India. A dual culture plate assay was used to evaluate the endophytes' antagonistic activity (Coskuntuna and Ozer, 2008). Discs of pathogen and test fungi, each measuring 5 mm in diameter, were inoculated on opposite corners of PDA plates alongside a 3 cm separation gap and then incubated at 28°C ± 2°C. The only inoculated pathogen plate was used as the control plate.

Using the formula provided by Fokkema (1976) and Yadav *et al.* (2021), the percentage of inhibition was determined,

$$\text{Inhibition } \% = C - T/C \times 100,$$

Here, 'C' denotes growth diameter of pathogen and 'T' denotes growth diameter of pathogen in presence of endophytic isolate.

2.6 Protease production assay

A modified procedure of Rodr'guez and Fraga (1999) was implemented to inoculate pure fungal endophytic isolates on Skim milk agar (Hi-Media, India), also incubated at 28 ± 2°C. Protease activity has been qualitatively indicated by visible clearance around the colony. The enzyme index was calculated by subtracting the fungal colony diameter from the total diameter of the zone.

2.7 Data analysis

Standard error of neam were calculated using analysis of variance (one-way ANOVA) in SPSS (22.0) for the data of antagonism evaluation and antibacterial activity. Duncan's multiple range test (DMRT) was utilized to identify statistically significant variations between the means at the $p \leq 0.05$ levels.

3. Results

3.1 Endophyte isolation

Endophytic fungi were recovered in various media from healthy leaves and stem samples of four distinct varieties of *O. sativa* After plating 290 stem and leaf pieces from four different rice plant types (Gitanjali, Hiranmayee, Khandagiri, and Lalat), 66 endophytic fungal isolates were isolated, 14 of which were chosen for further characterization based on morphological and functional features (Figure 1). They were unable to determine why two of the isolates did not sporulate or display any other signs of reproductive structure. These two isolates were given the name mycelia sterilia. Endophytic fungi were found to have a total colonization frequency (CF %) of 10.97% and 11.68% in healthy stem and leaf tissues of four cultivars of *O. sativa* (Table 1). Fungi from the genus *Aspergillus*-1 had the highest colonization frequency in the stem (3.44%), followed by the genera *Talaromyces* and *Aspergillus*-2 (1.72%), *Penicillium* (1.03%), *Aspergillus*-3, *Fusarium*, an unidentified genus, and *Rhizopus* (0.68%), and mycelia sterilia-1 (0.34%). In leaves, fungi from the genus *Aspergillus* colonized the most (3.79%), followed by *Aspergillus*-2, *Aspergillus niger*, and *Colletotrichum* (1.72%), *Talaromyces* (1.03%), *Penicillium* and mycelia sterilia-2 (0.68%), and an unidentified species-2 (0.34%), respectively. The genus *Aspergillus* has the highest colonizing frequency (%) and frequency dominance (%) (Table 1 and Figure 2).

