Isolation of Endophytic Fungi from Wild Medicinal plant *Helecteresisora* and their Antibacterial Properties

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Abstract
In the present investigation the diversity of endophytic fungi was studied associated with medicinaly important plant *Helecteresisora* in three different seasons. Secondary metabolites were produce from isolated endophytic fungi to investigate their antibacterial activity.Thefungal culture was extracted with ethyl acetate and crude extract was used to test against pathogenic bacteria viz. *Pseudomonas aeruginosa*,(MTCC 6458), *Escherichia coli* (MTCC 1698),and *Staphylococcus aureus*(MTCC 2639). A total of 13 fungal species viz.*Colletotrichumacutatum, Colletotrichumgloeosporiodes, Pithomyceschartarum, Aspergillusnidulance, Trimmatostromahughesii, Curvularialunata, Cladosporiumcladosporiodes, Nigrosporaoryzae, Alternariaalternata ,Aspergillusstellatus,Acremoniumkiliense,Fusariumoxysporum, Penicilliumchrysogenum*were isolated and identified based on the morphology of the fungal cultureand characteristics of the spores.

Key words;**Antibacterial potential, Seasonal variation, Amravati.**

Introduction

Endophytic fungi are microbes that colonize living internal tissues of plants without causingany harm to their host(Brown *etal* 1998).Many recentstudies have revealed the ubiquity of thesefungi, with an estimate of at least one millionspecies of endophytic fungi residing in plants (Dreyfuss*etal*. 1994). Endophytes particularly fungi have proved to be an potential source for bioactive compounds which have immense value in agriculture, medicine and industry (Tan et al., 2000; Tan and Zou,2001; Aly et al., 2010; Shankar and Krishnamurthy, 2010). Many important bioactive compounds with,cytotoxic, insecticidal, anticancer and antimicrobial potential have been successfully obtained from the endophytic fungi (Aly et al., 2010;Zhou et al., 2008).and recently numerous novel bioactive substances characterised from these microorganisms (Wagenaar et al., 2001; Li and Strobel et al., 2001; Brady, 2001; Shrestha et al., 2001; Kongsaeree et al., 2003).The study of endophytedistribution,biodiversity and their biochemical characteristics are of immense importance in plant biology to understand and to improve plant fitness. Medicinal plants are reported to harbourendophytes (Strobel, 2002), and have a potential to produce biologically active metabolites to protect their host from infectious agents and also provide adaptability to survive in adverse conditions .

The objective of this study was to isolate endophytic fungi from the medicinal plant *Helecteresisora*in three different seasons and to study the antimicrobial activity of the isolates against various pathogenic microorganisms.

Materials and Methods

Sample Collection

Medicinal Plant *Helecteresisora* was collected from various parts of Amravati district in three different seasons. The samples were brought to laboratory in sterile paper bags and storedat 4°C till further use.

Isolation of Endophytic Fungi

Collect sample were rinsed gently in running water to remove adhered dust and debris and
cut into 1-2 mm segments. Surface sterilization were done according to themethod described by (Suryanarayanan*et al*, 2011).The sterilized samples were placed in Petridishes containing potato dextrose agar (PDA) Petri dishes were sealed with parafilm and incubated at room temperature (25±2°C) for one week. The fungi growing out from the samples were sub cultured on fresh(PDA) medium to get pure culture.

Identification of Endophytic Fungi

The endophytic fungi were identified based on morphological characters of fungalculture and spores by standard mycological manuals (Barnett and Hunter 1998, Sutton1980,Subramanyam 1971).

Disc diffusion assay

The assay was conducted as per the procedure defined by Jorgensen and Turnidge (2007). The crude extracts were dissolved dimethyl sulfoxide (DMSO).The test organisms with theinoculum size of 10^5 colony-forming units (CFU)/ mL were streaked on the surface of the media Muller-Hinton agar (Hi-media) using sterile cotton swab.SterileWhatman paper disc impregnated with 20 µl of each extracts. DMSO was applied as a negative control to detect the solvent effects. The plates were incubated at 37°C for 24 h. The diameter of the clear zones surrounding the disc were measured.

Results

The plant materials of *Helecteresisora* was collected from different parts of Amravati district in three different seasons.. About 920 segments (320 segments of leaf,350 segments of stem, 250 segments of petiole) were processed for the isolation of endophytic fungi. A total thirteen endophytic fungi viz.*Colletotrichumacutatum, Colletotrichumgloeosporiodes, Pithomyceschartarum, Aspergillusnidulance, Trimmatostromahughesii, Curvularialunata, Cladosporiumcladosporiodes, Nigrosporaoryzae, Alternariaalternata ,Aspergillusstellatus,Acremoniumkiliense,Fusariumoxysporum, Penicilliumchrysogenum*were isolated.Colonizing frequency were high during monsoon and winter season where as lowest during summer(Table No.1).

