Major Research Project

Screening, Identification and Evaluation of Potential Biocontrol Fungal Endophytes of Lamiaceae Against Plant Pathogens

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Name of the Principal Investigator

Dr. Rahul B. Allapure

Head Dept. of Botany

Maharashtra UdayagiriMahavidyalaya ,Udgir

Dist. Latur 413517

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1. Introduction

Endophytic fungi have been described as fungi that asymptomatically colonize healthy plant tissues, eventhough they may, after incubation or a latency period,cause diseases (Petrini 1991; Stone et al. 2000). Diverseassociationswith host plants have been reported, rangingfrom mutualistic relationships (Schulz et al. 2002) and cryptic commensalism (Deckert et al. 2001) to latent andquiescent pathogens (Sinclair and Cerkauskas 1996).

The stability or the variability of the asymptomatic interaction depends on numerous factors such as environmentalstress, senescence of the hosts, virulence ofthe endophytes and the host defense response (Schulzand Boyle 2005). Endophytes may be beneficial to thehosts in conferring resistance to insects and herbivores(Clay 1988), drought tolerance (West 1994), protectionagainst pathogens (White and Cole 1985) and enhancedvegetative growth (Porter et al. 1979).Endophytic fungi have been encountered from anyplant ever investigated. In contrast, endophytic fungi inmedicinal plants, especially in tropical regions, are stillpoorly explored though they could represent a source of valuable new and bioactive compounds (Li et al.2001). Recently, endophytic fungi isolated from a Brazilian medicinal plant were shown to produce guignardic acid (Rodrigues et al. 2001). Likewise, newbioactive metabolites were produced by a *Colletotrichum sp*., an endophytic fungus in *Artemisia annua*, a traditional Chinese medicinal herb (Lu et al. 2000).

The study of fungal endophytes, and in particular their diversity and the understanding of the symbiotic interaction between fungal endophytes and their host, is related to several aspects of biology. For example, to investigate the evolutionary origins of these diverse symbiotic fungi, to determine the community structure and metabolic activity

of all fungal symbionts associated with plants across landscapes, to learn more about the contribution of endophytes to plant gene expression, and several questions concerning the biology of endophytes (Rodriguez, White et al. 2009).

Table 1 Symbiotic criteria used to characterize fungal endophytic classes

	Clavicipitaceos	Nonclavicipitaceous		
Criteria	Class 1	Class 2	Class 3	Class 4
Host range	Narrow	Broad	Broad	Broad
Tissue(s) colonized	Shoot and rhizome	Shoot, root and rhizome	Shoot	Root
In planta colonization	Extensive	Extensive	Limited	Extensive
In planta biodiversity	Low	Low	High	Unknown
Transmission	Vertical and horizontal	Vertical and horizontal	Horizontal	Horizontal
Fitness benefits*	NHA	NHA and HA	NHA	NHA

*Nonhabitat-adapted (NHA) benefits such as drought tolerance and growth enhancement are common among endophytes regardless of the habitat of origin. Habitat-adapted (HA) benefits result from habitat-specific selective pressures such as pH, temperature and salinity.

1.1 Properties of fungi and their role

Fungi are among the most widely distributed organisms on earth. Fungi are found in an enormous diversity of habitats and these habitats have diverse characteristics that determine the types of fungi found in them.

Fungi are organized into five phyla *Oomycota, Zygomycota, Ascomycota* (the largest phylum of fungi), *Basidiomycota, Deuteromycetes* and then into classes and orders (Guarro, Gene et al. 1999). They possess characteristic properties that are the key to their specialized lifestyle. All are chemoheterotrophic and obtain their nutrients by absorption, excrete a diversity of enzymes that digest the complex compounds outside the thallus “a type of body that is not differentiated into roots, stems, or leaves” , and then absorb the broken down products. The unit component of growth and development of fungi is the hypha. The hyphae can penetrate in media that gives them access to the nutrition that is inaccessible to other microorganisms. Fungal walls consist mostly of polysaccharides, where most of them have a complex fibrillar structure built primarily on chitin, chitosan, β- glucans and other Fungi play complex and important ecological roles in the ecosystem, as they continue the cycle of nutrients through ecosystems by breaking down dead organic material, and providing nutrients to plants. Saprophytic fungi are the primary decomposers of plants and woody debris. By decay of cellulose and lignified cellulose, they returned carbon to the atmosphere as carbon dioxide.

Mycorrhizal fungi that inhabit plants roots form mutualistic associations, where the fungal hyphae function is to absorb water and minerals from the soil and protect the roots from parasitic fungi, in return, the plants supplies fungus with carbohydrates (Bornemanand Hartin 2000). In terms of their role in humans, fungi, except those that have feeder roles, have proven to be effective curative agents and produce numerous secondary metabolites that have valuable therapeutic properties, such as the antibiotic penicillin derived from the fungi *Penicillium notatum* or the polyketides aflatoxin derived from fungi *Aspergillus flavus* and *A. parasiticus*, (Group 2005). Yeast fungus such as *Saccharomyces cereviseae* are the primary agents responsible for the fermentation.

In agriculture, fungi can provide a means to control plant pests (Luis C. Mejíaa 2008). On the other hand, fungi can also cause a number of devastating plant diseases that affect crop yields. An example of that is the fungus *Puccinia triticina*, the cause of wheat leaf rust (Bolton, Kolmer et al. 2008).

1.2 Characteristics of Fungal endophytes

A good definition of endophytes is provided by Petrini (1991); "All organisms inhabiting plant organs that at some time in their life can colonize internal plant tissues without causing apparent harm to the host".

Endophytic fungi are found in all divisions of fungi and the associations have evolved independently on many occasions (Sydney 2004). The most common endophytes are anamorphic (an artificial assemblage of asexual stages of *Ascomycetes* and *Basidiomycetes*) polysaccharides. In addition, proteins and lipids are found in the walls, usually in very small concentrations members of the

Ascomycetes and some are closely related to fungi known to cause disease in plants or animals.Unlike Mycorrhizae that colonize plant roots and grow out into the rhizosphere, endophytes reside entirely within plant tissues and may grow within roots, stems and/or leaves, emerging only to sporulate during plant or host-tissue senescence (Rodriguez 2009).

1.3 Importance of Fungal endophytes for plants

"All plants in natural ecosystems appear to be symbiotic with fungal endophytes" (Rodriguez 2009). Endophytes occur intercellularly within the leaves, stems, and reproductive organs of plants. A highly diverse group of fungi may offer significant benefits to their host plants by producing secondary metabolites that provide protection and survival value, such as conferring abiotic and biotic stress tolerance, increasing biomass and decreasing water consumption, enhancing insect and disease resistance. In some cases, endophytes decrease fitness by altering resource allocation (Rodriguez 2009).

Fungal endophytes have proved to be an important source for bioactive antimicrobial compounds such as alkaloids, peptides, steroids and phenol, which have a wide range of applications in the medical field (Strobel, Daisy et al. 2004) (Baby Joseph and R. Mini Priya and 2011). For example, naturally bioactive chemicals produced by the endophytic filamentous fungi *Fusarium spp*. and *Acremonium spp*show antimicrobial activity (Pannapa Powthong 2012). Another endophytic fungus *Pestalotiopsis* sp was isolated from the leaves of *Pinus caneriensis* and showed potent antimicrobial activity by inhibiting the growth of

all tested gram positive and gram negative bacteria (Bagyalakshmi Thalavaipandian A 2012).

1.4 Relationship between Fungal endophytes and plants

Research on fungal endophytes has largely focused on interactions with host plants, characterization of novel metabolites, and other topics related to endophytic symbioses(Seifert 2008). Phylogenetic evidence suggests that some endophytes have evolved from pathogens. The mechanisms of host recognition and colonization may be common among closely related endophytic and pathogenic fungi

1.5 Objectives:

1. The present study focuses on screening and identification of endophytic fungi isolated from members of Lamiaceae family.

2. The objective of this investigation is to determine the antagonistic potential (biological control) of fungal endophytes against selected plant pathogens.

3. Phylogenetic placement of the endophytic fungi isolated from plants and its possible evolutionary relationship with other fungal endophytes isolates.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Endophytic fungi were isolated from five lamiaceae family members from Udgir hilly areas .After plant selection, disease-free parts of the plant, that is, stem, root, and leaves, were excised with a sterile scalpel and placed in sterile plastic bags for storage at 4°C until use.The procedure of standardized and modified method described by Hallman *et al.* (2007).

2.2 Isolation of Endophytic Fungi

The endophytes were isolated using a modified method described by Arnold et al. The material was thoroughly washed in sterile water, surface-disinfected by soaking in 70% ethanol for 30 sec and 0.1% mercuric chloride ($HgCl_2$) solution for 2 min, and rinsed in sterile demineralised water. The plant material was subsequently rinsed in sterile demineralised water. Small pieces of inner tissues and needles were placed on aqueous agar (distilled water and 1.5% agar-agar) supplemented with antibiotic streptomycin (3 mg/100 mL) in petri plates and incubated at until fungal growth was initiated. The tips of the fungal hyphae were removed from the aqueous agar and placed on mycological medium, that is, potato dextrose agar (PDA: 300 g/L diced potatoes, 20 g/L dextrose and 20 g/L agar) or the Sabouraud agar (SA: 40 g/L dextrose, 10 g/L peptone, and 20 g/L agar). After several days of incubation, the purity of each fungal culture was assessed by examination of colony morphology. After purifying the isolates several times as described above, the final pure cultures were transferred to PDA slant

tubes. As controls, uncut, surface-disinfected, and non-disinfected pieces were also placed on the same agar to check for contaminated fungi.

2.3 Identification of Endophytic Fungi (Morphological Examination)

The fungi were identified based on morphological characteristics according to the methods described by Kong and Qi . Colony descriptions were based on observations on PDA under ambient daylight conditions. Growth rates at 20, 25, 30, 35, and 40°C were determined after 72 h following published protocols . Microscopic observations and measurements were made from preparations that were mounted in lactophenol. An Olympus CH i- 20 research microscope wasemployed for examination by light microscopy.