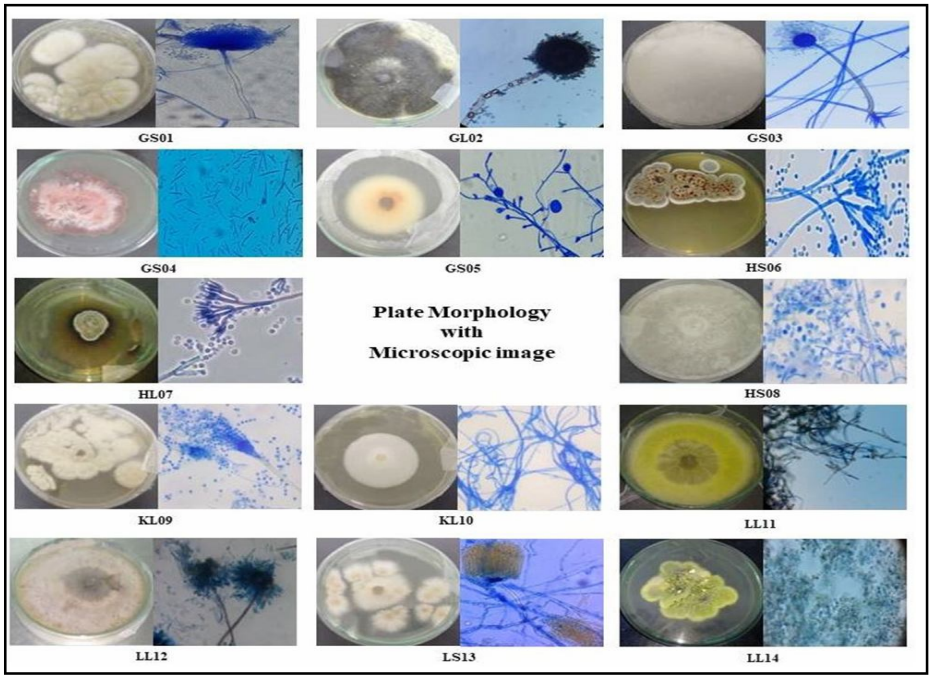

Figure 1: Image showing endophytic fungi from different parts of 4 varieties of rice plant.

Table 1: Occurrence, colonizing frequency (%) and frequency of dominance (%) of endophytic fungi isolated from different parts of *O.sativa*

Plant part	Endophytic fungi	Total no. of isolates	Colonizing frequency (%)
Stem	1. *Aspergillus* sp. (1)	10	3.44
	2. *Aspergillus* sp. (2)	5	1.72
	3. *Aspergillus* sp. (3)	2	0.68
	4. *Fusarium* sp.	2	0.68
	5. Unidentified sp.(1)	2	0.68
	6. *Penicillum* sp.(1)	3	1.03
	7. *Talaromyces* sp.	5	1.72
	8. Mycelia sterilia (1)	1	0.34
	9. *Rhizopus* sp.	2	0.68
	Total No. of isolates	32	10.97%
Leaf	1. *Aspergillus* sp. (1)	11	3.79
	2. *Aspergillus* sp. (2)	5	1.72
	3. *Aspergillus niger*	5	1.72
	4. *Penicillium* sp. (2)	2	0.68
	5. *Talaromyces* sp.	3	1.03
	6. Mycelia sterilia (2)	2	0.68
	7. Unidentified sp. (2)	1	0.34
	8. *Colletotrichum* sp.	5	1.72
	Total no. of isolates	34	11.68%

*The colony frequency was calculated based on 290 segments of plant parts plated.

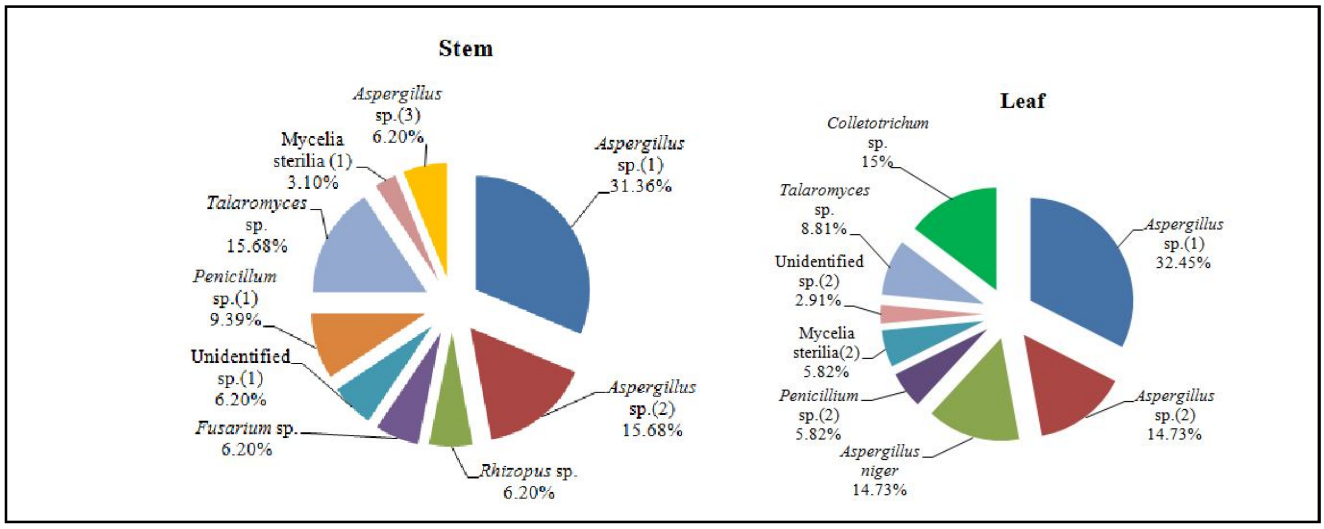

Figure 2: Frequency of dominant endophytic fungi (%) for four different rice plants as Gitanjali-V1, Hiranyamayee-V2, Khandagiri-V3, and Lalat-V4.

