

Exploring the Biotechnological Potential of Endophytic Fungi from *Acorus calamus*: Enzymatic Activity, Antimicrobial Properties, and Siderophore Production

Prabha Parki

Kumaun University

Himani Verma

Kumaun University

Babita Rana

HNB Garhwal University

Kapil Khulbe (✉ kapilkhulbe@gmail.com)

Kumaun University

Research Article

Keywords: Fungal endophytes, extracellular enzymes, antibacterial activity, siderophore

Posted Date: September 29th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3381750/v1>

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Abstract

The leaves and rhizome of *Acorus calamus* (Acoraceae) were investigated for the endophytic fungi associated with the plant. Two fungal endophytes were isolated and confirmed as *Cladosporium sphaerospermum* and *Plectosphaerella* sp. using molecular techniques. Both species were recorded as new isolates of *A. calamus*. The isolates were tested for extracellular enzyme production. The *Plectosphaerella* sp. demonstrated enzymatic activity in maximum instances, whereas *C. sphaerospermum* displayed positive results for three extracellular enzyme production (pectinase, cellulase, and tyrosinase) activities. The preliminary siderophore production assay was done, and *Plectosphaerella* sp. was found to have the potential for siderophore production. Both fungal isolates were screened for preliminary antibacterial activity by the agar-well diffusion method against plant and animal pathogens, in which *C. sphaerospermum* showed the maximum zone of inhibition against the animal pathogenic *Bacillus megaterium* (30.67 ± 1.64) while *Plectosphaerella* sp. against *Staphylococcus aureus* (16.67 ± 1.20). When tested against plant pathogenic strains, *C. sphaerospermum* showed the maximum zone of inhibition against *Xanthomonas phaseoli* (31.50 ± 1.76) and *Plectosphaerella* sp. against *Xanthomonas campestris* (15.33 ± 0.88).

Introduction

Endophytes are microorganisms, such as fungi or bacteria, that reside within the internal tissues of plants without causing any apparent harm to their host. These fungi have coevolved with plants, establishing a symbiotic relationship that can lead to the production of various secondary metabolites, which are chemical compounds not essential for the primary metabolic processes of the organism but often have beneficial properties. Over the last two decades, there has been a growing interest in exploring the potential of endophytic fungi as a source of valuable bioactive compounds with bioactive properties.

The secondary metabolites produced by endophytic fungi can display a wide range of biological activities, including enzymatic and antimicrobial properties. These compounds help the microorganism to establish itself and serve as a defence mechanism for the host plant, helping it ward off harmful pathogens. This natural protection has garnered significant attention for its potential application in industries and agriculture.

Due to the increasing resistance of harmful bacteria and fungi to chemically produced antimicrobial agents, infections brought on by microbial pathogens have become a major issue for the world and one of the most serious threats to human health. Endophytic fungi have effectively yielded a number of important bioactive substances with antibacterial and insecticidal properties in the last two decades. Endophytic fungi produce antagonistic substances (bioactive compounds/secondary metabolites) that may have agricultural applications for defending plants and crops from harmful phytopathogens (insects, pests, fungi, and bacteria). The extracellular microbial enzymes also play an important role in the food, wine, dairy, therapeutics, paper, leather and textile industries. In addition, they have several

advantages over chemically synthesized enzymes because microbial enzymes can withstand harsh environments like high pH and severe temperatures (Shubha and Srinivas 2017).

Recent research efforts have been focused on identifying and characterizing these bioactive compounds derived from endophytic fungi. Scientists are particularly interested in their potential as environmentally friendly alternatives to synthetic enzymes and antibiotics. By harnessing these natural compounds, it may be possible to reduce the reliance on synthetic chemicals, which can have harmful effects on the environment, non-target organisms, and even human health (Dos Reis et.al.,2022).

The process of discovering and utilizing bioactive compounds from endophytic fungi involves several steps, including isolation, identification, incubation, extraction, and testing for their biological activities. Overall, the exploration of endophytic fungi as a source of bioactive compounds has opened up promising avenues for eco-friendly industrial solutions, and sustainable agricultural processes. Though endophytic fungi have a wide variety of secondary metabolites and numerous species diversity (Sunil et al.,2018), their potential remains untapped. Therefore, attempts to characterize and identify endophytic fungi isolated from plants are escalating. The goal of the present study was to isolate and identify endophytic fungi from a semi-aquatic species of medicinal plant: *Acorus calamus* Linn. (Acoraceae) and to screen their potential for extracellular enzyme and siderophore production *in vitro*, including their preliminary antibacterial activity. Given that no authentic record of the bioactivity of fungal endophytes can be found, the present study gains relevance.

Materials and Methods

Collection of plant samples

The healthy plant of *Acorus calamus* was collected aseptically from the Pithoragarh district (29.58°N 80.22°E) of Kumaun Himalaya region during the month of October-November 2020. The collected plant sample was then brought to the laboratory aseptically and processed further within 24 hours.

Surface sterilization of plant samples

For the successful isolation of endophytic fungi, the surface sterilization process is an important step to maintain the integrity of internal microbes and for the removal of microbial epiphytes on the outer surface of the sample. The surface sterilization procedure of plant samples was carried out using the standard method of Petrini and Fischer (1990). The collected plant was washed thoroughly in slow-running tap water for 12–24 hours to remove the adhered soil particles in the plant. The plant was allowed to dry on blotting paper. The rhizomes of the collected plant were carefully cut with sharp sterile blades into small parts and subjected to surface sterilization under aseptic conditions in a Laminar air flow cabinet. The rhizome parts were treated sequentially with different disinfecting agents (a) 70% Ethanol-30 sec (b) Sterile distilled water sec ; (b) 1:3dilution of Sodium hypochlorite (NaOCl)- 5min ;(c) 70% Ethanol-30sec ; (d) 3 time rinsed in sterile distilled water-1-2min.

Isolation of endophytic fungi

The surface sterilized rhizome parts were air dried aseptically in Laminar airflow and then for the isolation of endophytic fungi, each sterilized part was placed in Petri dishes in such a way that their internal tissues were exposed to culture media (PDA) supplemented with antibacterial to suppress the growth of bacteria and incubated for 10–15 days at $25 \pm 2^\circ\text{C}$ temperature with observation in regular interval of time. Validation of surface sterilization was done by spreading 0.1ml of the last rinsed water on PDA plates and imprints of the rhizome parts were taken on PDA media (Schulz et al.,1993 and Qin et al.,2009). Newly growing hyphal tips of developing fungi were further cultured in fresh PDA medium to get pure isolates. By using the repeated sub-culturing method of Kjer et al.,2010 pure fungal endophyte colonies were obtained.

Identification of isolated fungal endophytes

Morphological identification

Morphological identification was done by slide culture method, in which small fragments of mycelia of isolated fungal endophytes were removed using sharp sterile needles and placed on a clean sterile glass slide and stained with lactophenol and cotton blue. Further, the slides were observed under the microscope.