The fungi which failed to sporulate were designated as “mycelia sterilia”. For colony chracteristics the mycelia were transferred in to PDA agar media. Colonization Frequency (CF) was calculated as described by Suryanarayanan et al. (2003).

Colonization frequency of endophytes Number of segments colonized by fungi X 100

Total number of segments analysed

2.4 Isolation and identification of fungal pathogens

Fungal strains used in the in vitro antagonism studies was *F. oxysporum*, isolated and identified as the known causative agents of wilt of Tur and Blight causing Alternaia

fungus. These organisms were sourced from a field near Udgir area and found to produce disease conditions on healthy plants from where they were re-isolated before selecting them as the test pathogens for the study.

The staining method with rose Bengal and microscopy examination under 63X magnification enabled us to detect fungal endophytes inside tissues. These techniques have a morphological concept, where endophytes are defined based on morphological characteristics and ideally by the differences among them.Rose Bengal as an easily water-soluble vital stain has shown ability for infiltrating the inside of the plant tissue to stain endophytes and to provide image contrast when green filter is used at microscopy examination (Norn 1962) One limitation of the rose Bengal stain may be that the solution dries out fast in contact with air, and the resulting stain can become spotty and disturb the microscopic examination of the sample.

2.5 DNA extraction

We used DNA extraction to obtain DNA in pure form intended for further analysis to identify the fungal endophytes species. DNA was extracted from hyphae that was catapulted into 70µl lysis buffer (see the contents of the buffer below) using a modified method of the protocol described in PCR protocols : A guide to methods and applications (Affiliation Cetus Corporation 1990, Ward 2008).

Lysis buffer 1.4 NaCl,

0.1 M Tris Hydrochloride,

0.2 20mM EDTA

2% CTAB

The samples were frozen and thawed twice at -80°C (20 min) and 65°C (10-45 min) respectively. They were incubated for 45 min, and the suspensions were extracted twice with 1 vol. (300 µl) of chloroform for 15 min centrifugation at 10000 g. The aqua phase was removed and the DNA was precipitated with one vol. (300 µl) of ice-cold isopropanol for 10 min. DNA was harvested by centrifugation for 20 min 13000 g and the pellet was washed with 1 ml cold 70% ethanol, and finally re-suspended in 60 µl dH2O .

2.6 Polymerase chain reaction (PCR)

Extracted and purified DNA from tissues infected with fungal endophytes is used as a template for polymerase chain reaction (PCR). First, a mixture is created (see table 2) consisting of:

• DNA template to be copied;

• Polymerase enzyme to synthesize new DNA;

• Primers, as a starting point that matches exactly the beginning and end of the DNA template;

• Deoxyribonucleotide triphosphates (dNTPs), as the building blocks from which the DNA polymerase can synthesize new DNA; and

• Magnesium chloride salt solution (MgCl2) a buffer to create an optimal environment for the reaction.

The reaction is performed in an automated machine (thermo cycler), which is capable of rapidly increasing and decreasing the temperature. For steps of PCR, see Table 3.

We know that DNA is a double stranded molecule linked together by weak hydrogen bonds. The DNA needs to be separated into single strands to be able to copy.

By heating the PCR mixture at 94°C the double helix of DNA is denatured. When temperature is lowered to 52°C, the primers anneal to their matching sequence on the original DNA strand. When the sample is reheated to 72°C, DNA polymerase adds complementary nucleotides to elongate DNA. During elongation, DNA polymerase uses the original single strand of DNA as a template to add complimentary dNTPs to the 3' ends of each primer. Repeated heating and cooling rapidly amplifies the DNA segment of interest, in our case the ITS region.

Table 1. The reagents and their volumes in the 25 µl DyNAzyme TM DNA Polymerase PCR reaction.

Sr No	Reagent	Volume (µl)
1	10X DyNAzyme TM buffer	2,5
2	10 mM dNTPs	0,5
3	25 mM MgCl2	0,5
4	25 pmol/µl ITS-5	0,5
5	Forward primer	0,5

6	25 pmol/µl ITS-4	0,5
7	Reverse primer	0,5
8	DyNAzyme TM DNA	0,5
9	Polymerase	0,5
10	Template	10
11	MQ water	10

PCR program; to generate the desired amount of amplification, steps are repeated 35 times*

Step	Temperature	Duration
First denaturation	94°C	3 min
Denaturation *	94°C	30 sec
Annealing *	52°C	30 sec
Extension*	72°C	1 min
Finish extension	72°C	10 min
Hold	4°C	∞

The ITS regions have been regarded as non-functional sequences, and regions that are often highly variable among fungal species. The multi copy characteristic of the rDNA repeat makes the ITS regions easy to amplify from small DNA samples (Gardes and Bruns 1993).

Because the internal transcribed spacer (ITS) region is a convenient target region for molecular identification of fungi, we have used ITS4 and ITS5 primers to amplify this region. The primers ITS4-ITS5 (T.M. White 1990) have been described schematically in Figure 6, whereas the length and order of primers nucleotides is shown in table 4; the

ITS4-ITS5 primers amplify 600bp-800bp at fungal ITS regions (Gardes and Bruns 1993).

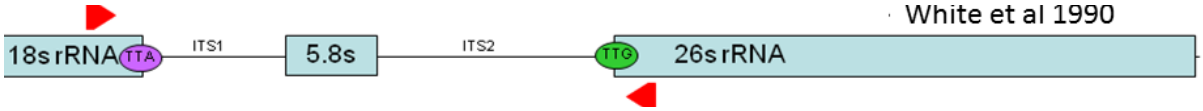

Figure 1. Schematic illustration of the internal transcribed spacers (ITS) numbered from 5' end. Red arrows indicate orientation and approximate position of primer sites. (Images modified from www.phytophthoradb.org - 960 × 720.)

.The primers used for amplification of rDNA by PCR process.

Primer	Sequence (5`→3`)
ITS-4 Reverse primer	TCCTCCGCTTATTGATATGC
ITS-5 Forward primer:	GGAAGTAAAAGTCGTAACA AGG

2.6 Agarose Gel Electrophoresis

To perform gel elctrophoresis, 0.8% agarose powder is solubilized in electrophoresis buffer (1xTAE)(http://bioinfoweb.com), and then heated in a microwave oven until completely melted. Subsequently, the mixture is poured into a gel frame and seven µl Ethidium Bromide is added to the gel to facilitate visualization of DNA after electrophoresis. One µl 6X Loading buffer was added to the samples (10 µl) to ensure that the samples sink to the bottom of the wells and allow for possible monitoring of their movement through the gel. In order to estimate DNA product sizes, DNA molecular weight markers GeneRulerTM DNA Ladder Mix are included in all gel runs. Agarose gels are run at 60 V for approximately one hour. DNA products are visualized using UV-light and photographed.

2.7 DNA Sequencing

DNA sequencing was done to find out which of fungal endophytes species was isolated.the three potent antifungal species like *Trichoderma , Chaetomium and Monilia* were subjected for the DNA sequencing .

To obtain the accurate base sequence from a PCR amplified piece of DNA, excess primers, nucleotides, and also residual alcohol, salts and phenol must be removed. It is important to have pure DNA, because impure template preparations can inhibit the cycle sequencing reaction, and provide incorrect results.

The purification of PCR products made using ExoSAP-IT, an enzymatic clean up method, prepares PCR products for sequencing application, see Table 5 (Corporation 2000).

Reagent and respective conditions needed to perform ExoSAP-IT method.

Reagent	Volume		Incubation temp/ time
ExoSAP-IT (A)		1µl	
PCR product(B)		15 µl	
Mix (A)+(B)	16 µl		37°C for one hour and 85 °C for 15 minutes.

After the ExoSAP-IT treatment of the PCR products, cycle sequencing was carried out in a thermal cycler. It is only a single primer (ITS-4 primer) used complementary to the 3`end of the strand, and only one strand is copied during sequencing.

BigDye Terminator v3.1 is suitable for performing fluorescence-based cycle sequencing reactions on single-stranded Sequencing buffer used to stabilize the reagents and

products in the sequencing reaction. The list of sequencing mixture and cycle sequencing can be found in Table 6 and Table 7, respectively.

Sequencing mixture

Reagent	Volume
BigDye Terminator v3.1	1µl
sequencing buffer (5X)	3µl
25 pmol/ µl ITS-4 primer	1µl
PCR product	3µl
MQ water	12 µl

PCR program for BigDye Terminator v3.1; Sequencing Cycle

Step	Temperature	Duration
First denaturation	96°C	1min
Denaturation *	96°C	10 sec *
Annealing *	50°C	10 sec *
Extension*	60°C	4 min *
Hold	4°C	∞

2.8 Phylogenetic analyses

MEGA (Molecular Evolutionary Genetics Analysis) is used as a tool for conducting sequence alignment. These sequence alignment are used to make phylogenetic trees.

The analyses used to construct phylogenetic tree were conducted with the following specifications: Nucleotides were used as substitutions type, maximum likelihood was used as the statistical method, bootstrap method with 2000 bootstrap replications was

used to test phylogeny, and finally the model method used was the Kimura 2-parameter model with the tree inference options Heuristic method - Close-Neighbor-Interchange.

Sequence similarity searches were performed for each of the 36 representative fungal sequences against the non-redundant database maintained by the National Center for Biotechnology Information (NCBI) using the "Local Alignment Search Tool" (BLAST) as the tool to infer functional and evolutionary relationships between sequences as well as help to identify members of gene families http://www.ncbi.nlm.nih.gov/About/primer/bioinformatics.html.

2.9 Study of antagonistic activity of fungal endophytes against Plant Pathogens

We used the dual culture method in order to investigate whether endophytic fungi have antagonistic activity against these pathogenic and non-pathogenic fungi. Mycelium discs (diameter, 5 mm) of 4-day-old fungi were placed in three corners of a potato dextrose agar (PDA) plate. Each of the eight endophytic fungi was inoculated at the center of each PDA plate. The plates were incubated at 25°C for 5 days. The percentage of inhibition was calculated from the following equation:

Inhibition (%) = [(growth diameter in the control sample – growth diameter in the sample with treated endophytes) × 100]/growth diameter in the control sample.

