# Bioactivity of fungal endophytes associated with ethno-medicinal plants used by tribal communities of Achanakmar-Amarkantak Biosphere Reserve

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# Bioactivity of fungal endophytes associated with ethno-medicinal plants used by tribal communities of Achanakmar-Amarkantak Biosphere Reserve

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The present study was conducted for screening of biological activity of fungal endophytes associated with medicinal plants of AABR (Achanakmar-Amarkantak Biosphere Reserve), CG, India. A total of twelve species of medicinal plants were selected as suggested by local tribes on the basis of their medicinal property. A total of 39 endophytic fungal isolates were obtained from 12 medicinal plants.The major isolates were *Alysidium* sp., *Taeniolella* sp., *Aspergillus* sp. and *Grallomyces* sp. The broad spectrum antimicrobial activity was shown by *Scytalidium* sp., *Taeniolella* sp., *Aspergillus* sp., and *Diplococcium* sp. against sets of human pathogens. Most of the fungal endophytic isolates were amylase positive and none of them were able to solubilize inorganic phosphate. The maximum production of IAA was observed in the isolate number AC-5/2 (*Fusarium* sp.). This study may be further used for isolation of novel antimicrobial and other bioactive compounds from endophytic fungi associated with medicinal plants used by local tribes.

**Key words:** Antimicrobial activity, endophytic fungi, medicinal plant, pathogen

## INTRODUCTION

Endophytes are all those microorganisms that colonize asymptomatically within healthy plant tissues and provide an effective protection to their hosts from biotic and abiotic stresses (Redman *et al.*2002). In general, endophytes are very important and viable components of microbial biodiversity. These microorganisms behave as latent pathogens and make symbiotic relationship with their host for partial or whole lifetime (Gond *et al.* 2010). It is also suggested that endophytic microorganisms may have lost their pathogenic nature.

Endophytic microbes are ubiquitous among terrestrial plants and are capable of growing within all tissue part of host. More than 8600 bioactive compounds have been reported from fungi with various beneficial usages (Berdy, 2005).The discovery of gibberellins in *Fusarium fujikuroi* and taxol from endophytic fungi, associated with *Taxus brevifolia* prove this assumption (Strobel and Daisy, 2003). Endophytic fungi are potentially more bioactive than soil fungi because more than 58% biologically active novel compounds are extracted and purified from fungal endophytes compared to only 38 % from soil fungi (Schulz *et al.* 2002).

The Achanakmar-Amarkantak Biosphere Reserve is located at the junction of Vindhya and Satpura ranges between Madhya Pradesh and Chhattisgarh state of Central India. Moist deciduous forests constitute 63% of the area and are famous due to rich diversity of endangered species of medicinal plants. Amarkantak region is popular for its forest product and ethnomedicinal plants. The economy and financial support of tribal comm-unities live here on its forest product like wood and medicinal plants. Tribal communities of this area are economically very poor thus cannot afford the high prices of modern healthcare and therefore the ethnomedicinal practices play an important role in their life (Bondya *et al.* 2006).The plants found in Achanakmar-Amarkantak Biosphere Reserve (AABR) are mostly used to cure seminal weakness, jaundice, antidote, anticancer, kidney stones, skin disease, liver and spleen enlargements etc (Tiwari *et al.* 2014).Several

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medicinal plants found in this region are used as antimicrobial, digestive disorders, anti-inflammatory, analgesic and antipyretic etc (Table 1). There have been a number of studies documented on ethno- medicinal plants in Amarkantak region, but only few studies are related to use of medicinal plants by native tribal communities of Central India (Madhya Pradesh and Chhattisgarh)(Malviya *et al.* 2012; Maravi*et al.* 2017).

The aim of the present study was to isolate endophytic fungi associated with some medicinal plants used by tribal communities of Achanakmar-Amarkantak biosphere reserve of Central India and screening for their antimicrobial potential and some other bioactivities like phosphate solubilization, amylase production and IAA production.