Identification of the test isolates by molecular methods

The molecular method based on the sequence of ITS regions¹ and 2 was employed for all the fungal strains isolated in the present study. For this study, the ITS regions 1 and 2 of the isolates were amplified using PCR with the primers 5'-GGTTAGGCCGGGTATTACC-3' and 3'-TTAATCCTTTCCGGCGGGTC-5'. The amplified products were sequenced using the Sanger method. The sequences thus obtained were BLAST analyzed for homology search online (www.ncbi.nlm.nih.gov). A phylogenetic tree was constructed by neighbour joining method using MEGA 11.

Screening of fungal isolates for extracellular enzyme activity

Endophytic fungi have the potential to produce different types of extracellular enzymes. Hence, to detect the extracellular enzyme activity, the pure fungal isolates were qualitatively screened for the production of extracellular enzymes (Amylase, Cellulase, Pectinase, Protease, Laccase, and Tyrosinase) by using the standard method of Hankin and Anagnostakis 1975. The fungal isolates were assayed by inoculating mycelial plugs of 10–15 days old culture in a solid media (GYP) containing their respective substrates and incubated at $25 \pm 2^\circ\text{C}$ temperature for 5–7 days, the halo zone around the fungal colony and colour produced by the fungal isolates signifies the presence of enzymatic activity.

Amylase

For Amylase activity the isolates were grown on glucose yeast extract peptone agar (GYP) media (glucose-1g, yeast extract- 0.1g, peptone-0.5g, agar-16g for 1L) with 0.2% soluble starch, pH6.0, incubated

for 5–7 days at $25 \pm 2^\circ\text{C}$ temperature, after incubation plates were observed for halo zone around the colony by pouring 1% iodine and 2% potassium iodide solution.

Cellulase

The fungal isolates were grown on glucose yeast extract peptone agar (GYP) media (glucose-1g, yeast extract- 0.1g, peptone-0.5g, agar-16g for 1L) containing 0.5% Carboxy-methylcellulose, pH6.0, after 5–7 days of incubation plates were flooded with 0.2% Congo red solution for 5-15min and then destained with 1M solution of NaCl, golden yellow zone around the colony appeared indicates the cellulolytic activity.

Pectinase

Pectin agar media (Pectin-5g Yeast extract-1g Agar-16g for 1L), pH6.0, was used for the pectinolytic activity of fungal isolates and incubated for 5–7 days. After the incubation period the plates were flooded with a 1% aqueous solution of hexadecyl trimethylammonium bromide (Citrimide), and the appearance of a clear halo zone around the fungal colony confirms pectinolytic activity.

Protease

8% gelatin solution was prepared separately and mixed 5mL per 100mL of GYP media (glucose-1g, yeast extract- 0.1g, peptone-0.5g, agar-16g for 1L), pH6.0, After the incubation of 5–7 days, plates were flooded with saturated aqueous ammonium sulfate solution, a clear halo zone around the fungal colony indicates the presence of proteolytic activity.

Laccase

Fungal isolates were grown on GYP media (glucose-1g, yeast extract- 0.1g, peptone-0.5g, agar-16g for 1L) containing 0.5g naphthol, pH6.0 after incubation the fungal colony turns into deep blue indicates the presence of Laccase activity.

Tyrosinase

The fungal isolates were grown on GYP media (glucose-1g, yeast extract- 0.1g, peptone-0.5g, agar-16g for 1L), pH6.0, incubated for 5–7 days, after incubation the 0.11% p-Cresol and 0.05% glycine solution were poured in plates, the reddish-brown colour of the fungal colony indicates the presence of tyrosinase activity.

Screening of fungal endophytes for siderophore production

The siderophore production assay was performed by following the method given by Schwyn and Neilands 1987. For the screening of the siderophore production potential of fungal isolates, the 8mm mycelial plugs of both the fungal endophytes from one week-old culture, were inoculated on Chrome Azurol S (CAS) agar media containing petriplates. These plates were then incubated for 5–7 days and examined the fungal colony for their orange colour halo zone on blue coloured media, which indicates the presence of siderophore production potential of fungal isolates.

Preliminary antibacterial assay

Test Microorganisms

Four plants and seven animal pathogenic bacterial strains (gram-positive and gram-negative) were obtained from MTCC Chandigarh, India, and were maintained by repeated culturing in the nutrient broth at 37°C. The selected test pathogens are given in Tables 1 and 2.

Table 1
Selected animal pathogenic bacterial strains

Animal pathogenic bacterial strains	Type	Disease caused
<i>Listeria monocytogenes</i> MTCC1143	Gram + ve	Food poisoning
<i>Salmonella typhi</i> MTCC3231	Gram-ve	Typhoid
<i>Bacillus cereus</i> MTCC1272	Gram + ve	Food poisoning
<i>Staphylococcus aureus</i> MTCC6908	Gram + ve	Skin infections
<i>Pseudomonas aeruginosa</i> MTCC15024	Gram + ve	Blood infections,Pneumonia
<i>Escherechia coli</i> MTCC443	Gram-ve	Diarrhoea
<i>Bacillus megaterium</i> MTCC2412	Gram + ve	Meningitis

Table 2
Selected plant pathogenic bacterial strains

Plant pathogenic bacterial strains	Type	Disease caused
<i>Xanthomonas campestris</i> ITCCBD0006	Gram-ve	Black rot of Crucifers
<i>Xanthomonas phaseoli</i>	Gram-ve	Bacterial blight diseases
<i>Ralstonia solanacearum</i> ITCCBH0007	Gram-ve	Bacterial wilt
<i>Agrobacterium tumefaciens</i> MTCC2250	Gram-ve	Crown gall disease

Agar Plug Diffusion Assay

The preliminary screening of the isolated endophytic fungi was performed through agar plug diffusion assay by using the protocol of Gulis and Stephnovich, 1999. The test pathogens were grown in a nutrient broth, 2 days before performing the assay. The bacterial suspension (100µl) of the test pathogen was then inoculated and spread equally with the help of an L-shaped spreader, on Petri plates containing nutrient agar (NA) media. Then, mycelial plugs (8mm) of actively growing fungal isolates from 5–7 days fresh culture, growing on a PDA media, were placed on the well, prepared at the center of the NA media containing Petri plates. Now, these petriplates were incubated in an incubator for 24 hours. at 37°C

temperature. After incubation, the zone of inhibition (ZOI) around the fungal colony was measured with the help of a ruler.

Results and Discussion

Morphological Identification

On the basis of the morphology of fungal colonies, a total of 4 fungal isolates (IACR 01, IACR 02, IACL 03, IACL 04) were isolated from the different segments of *A. calamus*. On the basis of the morphological characteristics of the four, two isolates each from leaf and rhizome were identified as *Cladosporium sphaerospermum* (CS) and *Plectosphaerella* sp. (PS), respectively.