3.2 Antibacterial activity of endophytic fungi

Endophytic fungal isolates were evaluated for antibacterial efficacy against eight clinically significant human infections. Based on the agar plug diffusion assay results, it was determined that 78.57% of the fungal isolates tested (out of a total of 14) were effective against at least two of the test pathogens (Table 2). In contrast, none of the pathogens were inhibited by the 3 fungal endophytes (HS06, HL07, and HS08). *S. typhi* and *V. cholerae* were the most inhibited (57.14% (8/14)) by the active endophytic strain, whereas *B. subtilis* was the least (21.42% (3/14) Table 2 and Figure 3). All eight bacterial

pathogens were inhibited by crude extracts from three fungal isolates: mycelia sterilia (KL10), *Aspergillus* sp. (LS13), and *Talaromyces* sp. (LL14). The inhibitory activity of KL10 was moderate throughout all pathogens. A crude ethyl acetate extract of LS13 and LL14 demonstrated strong inhibitory zones extending from 16 mm to 29 mm and 13 mm to 28 mm, respectively (Table 3). Against *Klebsiella pneumoniae* (29.70 ± 0.60^{F}) and *Salmonella typhi* (28.00 ± 1.00^{D}), isolate LS13 and LL14 showed the largest zone of inhibition, respectively. The best and most widely active crude extracts were those from *Aspergillus* sp., *Talaromyces* sp., and mycelia sterilia against both classes of pathogenic microbes (gram-positive and negative bacteria). However, the extracts displayed more effectiveness against gram-negative bacteria than positive bacteria (Figure 3 and Table 2).

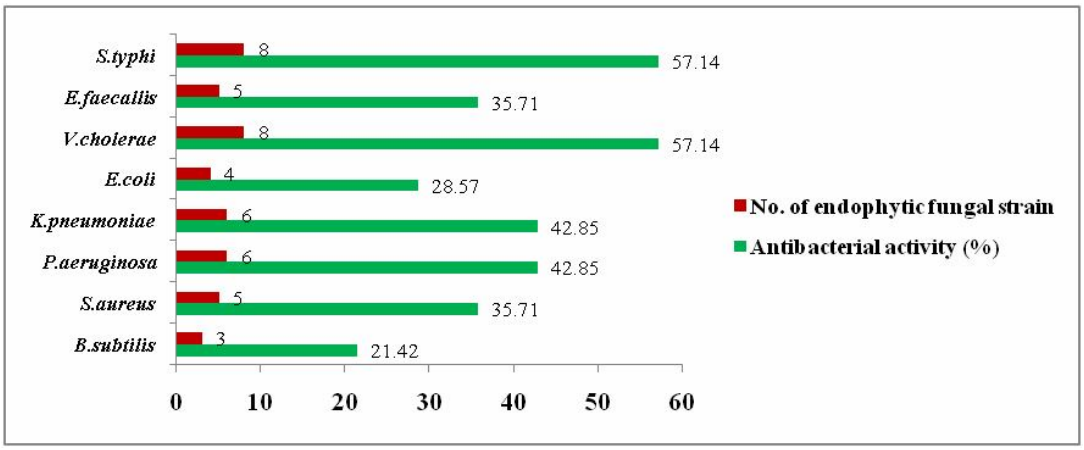

Figure 3: % of susceptible test bacteria to endophytic fungi.