The experiment was repeated twice in triplicates.

2.10 Plants selected for the study

1) *Ocimum sanctum L.*

Ocimum sanctum L. (Tulsi) is an erect, much branched sub-shrub 30-60 cm tall, with simple opposite green or purple leaves that are strongly scented and hairy stems. Leaves have petiole and are ovate, up to 5 cm long, usually somewhat toothed. Flowers are purplish in elongate racemes in close whorls .Tulsi is native throughout the world tropics and widespread as a cultivated plant and an escaped weed. It is cultivated for religious and medicinal purposes and for its essential oil. Tulsi is an important symbol in many Hindu religious traditions, which link the plant with Goddess figure. The name 'Tulsi in Sanskrit means 'the incomparable one'. The presence of a Tulsi plant symbolizes the religious bend of a Hindu family.

2) *Leucas aspera* species has many different common names depending on the region in which it is located, it is most commonly known as Thumbai. Found throughout India, it is known for its various uses in the fields of medicine and agriculture.

The leaves of the *Leucas aspera* can be obtuse, linear or linearly lanceolate or petiolate. They can reach up to lengths of 8.0 cm, and be 1.25 cm broad. The length of petioles is typically 2.5 to 6 mm long.The flowers are held together in auxiliary whorls or dense terminals. They contain 6 mm long bracts that are bristle-tipped, linear, acute and are "ciliate with long slender hairs".

The corolla on the *Leucas aspera* is 1 cm in length and the tube is 5 mm in length. It is annulate in the middle portion and pubescent on the upper region. The calyx is "densely white-woolly", upper lip is approximately 3 mm in length and the lower lip is approximately 6 mm in length. The middle lobe is rounded, obviate and the lateral lobes are subacute and small in size.

The fruit of the *Leucas aspera* is 2.5 mm long. They are nutlets that are brown, smooth and oblong in shape.

3) *Hyptis suaveolens*,(L). Poit. Pignut or chan, is a branching pseudocereal plant native to tropical regions of Mexico, Central, the West Indies, and South America, as well as being naturalized in tropical parts of Africa, Asia and Australia. It is generally 1–1.5 m (3.3–4.9 ft) tall, occasionally up to 3 m (9.8 ft). Stems are hairy, and square in cross section. Leaves are oppositely arranged, 2–10 cm (0.79–3.94 in) long, with shallowly toothed margins, and emit a strong minty odor if crushed. Flowers are pink or purple, arranged in clusters of 1-5 in the upper leaf axils.

4) *Leonotis nepetifolia* (L). R.Br. also known as lion's tail and wild dagga.The plant is a broadleaf evergreen large shrub native to South Africa and southern Africa, where it is very common.It is known for its medicinal and mild psychoactive properties. The shrub grows 3 to 6 ft (1 to 2 m) tall by 1.5 to 3.5 feet (0.46 to 1.07 m) wide. The medium-dark green 2–4 inches (5.1–10.2 cm) long leaves are aromatic when crushed. The plant has tubular orange flowers in tiered whorls, typical to the mint family, that encircle the square stems. They rise above the foliage mass during the summer season, with flowering continuing into winter in warmer climates.

5) *Anisomele smalabarica*

Perennial, semi-shrubby herb; stem to about 2 m high, much branched from base, subquadrangular, thickened below to 1.8 cm in diam., densely lanate. Leaves ovate-lanceolate to oblong-lanceolate, 3.6-16 x 1.3-7 cm, narrowed and rounded at base, acute, crenate-serrate, slightly bullate and velvety lanate above, densely so beneath; petioles 0.7-3.5 cm long, stout, lanate. Racemes to 34.5 cm long; verticils close, dense; peduncles densely lanate.Floral leaves 8-10 mm long, densely lanate.Bracts linear, to 5 mm long. Calyx to 9 mm long; tube to 5 mm long, lanate without, glabrous within; lobes lanceolate, to 4 mm long, acuminate. Corolla 1.4-2 cm long; tube to 9 mm long, white, glabrous without; throat pilose towards base of lower lip; Upper lip oblong, 4-6 mm long, obtuse at apex, slightly arched, whitish; lower lip to 1 cm across, coral pink, with 2 white streaks towards base, pilulose without with gland-tipped hairs, the lateral lobes shallowly rounded, the median one larger, broadly orbicular, 2-fid. Style glabrous; branches linear, unequal.Nutlets ovoid, 2.5 x 1.5 mm, trigonous, glabrous, blackish-brown and shining.

3.RESULTS AND DISCUSSION

3.1 Identification of Endophytic Fungi

Table No. 2 Taxonomic identification of the fungal endophytes isolated from *Oscimum sanctum*

Sr No	Name of Endophyte	*Oscimum sanctum*		
		Leaf	Stem	Root
1	*Alternaria alternata*	+	+	+
2	*Curvulari alunata*	+	+	+
3	*Fusarium oxysporum*	+	+	+
4	*Phoma sp.*	+	_	+
5	*Phyllosticta sp.*	+	_	_
6	*Phomopsis sp.*	+	_	_
7	*Pestalotiopsis sp.*	_	_	_
8	*Chaetomium indicum*	+	_	_
9	*Chaetomium globosum*	+	_	_
10	*Glomerella*	+	_	_
11	*Xylaria*	+	_	_
12	*Mycelia sterilia sp. 1*	+	+	_
13	*Aureobasidium sp.*	+	+	_
14	*Colletotrichum sp. 2*	+	+	_
15	*Drechslera andersenii*	+	+	_
16	*Cladosporium sp*	+	+	_
17	*Acremonium sp*	+	+	_
18	*Trichoderma sp*	+	+	+
19	*Monilia sp*	+	+	+
20	*Botrytis sp.,*	+	+	_
21	*Penicillium*	+	+	_
22	Unidentified Fungi	+	_	_
Total		21	13	06

Table No.3 Taxonomic identification of the fungal endophytes isolated from *Leucas aspera*

Sr No	Name of Endophyte	*Leucas aspera*		
		Leaf	Stem	Root
1	*Alternaria alternata*	+	+	+
2	*Curvulari alunata*	+	+	+
3	*Fusarium oxysporum*	+	+	+
4	*Phoma sp.*	_	_	_
5	*Phyllosticta sp.*	_	_	_
6	*Phomopsis sp.*	_	_	_
7	*Pestalotiopsis sp.*	_	_	_
8	*Chaetomium indicum*	_	_	_
9	*Chaetomium globosum*	+	_	_
10	*Glomerella*	+	_	_
11	*Xylaria*	_	_	_
12	*Mycelia sterilia sp.*	+	_	_
13	*Aureobasidium sp.*	_	_	_
14	*Colletotrichum sp.*	+	+	_
15	*Drechslera andersenii*	+	+	_
16	*Cladosporium sp*	_	_	_
17	*Acremonium sp*	_	+	_
18	*Trichoderma sp*	+	+	_
19	*Monilia sp*	+	_	_
20	*Botrytis sp.,*	+	+	_
21	*Penicillium*	+	+	+
22	Unidentified Fungi	+	+	+
Total		13	10	05

Table No.4 Taxonomic identification of the fungal endophytes isolated from *Hyptis suaveolens*

Sr No	Name of Endophyte	*Hyptis suaveolens*		
		Leaf	Stem	Root
1	*Alternaria alternata*	+	+	+
2	*Curvulari alunata*	_	+	+
3	*Fusarium oxysporum*	+	+	+
4	*Phoma sp.*	_	_	_
5	*Phyllosticta sp.*	_	_	_
6	*Phomopsis sp.*	_	_	_
7	*Pestalotiopsis sp.*	_	_	_
8	*Chaetomium indicum*	_	_	_
9	*Chaetomium globosum*	+	_	_
10	*Glomerella*	+	_	_
11	*Xylaria*	+	_	_
12	*Mycelia sterilia sp. 1*	+	+	_
13	*Aureobasidium sp.*	+	+	_
14	*Colletotrichum sp. 2*	+	+	_
15	*Drechslera andersenii*	+	_	_
16	*Cladosporium sp*	_	+	_
17	*Acremonium sp*	+	+	_
18	*Trichoderma sp*	+	+	_
19	*Monilia sp*	+	+	_
20	*Botrytis sp.,*	_	+	_
21	*Penicillium*	+	+	_
22	Unidentified Fungi	+	+	+
Total		16	13	04

Table No. 5 Taxonomic identification of the fungal endophytes isolated from *Leonotis nepetifolia*(L). R.Br.