The isolation of only two fungal endophytes is in contrast to other reports on fungal endophytes of medicinal plants (Barik et al., 2010; Mani and Audipudi 2016; Mohanta et al., 2007). In the present work, both plant parts were incubated for a period of 10–12 days and isolates were marked differently based on colony morphology. If microscopic techniques or longer incubation duration had been applied at the initial screening level, it is possible that additional fungal species would have been discovered in both plant organs. Fungi exhibit a wide range of growth rates, and some species require an incubation of 20 days (Li et al. 2016a, b; Yao et al. 2017; Du et al. 2020) to develop visible colonies under laboratory condition, which can be extended for up to 6 weeks if necessary (Yao et al. 2017; Dos Reis et al., 2022). Because we wanted to be able to do bioactive studies on endophytic fungi of *A. calamus*, we purposefully picked the culture morphology and 10–12 days incubation approach; our goal was not to come up with a species list.

The CS has olivaceous-grey colonies with a greenish-black reverse surface. The colonies have a crater-like shape, radially or regularly furrowed edges, and a wrinkled center. Conidiophores are smooth-walled, up to 300 µm long and 3–5 µm wide. Conidia are verrucose, single-celled, and have dimensions of 2.0–8.0 x 2.0–4.0 µm. They are spherical, ellipsoidal, or cylindrical with rounded ends.

Culture morphology and microscopic characteristics of PS were; branched and septate mycelium; hyaline, thin-walled, 1.5–2 µm broad hyphae. Hyaline, smooth, single, unbranched or rarely branched conidiophores with thin walls. Phialides can be terminal, lateral, cylindrical, subcylindrical, or ampulliform. They ranged in length from 11–41 µm, with a base width of 2.3–3.3 µm and prominent periclinal thickening at the conidiogenous locus. 2-celled, hyaline, thick and smooth and thick-walled septate conidia were ellipsoidal or cylindrical, having apiculate bases and obtuse apices, and grouped in slimy heads. One-celled, hyaline, aseptate conidia with cylindrical or elliptical shapes that are acute at the apex and base, measuring 5–8 x 2.1–3.3 µm.

Molecular Identification

Based on the BLAST homology search the fungal strains were identified as *Plectosphaerella* sp. and *Cladosporium sphaerospermum*. The phylogenetic tree as shown in Fig. 4a & b shows the relative

position of the test isolates compared to the closest related strains identified through BLAST.

Figure 4 (a & b) Phylogenetic position of the test isolates (shown in bold) with respect to the closest neighbours found based on BLAST homology

Table 3 Identification of fungal isolates with their gene accession numbers					
Host	Fungal isolates	Isolates identified	Gene bank Accession number	Phylum	Family
A.Calamus	IACR01	<i>Plectosphaerella</i> sp.	OR461558.1	Ascomycota	Plectosphaerellaceae
	IACR02	<i>Plectosphaerella</i> sp.	OR438756.1	Ascomycota	Plectosphaerellaceae
	IACL03	<i>Cladosporium sphaerospermum</i>	OR461556.1	Ascomycota	Cladosporeaceae
	IACL04	<i>Cladosporium sphaerospermum</i>	OR452986.1	Ascomycota	Cladosporeaceae

Screening for enzymatic activity

It is well recognized that microbial enzymes, which come from fungi and bacteria, are superior enzymes, especially for commercial-scale uses in industries. Rather than using chemical catalytic pathways, they carry out reactions in bio-processes in a cost- and eco-friendly manner. Tyrosinase, laccase, cellulase, protease, pectinase, and amylase activities were all examined in the two isolates. Figure 5,6 and Table 4,5 show the screening test findings, which were conducted on selective agar plates.

Oxidoreductase activity

The presence of tyrosinase and laccase activity was detected by observing the change in the colour of the fungal colony. Both the isolates showed positive results for tyrosinase activity, whereas laccase activity was recorded only for PS. Producing these extracellular enzymes may serve as a defence mechanism for the host against pathogenic invasion, a means of obtaining nutrients from the host, or a sign that the organism is a latent pathogen (Choi et al.,2005; Saikkonen et al.,1998; Saikkonen et.al.,2004). Moreover, both of these activities are of potential industrial use in removing toxic phenols (Argyeropoulos, 2001) and detoxification of pulp and paper plant effluents (D’Souza, 2001).

Hydrolytic activity

Using a selective medium together with the agar plate method, the hydrolytic enzyme production potential of isolated fungal endophytes was qualitatively assessed. Results revealed that PS has the maximum protease and pectinase activity with clear zones $7.3 \pm 0.75\text{mm}$ and $6.8 \pm 0.13\text{mm}$, respectively. On the other hand, the highest cellulase activity was recorded for the CS with a clear zone of $13.8 \pm 1.09\text{ mm}$

(Fig. 5,6 and Table 4,5.). Interestingly, both endophytic fungal isolates exhibited cellulase and pectinase activity; cellulase is considered the dominant hydrolytic enzyme of microbial origin among others, which helps endophytes penetrate the plant tissue through the lignocellulosic wall. Cellulase, pectinase, and protease, which are hydrolytic enzymes, are linked to hyperparasitic activity in this context and aid in the fungal penetration of plant cells. These enzymatic processes also increase the induced systemic resistance.

The hydrolytic enzymes released by fungal endophytes are employed in biotechnological applications to facilitate the industrial handling of the breakdown of proteins and polysaccharides. According to Kavamura et al. (2013), hydrolytic enzymes generated by endophytes may help plants flourish by preventing soil-borne pathogens from causing plant diseases.

Fungal enzyme production can vary depending on the growth stage and developmental phase of the fungus. This might explain the negative results for amylase activity by both the isolates and the absence of protease activity, in addition, to CS. Further, host-specific factors and interactions may affect the expression of fungal enzymes.

Table 4
Zone of clearance shown by fungal isolates.

Enzymes	Zone of clearance (in mm)	
	<i>Plectosphaerella sp.</i>	<i>Cladosporium sphaerospermum</i>
Cellulase	2.3 ± 0.27	13.8 ± 1.09
Pectinase	6.8 ± 0.13	5 ± 0.6
Protease	7.3 ± 0.75	ND
Siderophore	2.8 ± 1.09	ND

Data represent mean ± standard error mean (SEM) of three biological replicates. ND. Not Detected

Table 5
The potential of endophytic fungi to produce extracellular enzymes on solid media.