Table 2: Zone of inhibition of endophytic fungi isolated from *O. sativa* against different tested strains

Diameter of inhibition zone in (mm)								
Isolate code	**Bs**	**Sa**	**Pa**	**Kp**	**Ec**	**Vc**	**Ef**	**St**
GS 01	ND	ND	+	ND	ND	ND	ND	++
GL02	ND	ND	ND	+	+	+	ND	+++
GS03	ND	ND	ND	ND	ND	ND	ND	++
GS04	ND	ND	+	+	ND	+	ND	+++
GS05	ND	ND	ND	ND	ND	+	ND	+++
HS06	ND	ND	ND	ND	ND	ND	ND	ND
HL07	ND	ND	ND	ND	ND	ND	ND	ND
HS 08	ND	ND	ND	ND	ND	ND	ND	ND
KL09	ND	++	ND	ND	ND	ND	ND	ND
KL 10	+	+	++	+	+	+	+	+
LL11	ND	+	+	ND	ND	+	+	ND
LL12	ND	ND	+	+	ND	+	+	ND
LS 13	+++	+++	++	+++	+	+++	+++	+++
LL 14	++	++	++	++	+	+	++	++

(+) indicates zone of inhibition <15 mm; (++) indicates zone of inhibition between 15-20 mm; (+++) indicates zone of inhibition >20 mm, the isolates used for further study are marked bold, 'ND' – not detected.

(Bs-*Bacillus subtilis*, Sa-*Staphylococcus aureus*, Pa-*Pseudomonas aeruginosa*, Kp-*Klebsiella pneumoniae* Ec-*Escherichia coli*, Vc-*Vibrio cholerae*, Ef-*Enterococcus faecalis*, St-*Salmonella typhi*)

3.3 Analysis of antagonistic activity

Various patterns of interaction between the pathogens and endophytes were monitored and analyzed. The inhibition zones of GS01, HS08, LS13, KL10, KL09, and GS05 were depicted, and the percentage of growth-inhibiting antagonistic effects is summarized as follows: (Table 4). Data from statistical sources illustrates that *Pythium debarynum*, *Rhizoctonia solani*, and *Fusarium oxysporum* are effectively controlled by all tested endophytes, while *Aspergillus niger* is poorly controlled.

Table 3: Antibacterial activity of endophytic fungi (LS 13 and LL 14) against human pathogens

Isolates code	Diameter of inhibition zone [mean(mm) ± SE]							
	Test pathogens							
	B. subtilis	*S. aureus*	*P. aeruginosa*	*K.pneumoniae*	*E. coli*	*V. cholerae*	*E. faecallis*	*S. typhi*
LS 13	27.00 ± 0.10E	22.80 ± 0.70C	16.43 ± 0.51A	29.70 ± 0.60F	-	24.86 ± 1.95D	20.73 ± 0.64B	22.00 ± 1.00C
LL 14	13.83 ± 1.51A	14.73 ± 1.58A	20.83 ± 0.64B	24.20 ± 0.10C	-	27.13 ± 0.15D	19.70 ± 0.60B	28.00 ± 1.00D