Sr No	Name of Endophyte	*Leonotis nepetifolia*(L). R.Br.		
		Leaf	Stem	root
1	*Alternaria alternata*	+	+	+
2	*Curvularia lunata*	+	+	+
3	*Fusarium oxysporum*	+	+	+
4	*Phoma sp.*	+	+	+
5	*Phyllosticta sp.*	+	_	_
6	*Phomopsis sp.*	+	+	_
7	*Pestalotiopsis sp.*	_	+	_
8	*Chaetomium indicum*	+	_	_
9	*Chaetomium globosum*	+	_	_
10	*Glomerella*	+	_	_
11	*Xylaria*	_	+	_
12	*Mycelia sterilia sp. 1*	+	_	_
13	*Aureobasidium sp.*	+	+	_
14	*Colletotrichum sp. 2*	+	+	_
15	*Drechslera andersenii*	_	+	_
16	*Cladosporium sp*	+	+	_
17	*Acremonium sp*	_	_	_
18	*Trichoderma sp*	+	+	_
19	*Monilia sp*	+	+	_
20	*Botrytis sp.,*	+	+	_
21	*Penicillium*	+	+	+
22	Unidentified Fungi	+	_	+
Total		18	16	06

Table No.6 Taxonomic identification of the fungal endophytes isolated from *Anisomeles malabarica*

Sr No	Name of Endophyte	*Anisomeles malabarica*		
		leaf	Stem	root
1	*Alternaria alternata*	+	+	+
2	*Curvularia lunata*	+	+	+
3	*Fusarium oxysporum*	+	+	+
4	*Phoma sp.*	+	+	_
5	*Phyllosticta sp.*	_	_	_
6	*Phomopsis sp.*	+	_	_
7	*Pestalotiopsis sp.*	_	_	_
8	*Chaetomium indicum*	+	+	_
9	*Chaetomium globosum*	+	+	_
10	*Glomerella*	+	_	_
11	*Xylaria*	_	_	_
12	*Mycelia sterilia sp.*	+	_	_
13	*Aureobasidium sp.*	+	+	_
14	*Colletotrichum sp.*	+	+	+
15	*Drechslera andersenii*	+	_	_
16	*Cladosporium sp*	_	+	_
17	*Acremonium sp*	_	_	_
18	*Trichoderma sp*	_	+	+
19	*Monilia sp*	+	_	_
20	*Botrytis sp.,*	_	_	_
21	*Penicillium*	+	+	_
22	Unidentified Fungi	+	+	+
Total		15	12	06

3.2 Fungal endophytes isolated from Leaves, stem and roots of all plants

All five medicinal plants had a huge number of fungal assemblages. Altogether21 fungal endophytes genera were isolated from 120 segments of leaf, stem androot of the five medicinal plants of lamiaceae.

The fungal endophytes belonged to 21genera are given in (Table II to VI) . All these different endophytic fungal genera colonizedthe five medicinal plants *Ocimum sanctum, Leucas aspera,Hyptis suaveolens,Leonotis nepetifolia and Anisomeles malabarica* respectively. Previous studies also indicated similar results, where different numbers of endophytic fungi were isolated from some medicinal plants.

The highest (21) number of fungal endophytes was isolated from *O. sanctum* .Similarly, 13 genera of endophytic fungi were isolated from medicinal plant*Leucasaspera,*16 fungal genera were isolated from medicinal plant *Hyptis suaveolens,* 18 fungal genera were isolated from medicinal plant *Leonotis nepetifolia,* and 15 fungal genera were isolated from medicinal plant *Anisomeles malabarica.*

Most of the endophytic fungi colonized the leaves of all five medicinalplants. Leaves of *O. sanctum* were colonized by the maximum number of endophytes. This is a clear evidence for the tissue specificity of endophytes. Previous researchers also observed tissue specificity of endophytes in their studies .

Most of the endophytic fungi belonged to ascomycetes and fungi imperfecti, as shown in Table . Some results of characterization of colony and microscopic morphological study are shown in Figures 1 to 6 respectively. All isolates were identified as belonging to 21 genera, namely *Alternaria alternata,Curvularia lunata*

,Fusarium oxysporum,Phomasp.,Phyllosticta sp.,Phomopsis sp.,Pestalotiopsis sp.,Chaetomium indicum,Chaetomium globosum ,Glomerella,Xylaria,Myceliasterilia sp. ,Aureobasidium sp. ,Colletotrichum sp.,Drechslera andersenii, Cladosporium sp, Acremonium sp, Trichoderma sp, Monilia sp, Botrytis sp.,Penicillium and unidentified fungi.

The frequency of endophytic fungi isolated from *Leucas aspera* was lower as compared with *Oscimum sanctum*The low recovery rate of endophytic fungi from *Leucas aspera* might be due to the lack of essential specific growth substances such as plant raw material in the isolation medium. According to Mungo et al., isolation media that contain a blend of different parts of the plant raw material exhibit different efficiencies in fungi isolation. Each part of the plant such as the leaf, stem and rootwere collected and subjected to the endophytic isolation for diversity comparison. As shown in Table (II to VI)21 endophytic fungi were isolated from the leaves , followed by 13 from stem and 06 from root from *Oscimum sanctum*. The reason for low colonization by endophytic fungi in the root is unclear, but it could reflect the differences in microbial and physiological conditions in the different plant parts. Besides this, these results may be related to the possible available specific growth-promoting factors present in the different plant parts. On the other hand, some researchers suggested that endophytic fungi show a certain degree of tissue recurrence or specificity.

The dominant genera were *Alternaria alternata, Fusarium oxysporum, Penicillium*, while *Phomasp.,Phyllosticta sp.,Phomopsis sp.,Pestalotiopsis sp.. Cladosporium* sp. were least found in stem and root.

3.3 In vitro antagonism assay of endophytes as potential biocontrol agents

The in vitro studies showed that the endophytes produced inhibition of radial growth of the pathogens. The zone of inhibition of mycelial growth of *F. oxysporum*, ranged from 2.0 to 15.00 mm representing 1.00 to 80% compared to the control at 100%.

From the in vitro plate bioassay of different fungal endophytes with each of the host plant pathogen in five different types of media gave a clear idea about various types of interactions that can exist between them. Understanding endophyte-pathogen interaction is a vital for understanding the biodiversity of the plant tissue microflora compared to their chemodiversity. By macroscopic evaluation of the interaction and consulting with earlier reports on various endophyte-pathogen interaction types (Trejo-Estrada et al. 1998; Miles et al. 2012), we found different types of interactions.

The percentage antagonism (growth inhibition percentage) of each fungal endophyte was calculated against each of the two phytopathogens (Chamberlain and Crawford 1999). All the growth inhibition percentages along with their respective endophyte-pathogen interaction types are summarized in Tables 7 to 12 (against Fusarium & Alternaria).

Almost all the endophytic isolates were capable of inhibiting, to a varying extent on different media, one or both of the host-specific pathogens with a higher extent of antagonism against *Fusarium.*

According to the results, endophytic fungi communities isolated from Lamiaceae included 21 different genera. All identified taxa were subjected to a pilot study to investigate their potential antagonistic activity against two selected plant pathogens, *Fusarium* and *Alternaria* using dual-culture method on Malt yeast-Extract Agar (MYEA) and Potato-Dextrose Agar (PDA) media. Each endophytic isolate was cultured separately with *Fusarium* and *Alternaria* in two different Petri dishes and single cultures of endophyte, *Fusarium*, and *Alternaria* were used as controls. All cultures were prepared in 6 replicates and radial growth of colonies was measured within 15 days.

In control plates, *Fusarium* exhibited a competent growth rate on PDA with average vertical expansion as 0.2 mm/day, and 0.1 mm/day and 0.3 mm/day growth rate in diagonal and horizontal directions, respectively. *Alternaria* , however, had a relatively less vigorous growth rate on PDA in control plates where colonies had almost the same expansion towards each measured directions during 15 days (~0.07 mm/day).

Primary evaluation of antagonistic effect between endophytes and the selected pathogens demonstrated the potential ability of the candidate fungi species to inhibit the radial growth of both *Fusarium* and *Alternaria* on PDA cultures. Inhibition of growth was assayed by calculating growth rate (GR) and growth inhibition (GI) indices and results were tested regarding the significance of the differences by a one-way ANOVA test. Endophytes which were nominated for antagonistic test had the most vigorous growth on PDA, therefore colonies could last long enough to measure growth factor in

dual-cultures with the pathogen. All values were defined as mean of measurements for six replicates of every dual-culture and single culture of pathogens and each endophyte were used as controls.

As a conclusion, a decline in growth rates of both *Fusarium* and *Alternaria* was observed in all dual-cultures after the incubation period (15 days), however neither endophytic fungus showed any defect in growth rate while confronting the pathogen in compare with control samples. According to the growth rate, *Alternaria* had comparatively less radial extension toward horizontal and diagonal dimensions but such a difference was not found in vertical growth of the pathogen . Expansion of *Fusarium* colonies was dominated in all measured dimensions while confronting the endophyte in dual-cultures.

Radial growth of colonies was applied to assess the growth inhibition value in dual-cultures. All measurements were recorded from day 0 to day 15, and results were compared. Accordingly, *Trichoderma* had the strongest antagonistic effect on *Fusarium* while *Monilia s*howed a relatively feeble effect on this pathogen particularly against vertical extension of the colonies. In compare with other endophytes, *Curvularia* had also weaker antagonistic effect on growth of *Fusarium* although other fungi showed almost the same growth inhibitory effect in dual-cultures with this pathogen .

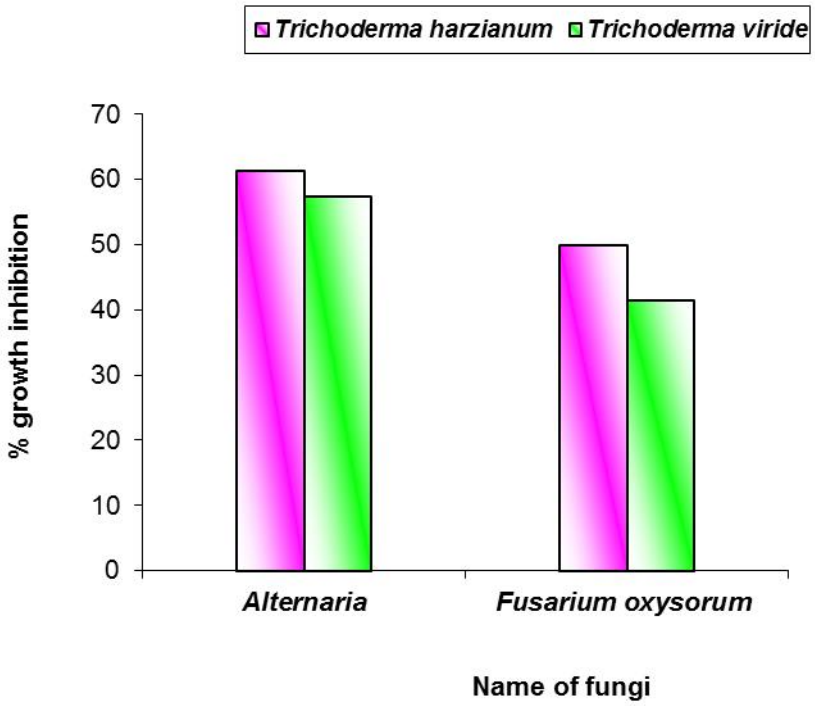

Fig.1 % growth inhibition against fungi shown by *Trichoderma* species.