Enzymes	Endophytic fungi	
	<i>Plectosphaerella sp.</i>	<i>Cladosporium sphaerospermum</i>
Amylase	ND	ND
Cellulase	+	+
Pectinase	+	+
Protease	+	ND
Tyrosinase	+	+
Laccase	+	ND
ND : Not detected		

Siderophore activity

Due to the potential use of these chelators in both agricultural and medical applications, microbial siderophore synthesizers have drawn greater attention (Neilands 1993, 1995). In this preliminary study among the two isolates, PS showed an orange halo zone of 2.8 mm around the mycelia plug, indicating its ability to produce siderophore, whereas CS exhibited a negative result (Fig. 6). Previously, Chowdappa.al.,2020 and Aramsirujiwet et al.,2016 also reported that about half of the tested fungi demonstrated the ability to produce siderophore and scavenge iron.

Figure 7A. Orange halo zone of fungal colony indicates the Siderophore production potential by the isolate *Plectosphaerella sp.***B.***Cladosporium sphaerospermum* showing negative result.

Antibacterial activity

To address multidrug-resistant microbial pathogens, there is an urgent need for novel drugs to treat infectious diseases in plants and animals including humans. (Monciardini et.al., 2014). The investigation of the antimicrobial activity of the isolated fungal endophytes using dual culture assay revealed antibacterial activity against plant and animal pathogens. Amongst the two isolates, CS demonstrated antibacterial activity against all the animal and plant pathogens used with maximum zone of inhibition against *B. megaterium* (30.67 ± 0.33), *X. phaseoli* (31.50 ± 1.76), *A. tumefaciens* (27.17 ± 0.44), *X. campestris* (26.50 ± 1.04), *L. monocytogenes* (25.83 ± 1.74), and *S. typhi* (24.33 ± 1.76). Another isolate i.e. PS, though not active against all tested pathogens exhibited good antibacterial activity with maximum zone of inhibition against *S. aureus* (16.67 ± 1.20), *X. campestris* (15.33 ± 0.88), *E. coli* (14.83 ± 0.44), and *B. megaterium* (13.17 ± 0.73). These findings are extremely important, especially in light of the rising drug resistance of microorganisms. Thus, using these endophytes as a source of bioactive

substances may be promising for the development of new medications that could combat infectious diseases.

The present study's results are encouraging, and they strongly urge additional research, such as the isolation or fractionation of extracellular enzymes from these endophytes, their characterization, and confirmation of the mechanism involved. Previously Barik et al., (2010); Mani and Audipudi (2016), and Mohanta et al. (2008) have reported endophytic fungi from rhizomes of *A. calamus*. Furthermore, among the two isolates, *Cladosporium sphaerospermum* was recorded as a growth-promoting endophyte from *Glycine max* (Hamayun et al., 2009), whereas *Plectosphaerella* sp. was identified as soil-borne and pathogenic (su et.al., 2017). This is the first report on the enzymatic, siderophore, and antimicrobial activity of any endophytes from the investigated plant, to the best of our knowledge.

Table 6
Antibacterial activity of *Plectosphaerella* sp. and *C. sphaerospermum* against pathogenic bacterial strains. Data represent mean $\pm$ standard error mean (SEM) of three biological replicates. ND. Not Detected

Bacterial strains	Zone of inhibition (mm)	
	<i>Plectosphaerella</i> sp.	<i>C. sphaerospermum</i>
<i>Listeria monocytogenes</i>	9.17 $\pm$ 0.83	25.83 $\pm$ 1.74
<i>Salmonella typhi</i>	9.50 $\pm$ 0.76	24.33 $\pm$ 1.76
<i>Bacillus cereus</i>	13.33 $\pm$ 1.59	22.40 $\pm$ 1.86
<i>Staphylococcus aureus</i>	16.67 $\pm$ 1.20	7.83 $\pm$ 1.86
<i>Pseudomonas aeruginosa</i>	ND	11.17 $\pm$ 1.59
<i>Escherichia coli</i>	14.83 $\pm$ 0.44	8.17 $\pm$ 0.33
<i>Bacillus megaterium</i>	13.17 $\pm$ 0.73	30.67 $\pm$ 1.64
<i>Xanthomonas campestris</i>	15.33 $\pm$ 0.88	26.50 $\pm$ 1.04
<i>Xanthomonas phaseoli</i>	ND	31.50 $\pm$ 1.76
<i>Ralstonia solanacearum</i>	10.67 $\pm$ 0.8	22.83 $\pm$ 0.44
<i>Agrobacterium tumefaciens</i>	ND	27.17 $\pm$ 0.44

Conclusion

Some key insights are provided by our current research into the bioactive potential of two new endophytes from the medicinal plant *A. calamus* as an alternative source of many biologically active compounds. This is the first account of two new records of endophytic fungi in this medicinal plant, *A. calamus*, and their valuable bioactive potential, i.e., enzymatic, siderophore, and antimicrobial activity. This investigation provides an avenue for taking up future research and development activities on these

fungal endophytes as an alternative source of various valuable compounds. An in-depth investigation of these species is required to assess their potential to be used in the industrial and pharmacology sectors.

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Figures

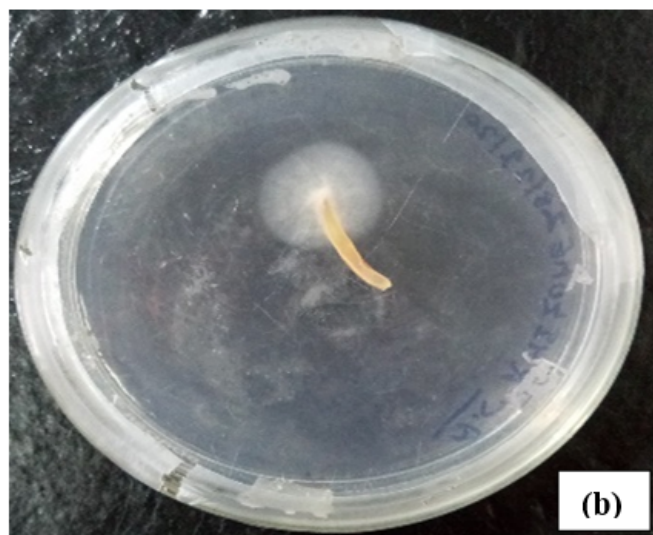
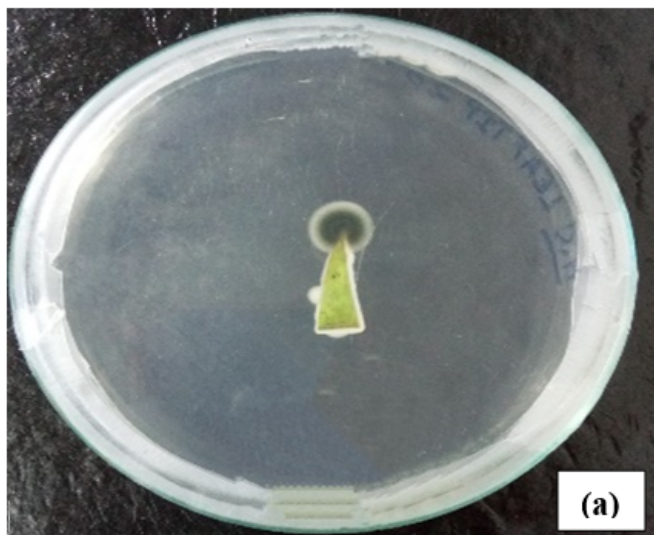