Table 4: Antagonistic and antifungal assessment of endophytic fungal isolates

Antagonistic activities						Antifungal mechanism	
Growth inhibition percentage (%)							
Isolates code	Stem/leaf endophytic fungi	An	Rs	Fo	Py	Protease	PEI
GS 01	***Aspergillus* sp.1**	44.43 ± 0.993^{a}	58.88 ± 4.972^{b}	47.97 ± 8.086^{a}	67.06 ± 0.817^{b}	_	_
GL02	***Aspergillus niger***	0.00	0.00	0.00	0.00	+	1.15 ± 0.005^{A}
GS03	***Rhizopus* sp.**	54.49 ± 7.288	49.13 ± 12.021	53.86 ± 7.431	50.03 ± 12.728	-	-
GS04	***Fusarium* sp.**	41.79 ± 1.549^{a}	59.28 ± 2.403^{b}	44.05 ± 6.547^{a}	52.24 ± 3.248^{b}	+	1.24 ± 0.003^{B}
GS05	**Unidentified sp.**	44.46 ± 1.966^{a}	64.02 ± 1.367^{b}	53.43 ± 5.832^{b}	50.89 ± 2.508^{a}	+	1.41 ± 0.005^{C}
HS06	***Penicillium* sp.1**	_	_	_	_	+	1.51 ± 0.003^{D}
HL07	***Penicillium*sp.2**	30.03 ± 1.443^{a}	39.96 ± 1.299^{b}	43.90 ± 1.408^{b}	42.79 ± 1.002^{b}	+	1.49 ± 0.005^{E}
HS 08	**Mycelia sterilia sp.1**	47.36 ± 1.521^{a}	52.93 ± 1.246^{b}	63.41 ± 1.408^{c}	42.25 ± 2.176^{a}	_	_
KL09	***Aspergillus*sp.2**	30.00 ± 1.443	39.96 ± 1.299	57.73 ± 1.299	49.16 ± 5.218	_	_
KL 10	**Mycelia sterilia sp.2**	42.53 ± 1.472^{a}	53.03 ± 1.507^{b}	46.60 ± 1.270^{a}	44.40 ± 1.270^{a}	_	_
LL11	**Unidentified sp.**	27.58 ± 0.995^{a}	49.92 ± 0.648^{c}	36.18 ± 1.261^{b}	24.99 ± 1.925^{a}	_	_
LL12	***Colletotrichum* sp.**	12.64 ± 1.150^{a}	48.13 ± 2.661^{c}	32.85 ± 3.595^{b}	19.93 ± 3.333^{a}	+	1.32 ± 0.005^{F}
LS 13	***Aspergillus*sp.3**	18.38 ± 1.520^{a}	48.32 ± 0.320^{b}	42.37 ± 1.257^{b}	33.32 ± 13.886^{a}	_	_
LL 14	***Talaromyces* sp.**	39.42 ± 0.406	0.00	46.34 ± 1.408	54.54 ± 1.310	+	1.66 ± 0.003^{G}

3.4 Protease activity

Seven (50%) of the 14 entophytic isolates tested positive for protease activity, such as GL02, GS04,GS05, HS06, HL07, LL12 and LL14 (Table 4), produced protease activity, due to the establishment of a distinct halo zone surrounding the fungal colony. Among the fourteen tested isolates, LL14 demonstrated the highest protease activity with an enzyme index (EI) of 1.66 ± 0.003^{G}, while, GL02 produced the least protease with an enzyme index of 1.15 ± 0.005^{A}.

4. Discussion

Throughout the world, endophytic fungi are found naturally dispersed among plants from both temperate and tropical climates. As relatively diminutive research has been conducted on endophytic fungi associated with *O. sativa* the primary objectives of the current work are to examine the existence of the endophytic fungus sheltering healthy leaf and stem tissues of the plant, to investigate the antibacterial potential and antagonistic study of those endophytic fungi. The findings revealed the presence of an endophytic fungal community inside the leaves and stems of *O. sativa* and from 290 surface-sterilized leaves and stem, fragments plated on various media, 66 isolates from 10 distinct genera of endophytic fungi were recovered. The largest proportion of endophytes recovered from such a plant's leaf was observed. Fungi belonging to the Ascomycota division were found to be predominant in both the stem and leaf tissues (77.16%). Although, fungi from the classes Sordariomycetes and Eurotiomycetes were equally distributed across rice plants; fungi from the class Duteromycetes were found to be the least dominant (2.82%) in stem samples and unidentified genera (2.91%) in leaf samples, accounting for a small percentage of the total isolate. According to a diversity analysis, *Aspergillus* sp. was frequently isolated. *Aspergillus* and *Penicillium* sp. were also among the most prevalent endophytes, according to a report on fungal endophytes isolated from healthy paddy plants in China reported by Tian *et al.* (2004) and John *et al.* (2019), which is merely similar to our findings.

In this study, the crude metabolites of 11 out of 14 (78.57%) fungal strain revealed noticeable antibacterial activity against at least one of

all the test pathogens examined. *Aspergillus* sp. (LS13) was the most effective isolate against all of the test pathogens. This was followed by the *Talaromyces* sp. (LL14) and mycelia sterilia-1(KL10). Endophytic *Aspergillus* sp. and *Talaromyces* sp. fungi have both been found to have antimicrobial activity against various pathogens and thus the results collaborate with the findings of some previous works (Rustamova *et al*., 2020; Mishra *et al*., 2021 and John *et al*., 2022). Secondary metabolites including steroids, terpenes, or terpenoids may be responsible for the antimicrobial activities seen in the ethyl acetate extracts of fungal isolates used in our study.