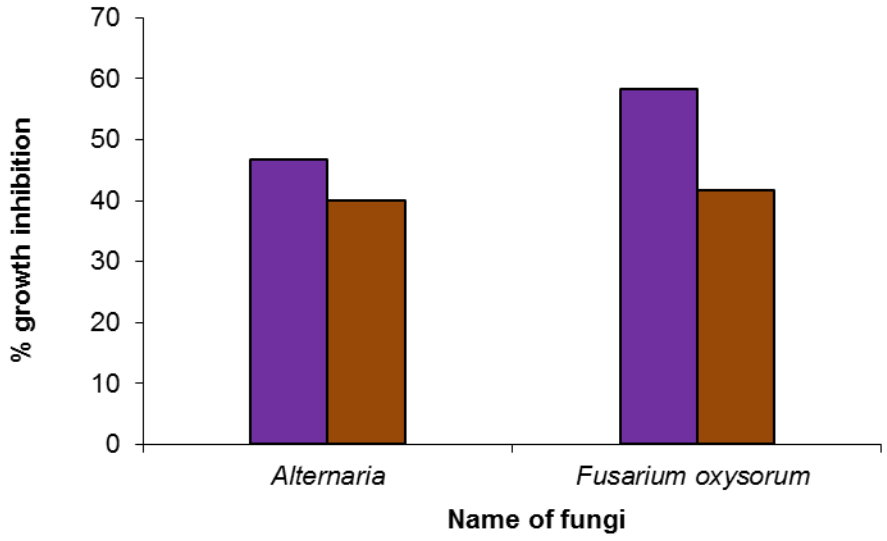

Fig.2. % growth inhibition against fungi shown by *Curvularia.*

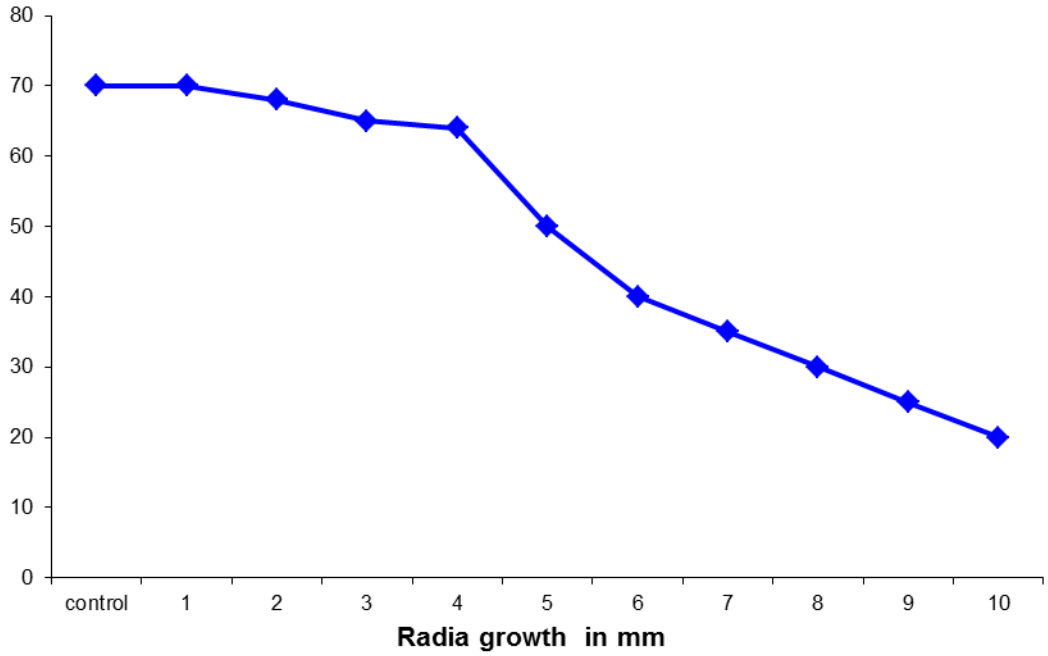

Fig.3. Effect Fungal endophyte *Monilia* on Plant pathogen *Fusarium*

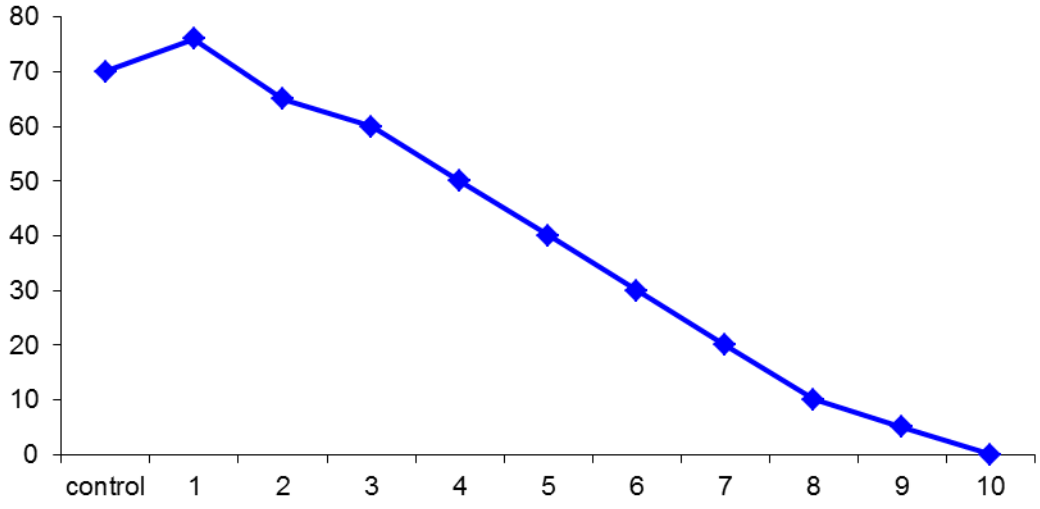

Fig.5. Effect of Fungal endophyte *Botrytis* on Plant Pathogen *Alternaria*

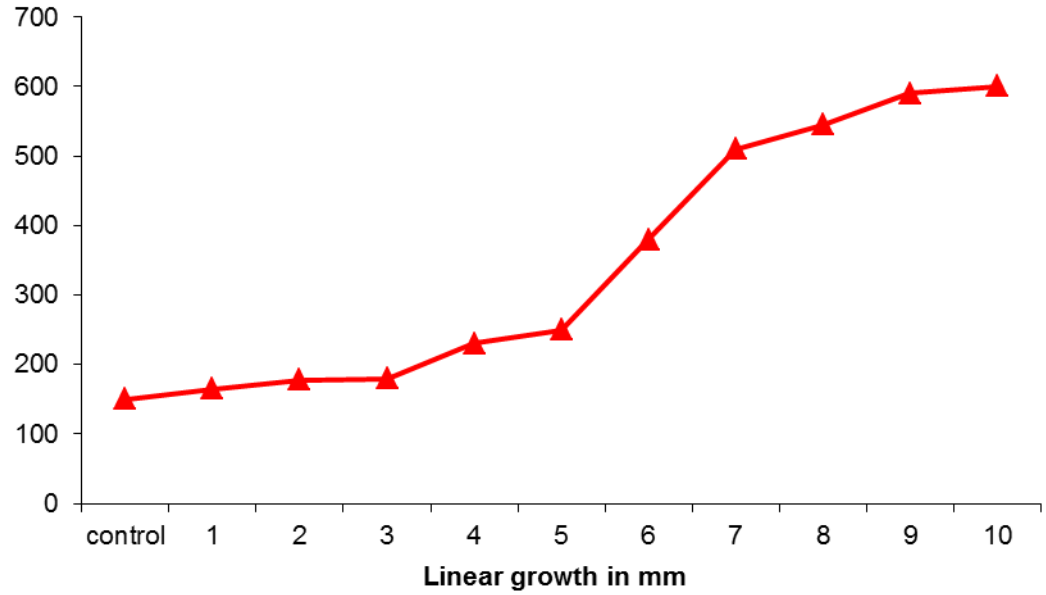

Fig.6. Effect of Fungal Endophyte *Phoma* on Plant pathogen *Fusarium*

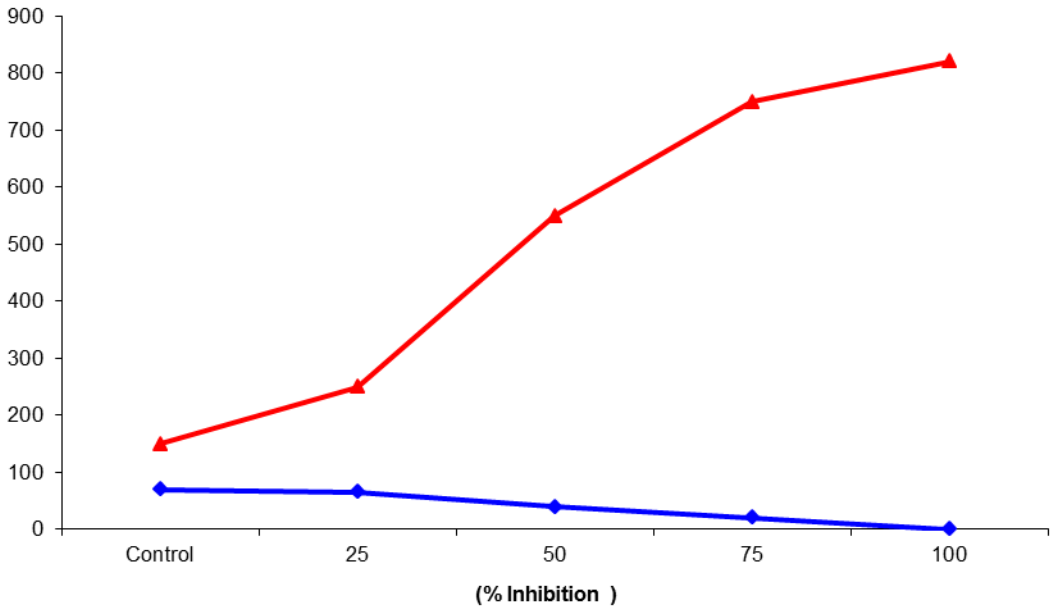

Fig.7. Inhibition of fungal pathogen *Fusarium* by Fungal endophyte *Collectotrichum*