Figure 1

Infected fragments of leaf and rhizome by endophytic fungi in PDA medium **(a)** Leaf **(b)** Rhizome

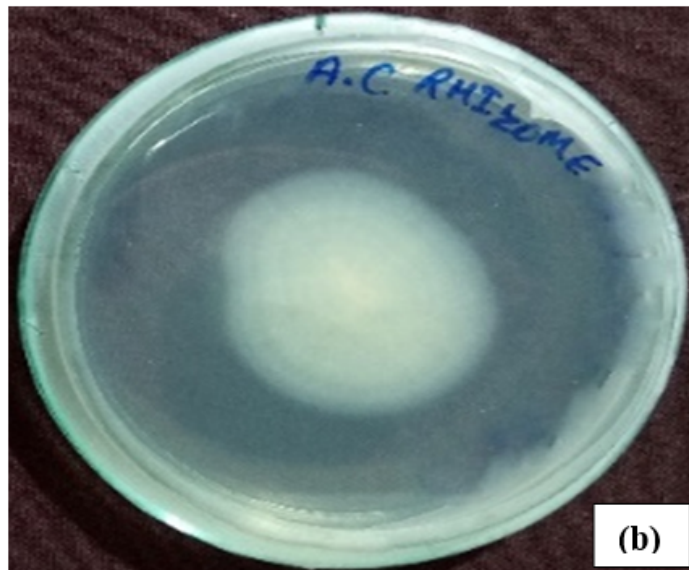
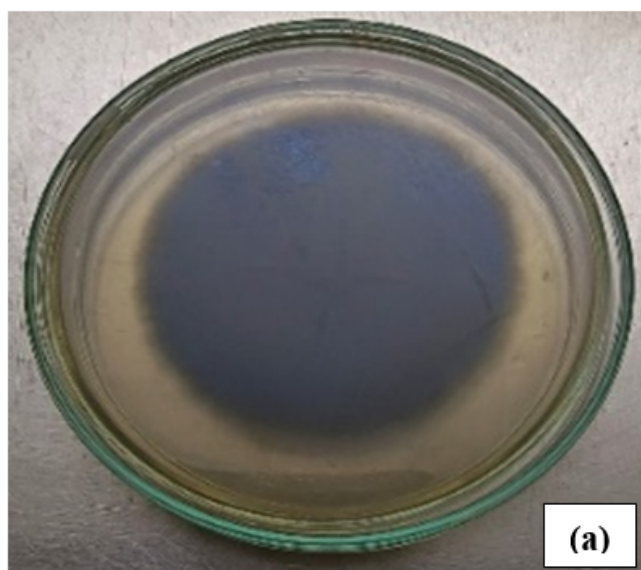


Figure 2

Morphological images of fungal isolates on PDA **(a)** *Cladosporium sphaerospermum* **(b)** *Plectosphaerella* sp.

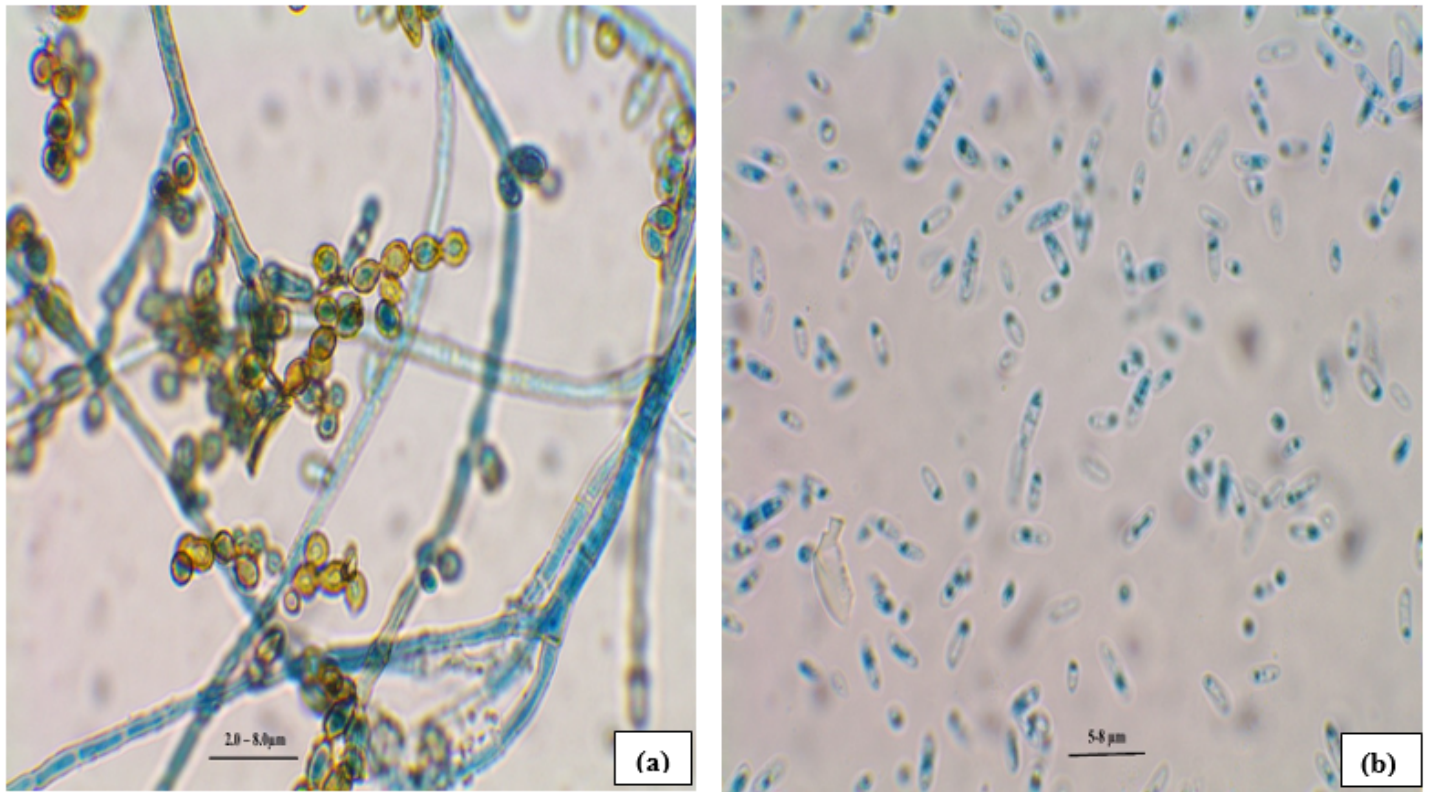
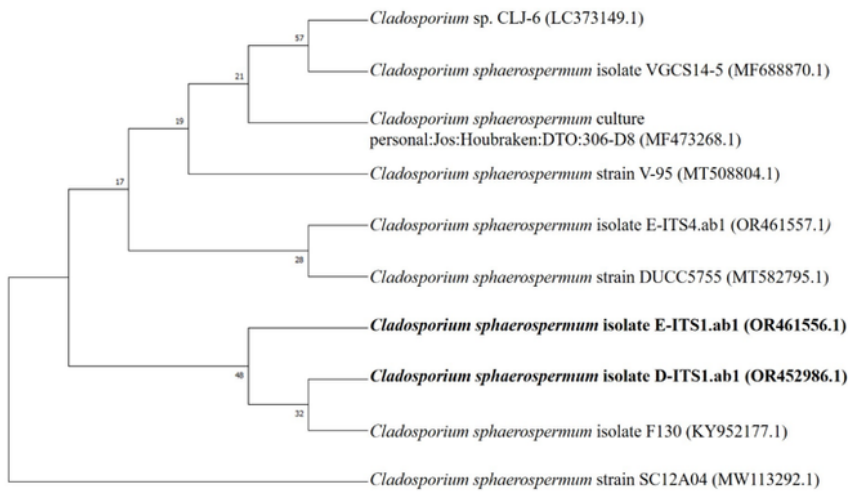
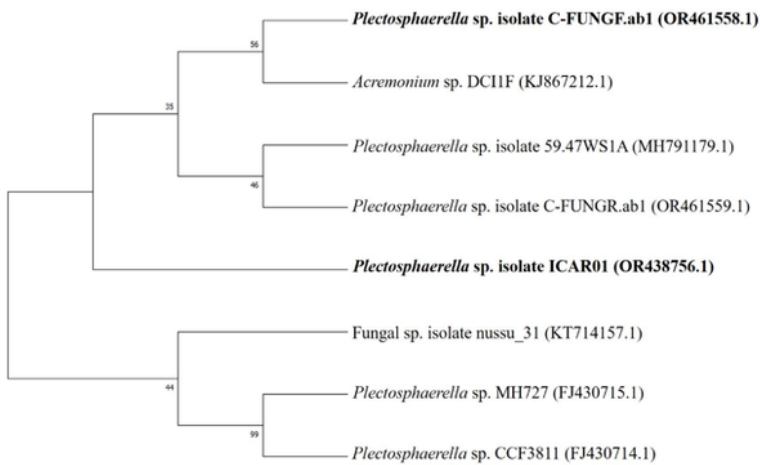