Antibacterial activity of the endophytic fungal extracts against three pathogenic bacteria such as *Pseudomonas aeruginosa*,(MTCC 6458), *Escherichia coli* (MTCC 1698),and *Staphylococcus aureus*(MTCC 2639) were tested by using disc diffusion method. Most of the fungal extracts showed antibacterial activity on test bacteria(Table 2).Ethyl acetate extract of the tested fungal isolates exhibits highest zone of inhibition against *Pseudomonas aeruginosa*, (MTCC 6458) and *Staphylococcus aureus*(MTCC 2639). whereasweak against *Escherichia coli* (MTCC 1698).(Fig.1-3).

Table No .1.Colonization frequency of endophytic fungi isolated from the different parts of *Helecteresisora*

Sr.No.	Endophytes	Seasons								
		Monsoon			Winter			Summer		
		Stem	Leaf	Petiole	Stem	Leaf	Petiole	Stem	Leaf	Petiole
	Penicilliumchrysogenum	_	_	_	_	_	_	9.78	4.94	2.56
	Fusariumoxysporum	_	_	_	_	_	_	5.23	10.02	1.96
	Acremoniumkiliense	33.65	24.35	22.12	_	_	_	_	_	_
	Aspergillusstellatus							7.58	3.88	6.05
	Alternariaalternata	49.51	31.27	25.45	_	_	_	_	_	_
	Nigrosporaoryzae	20.81	19.22	27.24	27.04	18.23	21.56	_	_	_
	Cladosporiumcladosporiodes	_	_	_	24.16	26.33	48.33	_	_	_
	Curvularialunata	16.90	35.83	37.54		37.73	41.22	_	_	_
	Trimmatostromahughesii	46.20	34.52	29.12	31.05	33.96	_	_	_	_
	Aspergillusnidulance	_	_	_	_	_	_	10.32	6.38	5.40
	Pithomyceschartarum	_	_	_	_	_	_	_	_	_
	Colletotrichumgloeosporiodes	20.47	15.44	29.64	16.20	18.19	16.48	_	_	_
	Colletotrichumacutatum	21.33	17.80	30.16	19.88	31.24	19.30	_	_	_

Table 2.Antibacterial activity of endophytic fungi isolated from *Helecteresisora*

Sr.No.	Name of the fungi	Zone of inhibitions in mm		
		Sa	Pa	E.coli
	Fusariumoxysporum	9.33	15.66	16.33
	Acremoniumkiliense	14.33	0.00	7.00
	Aspergillusstellatus	13.66	10.00	15.33
	Alternariaalternata	17.33	12.66	18.25
	Nigrosporaoryzae	7.00	17.33	8.66
	Cladosporiumcladosporiodes	12.20	14.56	15.20
	Curvularialunata	16.30	15.14	9.22
	Colletotrichumacutatum	10.23	0.00	6.36

Sa :*Pseudomonasaeruginosa*,E.coli:*Escherichia coli*, Pa:*Staphylococcusaureus*

Discussion

Endophytesare organisms that colonize the living tissues of their hosts without showing any symptoms of its presence. Several endophytic fungi have been isolated from a variety of plant which have proved as a rich source of biologically active metabolites. (Devi etal2013). Medicinal plants have been considered potential source of endophytes synthesizing associated plant natural products.(Strobel and Daisy2003).In this study, six endophytic fungi ie.*Phomacrysanthemicola*,*Verticilliumalbo-atrum* ,*Acremoniumaff.kiliense*,*Aspergillusstelatus*,*Fusariumoxysporum*,*Epicoccumnigrum*have been isolated, from the stem,leaf and petiole of the *Helecteresisora*.

Many studies are evaluating metabolic activity from endophytic fungi isolated, mostly, of plants with properties medicinal. (Pileggi*et al.* 2002), studied antimicrobial action in endophyte fungi isolated form medicinal plant *Simphytumofficinale*, popularly known as confrei. These isolated inhibitgrowing of pathogenic bacteria *S. aureus*..(Souza *et al.* 2004), tested the antimicrobial activity ofendophytes from Amazonian toxic plants *Palicourealongiflora* and*Strychnoscogens.* From total of 79 fungal isolates whose metabolites were tested, 19 inhibited at least one of the pathogenic microorganisms tested: *Bacillus* sp., *B.subtilis, S.aureus, E. coli,Candida albicans, Trichoderma*sp., and *Aspergillusflavus.*(Sutjaritvorakul*et al.* 2011) used the paper disk susceptibility test to evaluate the antimicrobial activity of metabolites produced by fungal endophytes against five reference human pathogenic microorganisms (*S. aureus, B. subtilis, Pseudomonas aeruginosa, E. coli* and *C. albicans*).