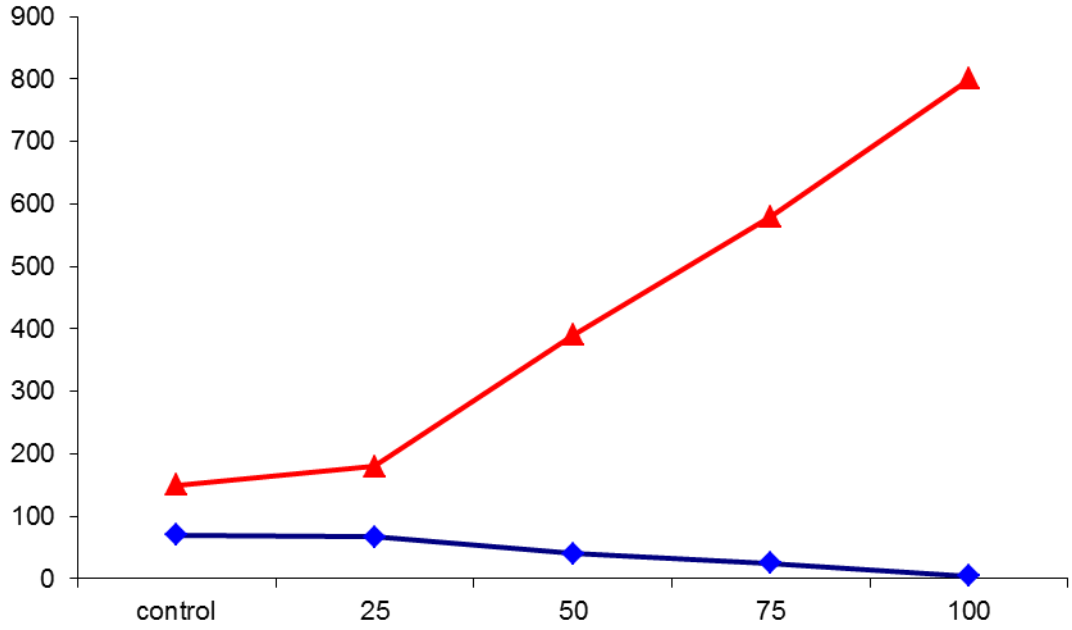

Fig.8. Inhibition of Fungal Pathoen *Alternaria* by Fungal endophyte *Penicillium*

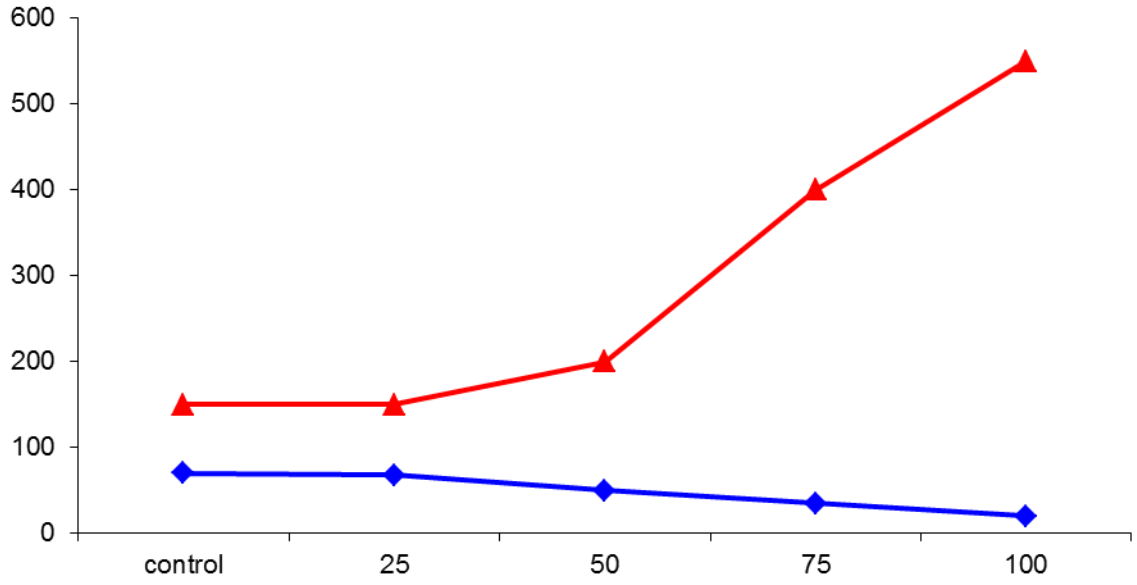

Fig.9. Inhibition of Fungal Pathogen *Fusarium* by Fungal Endophyte *Acremonium*

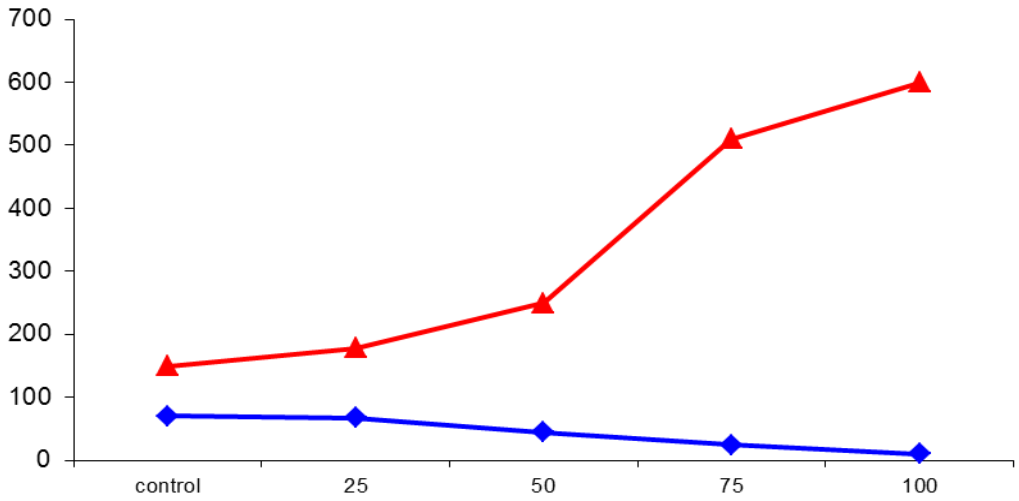

Fig.10 Inhibition of Fungal Pathogen *Fusarium* by Fungal Endophyte *Phyllosticta*

Table : 7 In vitro antagonism of the Fungal isolates after four days of incubation against plant pathogenic fungi

Sr No.	Name of Fungal Endophyte isolate from *Ocimum sanctum* L.	Antagonism against	
		Alternaria	*Fusarium*
1	*Trichoderma*	+++	+++
2	*Curvularia*	++	+
3	*Monillia*	++	+++
4	*Chaetomium*	+	+
5	*Botrytis*	+	+
6	*Phoma*	++	++
7	*Collectotrichum*	+	+
8	*Penicillium*	+	+
9	*Acremonium*	+	+
10	*Phyllosticta*	+	+

Antifungal activity:

+, < 2mm;

++, 2-10mm;

+++, >10mm.

Table. 8 In vitro antagonism of the Fungal isolates after four days of incubation against plant pathogenic fungi

Sr No.	Name of Fungal Endophyte isolate from *Leucas aspera*	Antagonism against	
		Alternaria	*Fusarium*
1	*Trichoderma*	+++	+++
2	*Curvularia*	+	+
3	*Monillia*	++	+++
4	*Chaetomium*	+++	++
5	*Botrytis*	+	+
6	*Phoma*	+	+
7	*Collectotrichum*	++	+
8	*Penicillium*	+	++
9	*Acremonium*	++	+
10	*Phyllosticta*	+	+

Antifungal activity:

+, < 2mm;

++, 2-10mm;

+++, >10mm.

Table. 9 In vitro antagonism of the Fungal isolates after four days of incubation against plant pathogenic fungi

Sr No.	Name of Fungal Endophyte isolate from *Hyptis suaveolens*	Antagonism against	
		Alternaria	*Fusarium*
1	*Trichoderma*	+++	+++
2	*Curvularia*	++	+++
3	*Monillia*	+	+
4	*Chaetomium*	++	++
5	*Botrytis*	+	+
6	*Phoma*	+	++
7	*Collectotrichum*	++	+
8	*Penicillium*	+	+
9	*Acremonium*	+	+
10	*Phyllosticta*	+	+

Antifungal activity:

+, < 2mm;

++, 2-10mm;

+++, >10mm.

Table. 10 In vitro antagonism of the Fungal isolates after four days of incubation against plant pathogenic fungi

Sr No.	Name of Fungal Endophyte isolate from *Leonotis nepetifolia*	Antagonism against	
		Alternaria	*Fusarium*
1	*Trichoderma*	+++	+++
2	*Curvularia*	++	++
3	*Monillia*	+++	++
4	*Chaetomium*	+	+
5	*Botrytis*	+	+
6	*Phoma*	++	+
7	*Collectotrichum*	++	+
8	*Penicillium*	+	+
9	*Acremonium*	+	+
10	*Phyllosticta*	+	+

Antifungal activity:

+, < 2mm;

++, 2-10mm;

+++, >10mm.