Figure 3

Microscopic images of fungal isolates on PDA **(a)** *Cladosporium sphaerospermum* **(b)** *Plectosphaerella* sp.



(a)



(b)

Figure 4

(a & b) Phylogenetic position of the test isolates (shown in bold) with respect to the closest neighbours found based on BLAST homology

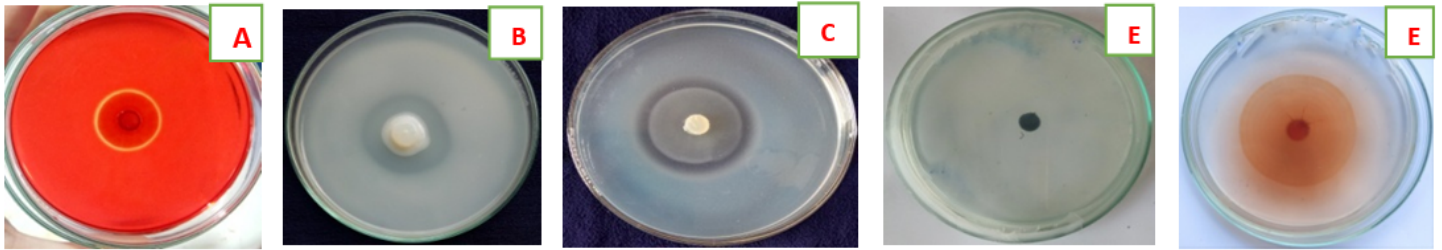


Figure 5

Enzymatic activity by *Plectosphaerella* sp. **A.**Cellulase **B.**Pectinase **C.**Protease **D.** Laccase **E.**Tyrosinase

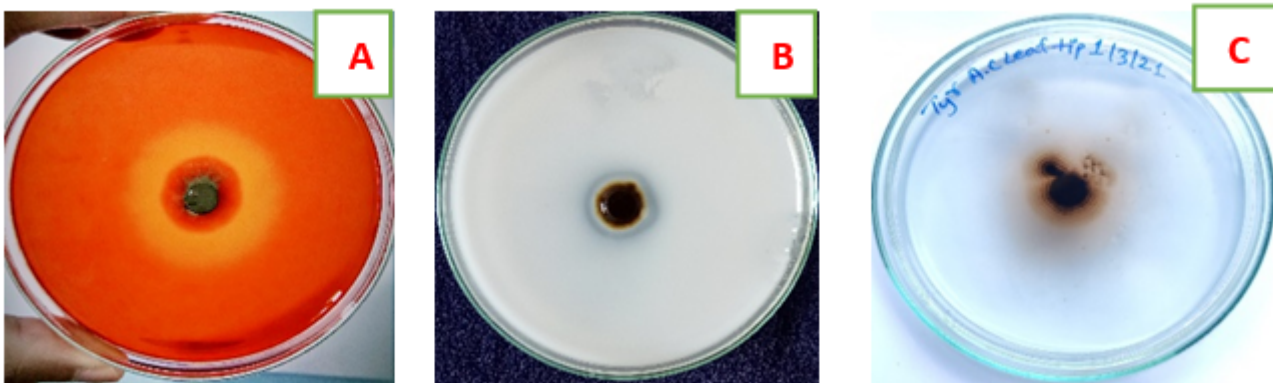
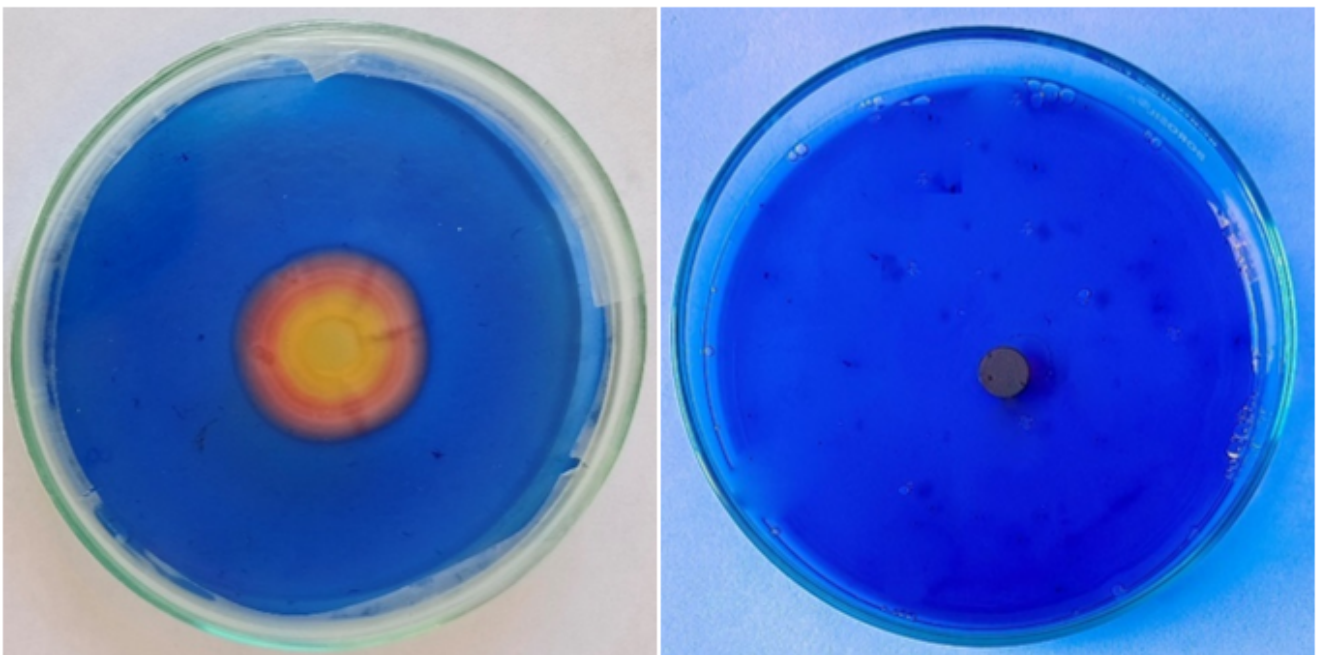