Similarly to the present study, (Teles*et al.* 2006) also extracted secundary metabolites from *Periconiaatropurpurea*, an endophyte from *Xylopiaaromatica*, using ethyl acetate. Besides identifying these compounds, the authors tested their biological activity, proving their cytotoxic and antifungal potential.

The results of this study demonstrate the antimicrobial potential of endophytic fungi isolated from *Helecteresisora*agaist bacterial pathogens.Ethyl acetate crude extracts produced by the endophytic isolates showed promising results for growth inhibition of pathogenic bacteria. Therefore, it suggests that these endophytes can be important sources of bioactive substances.

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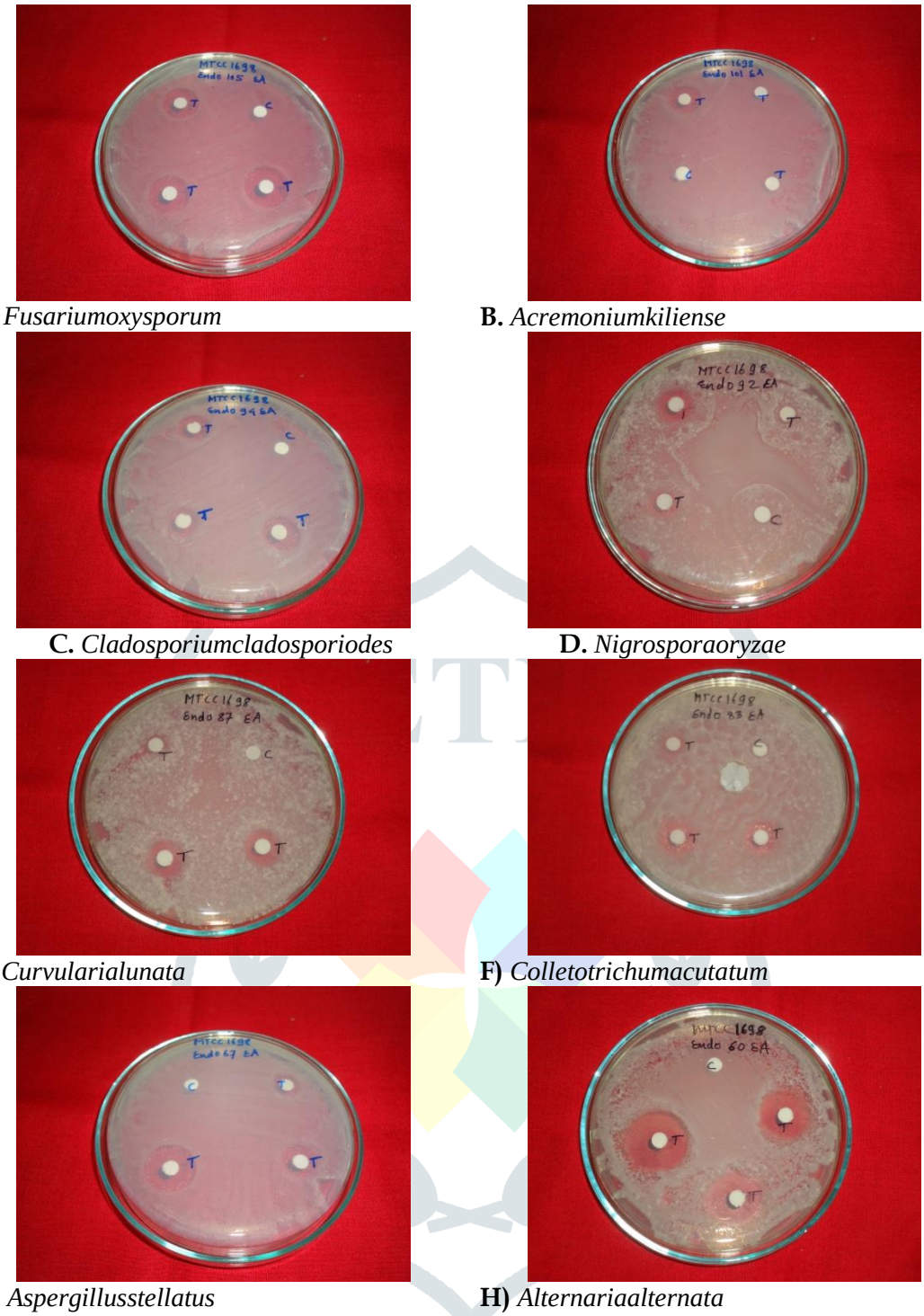