Table. 11 In vitro antagonism of the Fungal isolates after four days of incubation against plant pathogenic fungi

Sr No.	Name of Fungal Endophyte isolate from *Anisomeles malabarica*	Antagonism against	
		Alternaria	*Fusarium*
1	*Trichoderma*	+++	+++
2	*Curvularia*	+++	+
3	*Monillia*	+++	+++
4	*Chaetomium*	+	+
5	*Botrytis*	+	++
6	*Phoma*	+	+
7	*Collectotrichum*	++	+
8	*Penicillium*	+	+
9	*Acremonium*	+	++
10	*Phyllosticta*	+	+

Antifungal activity:

+, < 2mm;

++, 2-10mm;

+++, >10mm.

Table 7 to 11 summarizes antifungal activity by Fungal endophytes . About Ten positive Endophytic fungi were screened for their antifungal activity against pathogenic fungi.

Isolate *Trichoderma* from Leaf of *O. sanctum, Phoma* from stem of *Hyptis* and *Chaetomium* from leaf of of *Anisomeles malabarica* are potent as per antifungal activity was concerned this is followed by isolates *Monillia, Phyllosticta* etc. from *Leonotis nepetifolia,* rest other endophytic fungi are less effective. Highest antifungal activity was recorded in endophytic fungi *Trichoderma* .Thus the activity spectra of endophytes were greatly different depending upon isolates, suggesting that several substances participated in antimicrobial activity.

Discussion

Over the last decades, endophytic microorganisms have gained immense importance as valuable natural resources for imminent utilization in diverse areas such as agriculture and biotechnology (Aly et al. 2011; Rajulu et al. 2011; Kusari and Spiteller 2011; Li et al. 2012).

A number of bioprospecting strategies could be engaged in order to discover competent endophytes with desirable traits. For instance, endophytes could be isolated from randomly sampled plants from different population, or initially performing a detailed investigation of an ecosystem in order to determine its features with regard to its natural population of plant species, their relationship with the environment, soil composition, and biogeochemical cycles, followed by endophyte isolation and characterization (Debbab et al. 2012; Kusari and Spiteller 2012). Another approach could be to evaluate the evolutionary relatedness among groups of plants at a particular sampling site, correlating to species, genus, and populations, through morphological data matrices and molecular sequencing, followed by isolation of endophytes from the desired plants. Medicinal plants could also be bioprospected for endophytes, especially those plants capable of producing phytotherapeutic secondary metabolites (Aly et al. 2011; Debbab et al. 2012).

Herein we report for the first time, the isolation and incidence of endophytic fungi harbored in different tissues of different plants of lamiaceae family . Fungal endophytes producing a plethora of bioactive compounds, even the ones exclusive to the associated plant, thereby assisting in the chemical defense of the host against invading

pathogens (Strobel and Daisy 2003; Strobel et al. 2004; Arnold et al. 2003; Rodriguez et al. 2004, 2008; Zhang et al. 2006; Gunatilaka 2006; Staniek et al. 2008; Suryanarayanana et al. 2009; Aly et al. 2010; Kharwar et al. 2011; Porras-Alfaro and Bayman, 2011; Debbab et al. 2012;).

Thus, in order to screen for the most promising endophytes, we estimated the potential of the isolated endophytic fungi as biocontrol agents by challenging them with two major fungal pathogens of the host plant, *Alternaria* and *Fusarium*. The isolated endophytic fungi were challenged by the host-specific phytopathogens on five different media, namely PDA and NA.

It is imperative that any plant-fungal interaction is always preceded by a physical encounter between a plant and a fungus, followed by several physical and chemical barriers that must be overcome to efficaciously establish a plant-endophyte association (Kusari et al. 2012b). It is mostly by chance encounters that particular fungi establish as endophytes for a particular ecological niche, or plant population, or plant tissue, either in a localized and/or systemic manner (Hyde and Soytong 2008). Thus, even a fungus that is pathogenic in one ecological niche can be endophytic to plant hosts in another ecosystem. It has been established for a plethora of fungi that pathogenic-endophytic lifestyles are interchangeable and are due to a number of environmental, chemical and/or molecular triggers. (Schulz et al. 1999; Hyde and Soytong 2008; Eaton et al. 2011

Conclusion

Endophytes are a poorly investigated group of microorganisms that represent an abundant and dependable source of bioactive and chemically novel compounds with potential for exploitation in a wide variety of medical, agricultural, and industrial arenas. The mechanisms through which endophytes exist and respond to their surroundings must be better understood in order to be more predictive about which higher plants to seek, study, and spend time isolating microfloral components. This may facilitate the product discovery processes. The results presented in this review show that, as expected, great diversity has been found among endophytes isolated from plant hosts. They play important roles for protecting plants against diseases. Certainly, one of the major problems facing the future of endophyte biology and natural-product discovery is the rapid diminishment of rainforests, which hold the greatest possible resource for acquiring novel microorganisms and their products. The role of endophytes protecting plants against diseases has been quite well studied. However, Countries need to establish information bases of their biodiversity and at the same time begin to make national collections of microorganisms that live in these areas.

The potential of inimitable fungal endophytes adept in biosynthesizing bioactive metabolites, occasionally those imitative to their host plants, has irrefutably been recognized. Endophytes can be accepted as new sources for gene- and drug discovery in medical sciences and will provide, by distinct genomic blueprints, new insights in gene assembly and expression control. Nonetheless, there is still no known breakthrough in the biotechnological production of these bioactive natural products using endophytes. It is imperious to expound the metabolome in endophytes correlating to their host plants

on a case-by-case basis to comprehend how the biogenetic gene clusters are regulated and their expression is affected in planta and ex planta (i.e., by environmental changes and axenic culture conditions). Only a deeper understanding of the host-endophyte relationship at the molecular level might help to induce and optimize secondary metabolite production under laboratory conditions to yield desired metabolites in a sustained manner using endophytes. This can be achieved by challenging the endophytes by specific and non-specific pathogens, especially those attacking their host plants, by devising suitable co-culture and dual-culture setups (qualitative, followed by suitable quantitative experiments).

Once the production of a target or non-target natural product with a desired biological activity has been achieved, techniques like genome mining, metabolic engineering and metagenomics could be utilized to influence the manipulation of secondary metabolite production by endophytic fungi or the plant itself by directed infection with beneficial endophytes. Such directed investigation with the scientific rationale of mimicking the natural

plant-endophyte-pathogen interactions should be pursued to warrant a virtually incessant discovery and sustained supply of bioactive pro-drugs against the current and emerging diseases.

Summary

1. Endophytic fungi have been described as fungi that asymptomatically colonize healthy plant tissues, eventhough they may, after incubation or a latency period,cause diseases.
2. A good definition of endophytes is provided by Petrini (1991); “All organisms inhabiting plant organs that at some time in their life can colonize internal plant tissues without causing apparent harm to the host”.
3. Fungal endophytes have proved to be an important source for bioactive antimicrobial compounds such as alkaloids, peptides, steroids and phenol, which have a wide range of applications in the medical field.
4. Endophytic fungi were isolated from five lamiaceae family members from Udgir hilly areas.
5. The endophytes were isolated using a modified method described by Arnold et al.
6. Fungal strains used in the in vitro antagonism studies was F. oxysporum, isolated and identified as the known causative agents of wilt of Tur (Pigeon pea).
7. DNA sequencing was done to find out which of fungal endophytes species was isolated.the three potent antifungal species like Trichoderma , Chaetomium and Monilia were subjected for the DNA sequencing
8. MEGA (Molecular Evolutionary Genetics Analysis) is used as a tool for conducting sequence alignment. These sequence alignment are used to make phylogenetic trees.

9. *Ocimum sanctum,Leucas aspera ,Hyptis suaveolen, Leonotis nepetifolia* and *Anisomeles malabarica* plants were selected for the isolation of fungal endophytes.
10. According to the results, endophytic fungi communities isolated from Lamiaceae included 21 different genera. All identified taxa were subjected to a pilot study to investigate their potential antagonistic activity against two selected plant pathogens, Fusarium and Alternaria using dual-culture method on Malt yeast-Extract Agar (MYEA) and Potato-Dextrose Agar (PDA) media.
11. The in vitro studies showed that the endophytes produced inhibition of radial growth of the pathogens. The zone of inhibition of mycelial growth of F. oxysporum, ranged from 2.0 to 15.00 mm representing 1.00 to 80% compared to the control at 100%.
12. About Ten positive Endophytic fungi were screened for their antifungal activity against pathogenic fungi.
13. Isolate *Trichoderma* from Leaf of *O. sanctum, Phoma* from stem of *Hyptis* and *Chaetomium* from leaf of of *Anisomeles malabarica* are potent as per antifungal activity was concerned this is followed by isolates *Monillia*, *Phyllosticta* etc. from *Leonotis nepetifolia*, rest other endophytic fungi are less effective. Highest antifungal activity was recorded in endophytic fungi *Trichoderma* .
14. Molecular analysis and phylogenetic analysis (NCBI)showed that the fungal endophyte *Trichoderma* is close resemblance with the *T. longibrachiatum* showing highest antifungal activity .
15. Thus, even a fungus that is pathogenic in one ecological niche can be endophytic to plant hosts in another ecosystem. It has been established for a plethora of

fungi that pathogenic-endophytic lifestyles are interchangeable and are due to a number of environmental, chemical and/or molecular triggers.