Figure 6

Enzymatic activity by *Cladosporium sphaerospermum* **A.**Cellulase **B.**Pectinase **C.**Tyrosinase.



A. *Plectosphaerella* sp.

B. *C. sphaerospermum*

Figure 7

A. Orange halo zone of fungal colony indicates the Siderophore production potential by the isolate *Plectosphaerella* sp. B. *Cladosporium sphaerospermum* showing negative result.

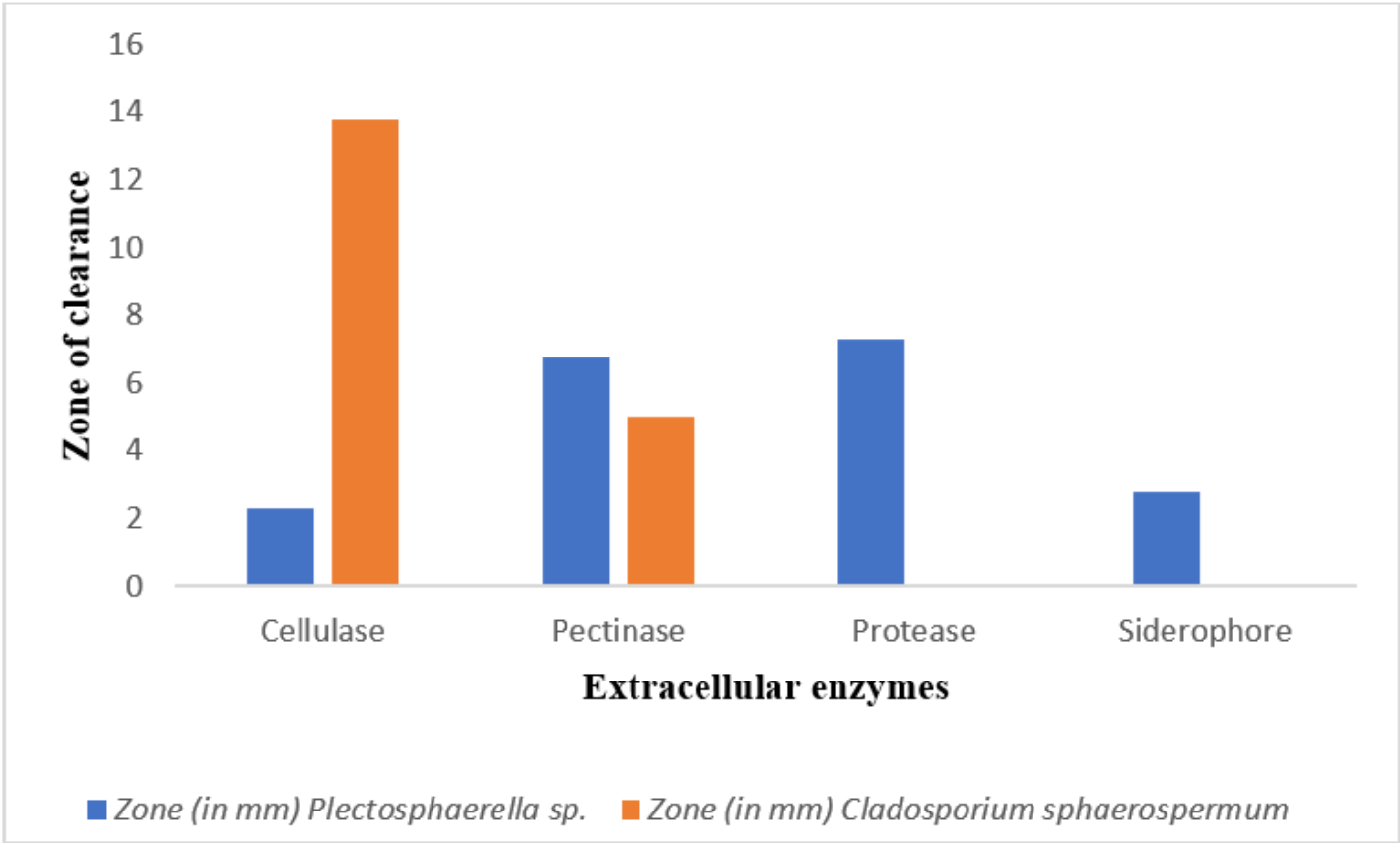
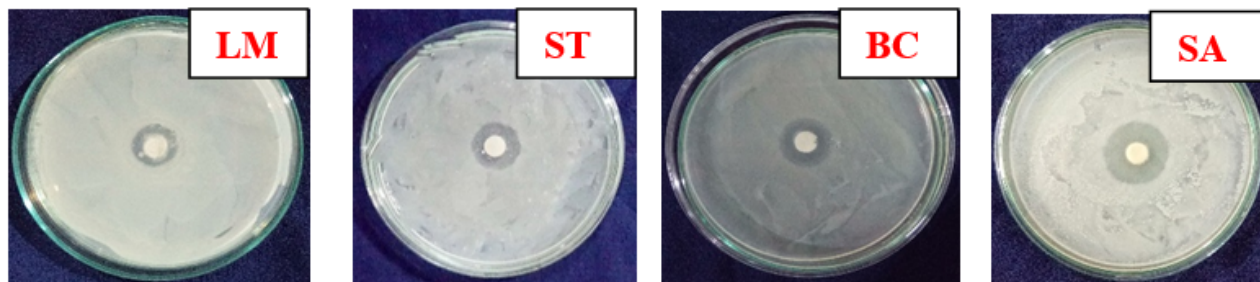
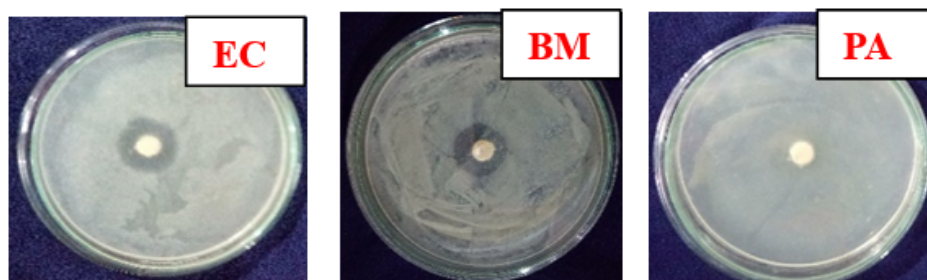