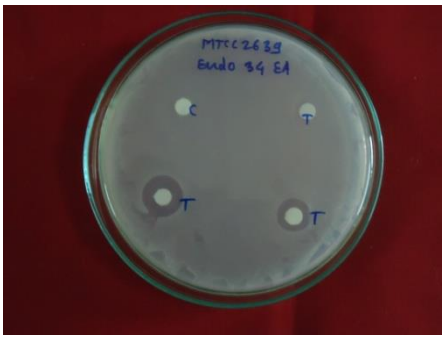
A. *Fusariumoxysporum* **B.** *Acremoniumkiliense*

C. *Cladosporiumcladosporiodes* **D.** *Nigrosporaoryzae*

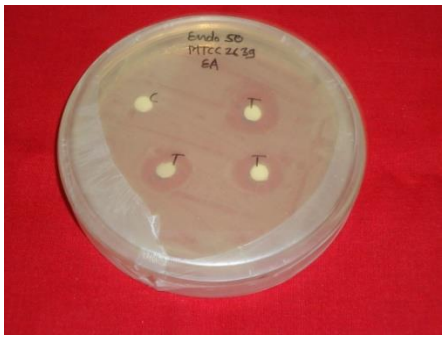
E) *Curvularialunata* **F)** *Colletotrichumacutatum*

G) *Aspergillusstellatus* **H)** *Alternariaalternata*

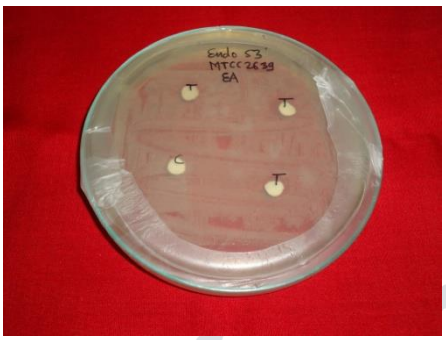
Fig.1.Antibacterial activity of isolated Endophytic fungi against *Escherichia coli*



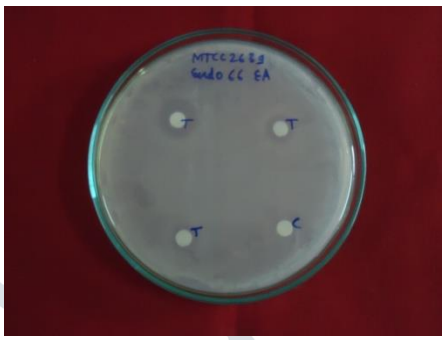
A. *Curvularialunata*



B. *Alternariaalternata*

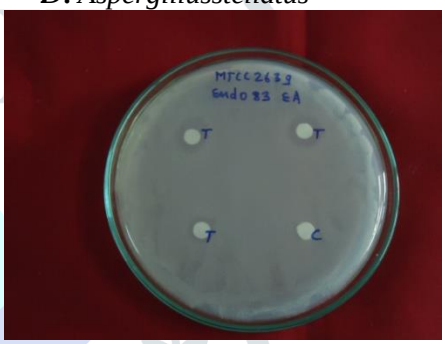


C. *Nigrosporaoryzae*



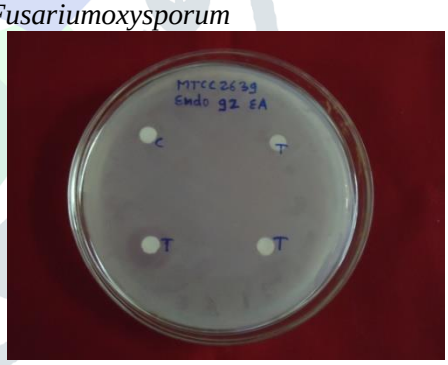
D. *Aspergillusstellatus*

E*Acremoniumkiliense*



F) *Fusariumoxysporum*

G) *Colletotrichumacutatum*



H) *Cladosporiumcladosporiodes*

Fig.2.Antibacterial activity of isolated Endophytic fungi against *Staphylococcus aureus*

C. *Fusariumoxysporum*

D. *Cladosporiumcladosporiodes*

E) *Alternariaalternata*

F) *Nigrosporaoryzae*

G) *Aspergillusstellatus*

H) *Curvularialunata*

Fig.3.Antibacterial activity of isolated Endophytic fungi against *Pseudomonas aeruginosa*