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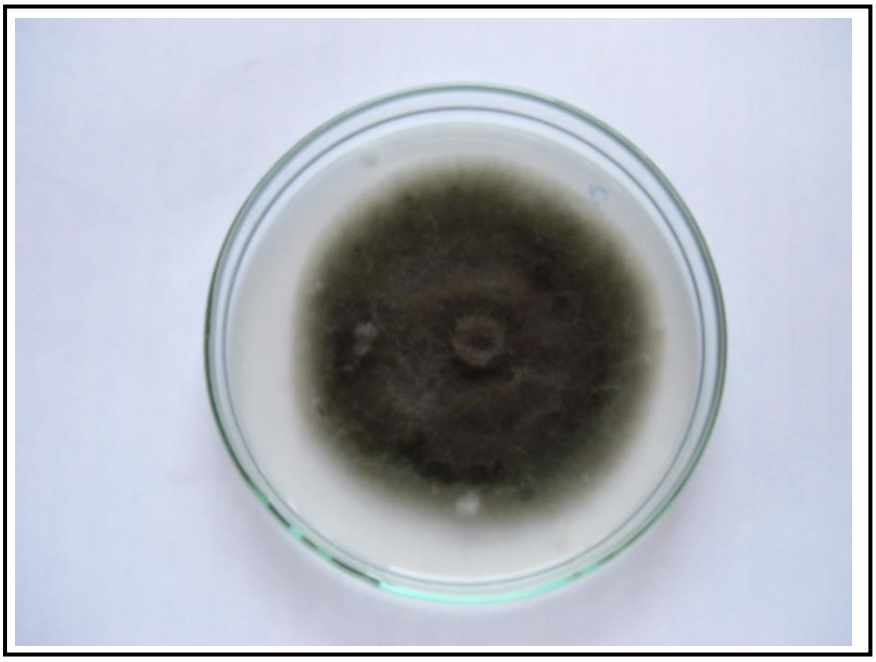
Appendix –I

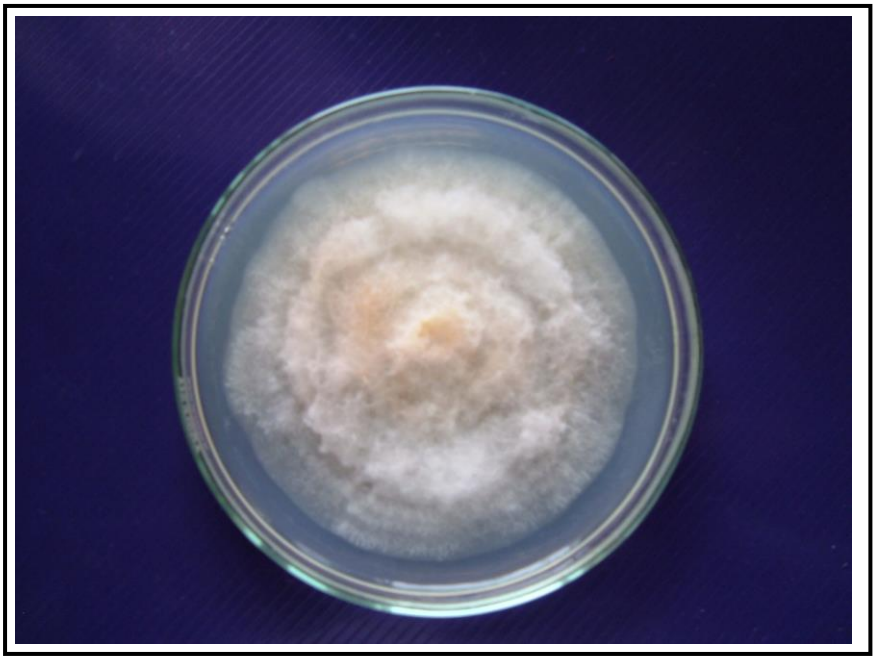
PhotoPlates

Fungal Endophytes

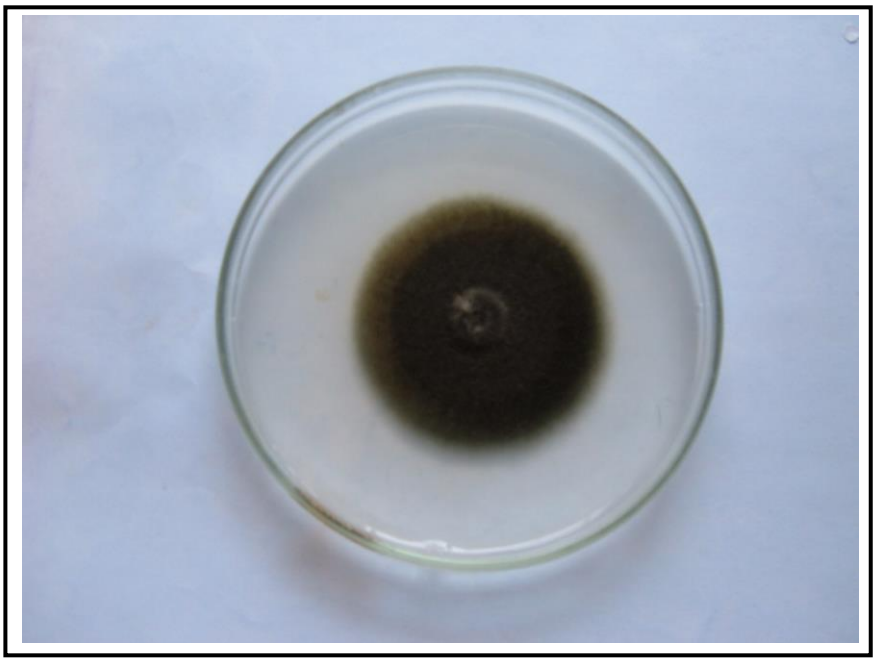
Alternaria alternata



Fusarium oxysporum



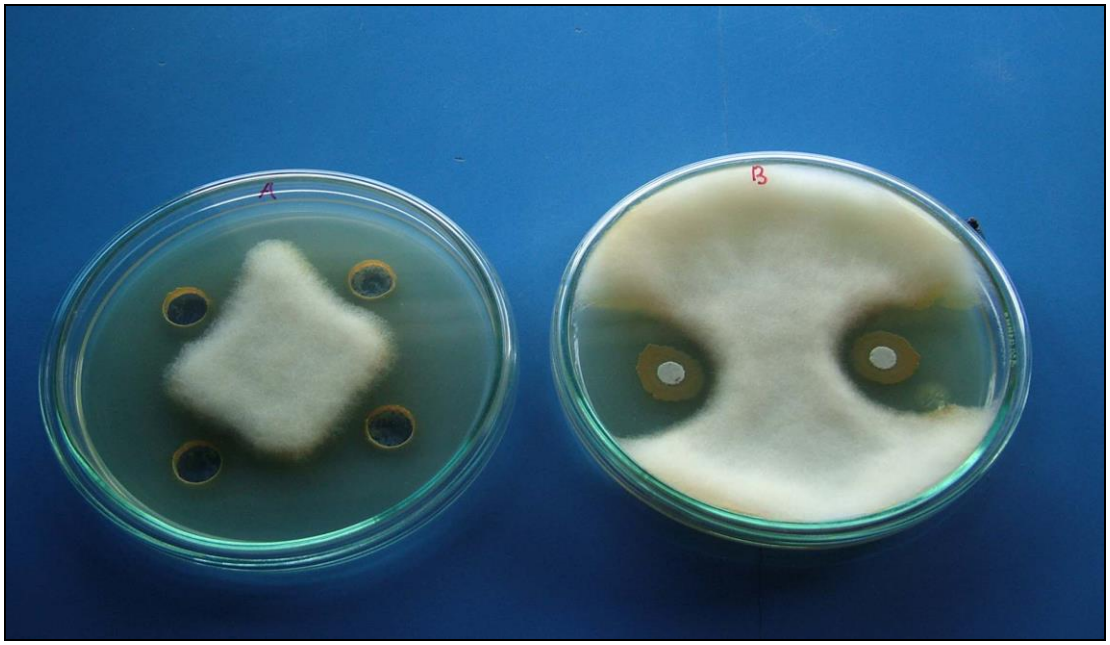
Aspergillus niger



Curvularia lunata

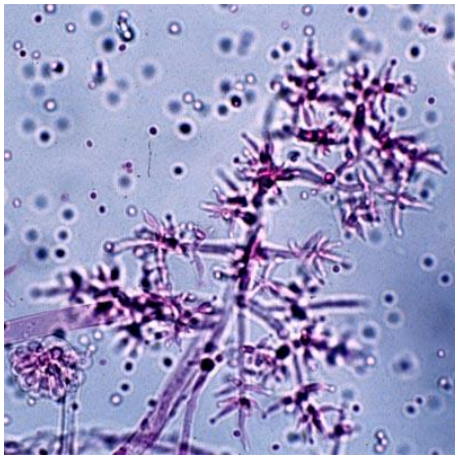
In vitro effect of endophytic fungi (*Trichoderma)*on mycelia growth of pathogens (*Fusarium*)

. In vitro antifungal activity of *Curvularia* against *Fusarium*

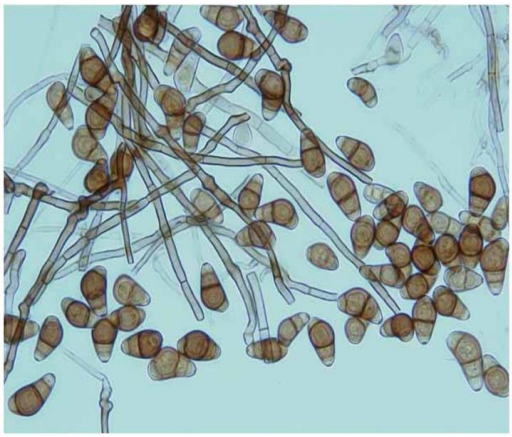


In vitro effect of endophytic fungi (*Trichoderma)*on mycelia growth of pathogens (*Alternaria)*

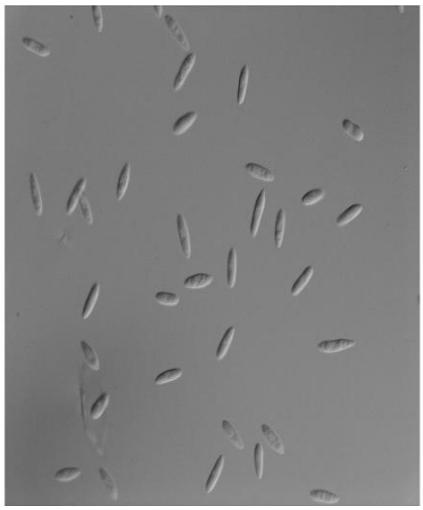
Trichoderma



Curvularia



Collectotrichum



Aspergillus