Figure 8

Enzymatic and siderophore production activities of fungal isolates.



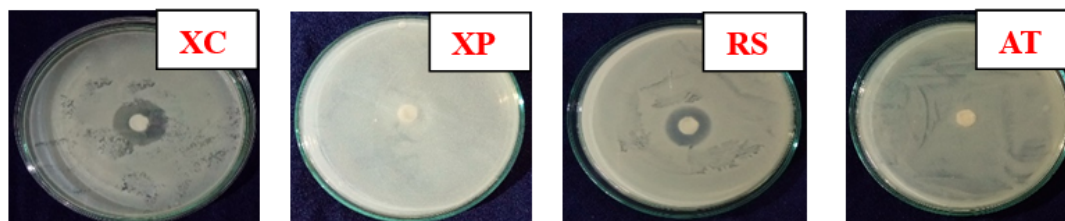
LM *Listeria monocytogenes* **ST** *Salmonella typhi* **BC** *Bacillus cereus* **SA** *Staphylococcus aureus*



EC *Escherichia coli* **BM** *Bacillus megaterium* **PA** *Pseudomonas aeruginosa*

Figure 9

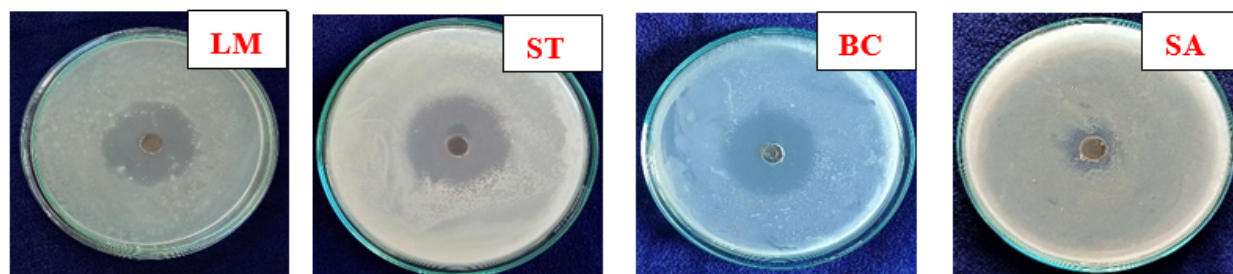
Antibacterial potential of *Plectosphaerella* sp. against animal pathogen by Agar well diffusion method.



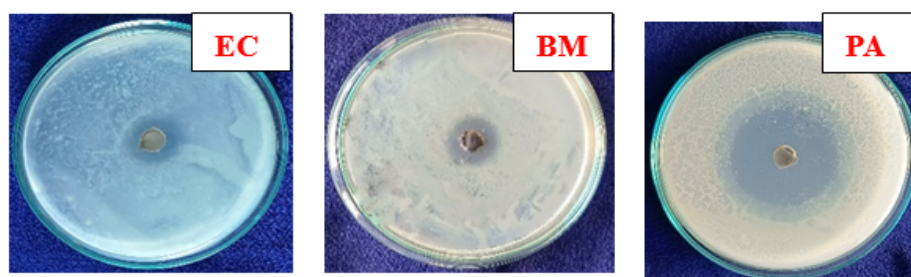
XC *Xanthomonas campestris* **XP** *Xanthomonas phaseoli* **RS** *Ralstonia solanacearum* **AT** *Agrobacterium tumefaciens*

Figure 10

Antibacterial potential of *Plectosphaerella* sp. against plant pathogens by Agar well diffusion method.



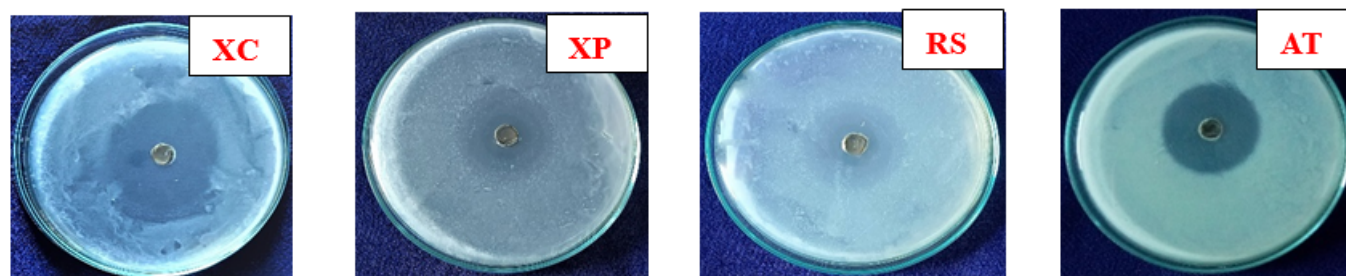
LM *Listeria monocytogenes* **ST** *Salmonella typhi* **BC** *Bacillus cereus* **SA** *Staphylococcus aureus*



EC *Escherichia coli* **BM** *Bacillus megaterium* **PA** *Pseudomonas aeruginosa*

Figure 11

Antibacterial potential of *C. sphaerospermum* against animal pathogens by Agar well diffusion method.



XC *Xanthomonas campestris* **XP** *Xanthomonas phaseoli* **RS** *Ralstonia solanacearum* **AT** *Agrobacterium tumefaciens*

Figure 12

Antibacterial potential of *C. sphaerospermum* against plant pathogens by Agar well diffusion method.

Supplementary Files

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