When two or more strains of microbes are co-grown (a "dual culture"), they are forced to interact with one another, which may lead to the synthesis of compounds that have not been seen when the strains have been cultured separately. Using a dual-culture method like this, one might be a crucial step in finding new secondary metabolites (Holguin and Bashan, 1996; Long and Azam, 2001; Maurya *et al*., 2020; Pandey *et al*., 2022).

Rhizoctonia solani, *Pythium debarynum*, *Fusarium oxysporum*, and *Aspergillus niger* are the four phytopathogens utilized in this study, are globally prevalent among cereal crops and possess distinctive characteristics. During our study of antagonistic properties of the isolates against these fungi, we observed GS 01 (*Aspergillus* sp. 1), HS 08 (mycelia sterilia sp.1), LS 13 (*Aspergillus* sp.3), KL 10 (mycelia sterilia sp. 2), and KL09 (*Aspergillus* sp.2) were among the dominant antagonists. Several endophytic fungal isolates exhibited antifungal activity against multiple pathogens too. The development of biologically active compounds in the media might well be accountable for the antagonism (Castillo *et al.,* 2002). Several authors have documented the capability of *Aspergillus* species to inhibit *Fusarium* isolates. Antibiosis initiated the lysis of *F. oxysporum* mycelium by *A. niger* (Dwivedi and Enespa, 2013). *Fusarium sambucinum* and *Phytophthora erythroseptica* were effectively suppressed by *A. niger*, *A. tamarii*, and *A. terreus* (Abdallah *et al*., 2015; Maurya *et al*., 2020). Fascinating studies have demonstrated that non-toxigenic strains of *Aspergillus flavus* have the capability to suppress mycotoxigenic *Aspergillus* in cereals (Sarocco and Vannacci, 2018). These findings are in correlation with our findings that *Aspergillus* sp. and mycelia sterilia sp. are potent bioagents that can be used from an agricultural point of view. Due to their low environmental impact and low cost, botanical and bioagent uses are preferable to fungicides for disease management. Our results corroborate those of Patel *et al*. (2021) and Maurya *et al*. (2021) who found that *Trichoderma harzianum* and *Trichoderma viride* significantly improved germination, reduced the percentage of mortality, and disease manifestation when used as a bioagents.

Protease activity was detected in 50% of the isolates. Protecting the host from various pathogens, such as insects, pests, and nematodes is the sole objective of the protease enzyme. A study was conducted that *Talaromyces* sp. produced a high concentration of protease enzymes (Haggag *et al*., 2006), as observed in the present investigation indicating its capability to protect the host from phytopathogen attack.

According to our findings, endophytic fungi isolated from *O. sativa* leaves and stems yield antimicrobial compounds that impede the growth of pathogenic microorganisms, suggesting they may have pharmaceutical potential. The production of bioactive, pharmaceutically important, and economically significant antimicrobial compounds might benefit from further investigation of the bioactivity of the strains produced in this work. Therefore, more research is now required to pinpoint the active substances produced in order to discover novel drugs having antimicrobial attributes.

5. Conclusion

In light of these findings, endophytes may be well-thought-out as a possible source of new bioactive compounds. This research showed that *Aspergillus* sp., *Talaromyces* sp., and mycelia sterilia were among the endophytic fungi isolated from rice that exhibited antibacterial properties in their respective extracts. Additionally, this finding suggests endophytic fungi might be used as an efficient antagonistic agent against key fungal phytopathogens. These species support plant development in a variety of ways, including the synthesis of a hydrolytic enzyme-like protease and the protection of plants from phytopathogens. Because of this, endophytic fungi are crucial in the quest for natural compounds. The endophytic fungus may potentially be an alternate source for the production of therapeutic compounds and bioactive metabolites that are difficult to get through chemical synthesis and have great activity against pathogenic microbes. This research, however, will only be a stepping stone to more indepth analyses of the bioactive natural products generated by these endophytes in terms of their chemistry and biology. Endophytes can be studied further to see whether they can be used as biocontrol agents or as novel pharmacological agents.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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