## MATERIALS AND METHODS

### *Sample collection*

Medicinal plants were collected randomly from different locations (transition zone and buffer zone) of Achanakmar wildlife sanctuary, Bilaspur (Latitude: 21°15'N - 22°58'N Longitude: 81°25'E - 82°5'E Central point: 22°062 313 N - 81°442 533 E). Plant samples were collected according to medicinal uses as suggested by the local tribe (ThoonnuGond, a Vaidya by profession and others) of Amarkantak (Table 1). A total of 12 plant samples were collected from this region. All plant materials were separately kept in zip lock plastic bags and labeled. All samples were stored in an icebox at 4 °C and brought to the laboratory. All samples were used within 48 hrs. of collection.

### *Isolation and identification of endophytes*

The plant samples were washed thoroughly in running tap water for 30 min to remove the debris adhered and then rinsed with double distilled water before processing. To eliminate all the epiphytic microorganisms, healthy plant tissues initially surface sterilized using standard methodology (Petrini*et al.* 1993). The samples were immersed in 70% ethanol for 2–3 min, then sterilized with aqueous sodium hypochlorite solution (4% available Chlorine) for 2–5 min followed by rinsing in 70% ethanol for 30s. The tissues were finally rinsed in sterile double distilled water for three times and allowed to surface dry under sterile conditions.

The effectiveness of surface sterilization was checked by leaf imprint method (Schulz *et al.* 1993). The samples were cut into small pieces (leaves 0.5×0.5 cm squares, root 0.25 cm thick sections and bark 0.5×0.5 cm squares) using a sterile blade. Five segments of plant tissue were placed on each potato dextrose agar (PDA) plate containing streptomycin sulphate (150 mg/L)for isolation of fungi. A total of fifteen tissue segments from each medicinal plant(host) were plotted for endophyte isolation.

Petri plates were sealed with Parafilm to avoid air contamination. Petri plates were incubated in abiological oxygen demand (BOD) incubator (Super Tech Instruments) for up to 1 week at 26±2 °C.Actively growing fungal tips emerging out from plant tissues were sub-cultured on fresh PDA plates for identification, documentation and antimicrobial study.Endophytic fungal genera were identified, according to their colony morphology and microscopic structures such as mycelium characteristics, fruiting structures and spores morphology, using standard taxonomic manuals (Ellis 1971, 1976).

### *In vitro* antimicrobial (bacterial / fungal) activity of endophytic isolates

Endophytic fungal isolates were cultured in 500 ml Erlenmeyer flasks containing 250 ml potato dextrose broth (PDB) and incubated at 26±2 °C for 21 days in a BOD incubator. The broth from each flask was taken in 2.0 mL centrifuge tubes separately and centrifuged at 10,000 RPM for 10 min to get cell free supernatant. The 100 µl of the cell free broths were used to test their antimicrobial activity against clinical isolates of human pathogens i.e. Bacterial pathogens *Shigella boydii* (IMS/GN2), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae*, *Escherichia coli* (ATCC-25922), and *Enterococcus faecalis* (IMS/GN7) and fungal pathogens *Microsporum gypseum* (IMS/A-014), *Trichophyton mentagrophytes* and, *Candida albicans*. The 50 µl overnight grown pathogenic bacterial culture was spread evenly with a glass spreader onto the surface of solidified Mueller-Hinton (MH) Agar petri plates. On these MHA plates, wells of 6 mm diameter were cut out with the help of cork borer and filled with 100 µl of 21 days old fermented cell free broth of endophytic fungal isolates. The plates were incubated at 37°C for 24 hours and zone of inhibition against pathogens

were observed. The respective zones of inhibition against bacterial pathogens were measured in centimeters. In case of fungal pathogen, in which 4 wells of 6 mm were cut out with help of cork borer from the PDA plate containing a pathogenic fungal growth and filled with 100 µl of cell free fermented broth of endophytic isolates. The plates were incubated at 25 ±2 °C for 1 week and observed for antifungal activity.

***Phosphate solubilization activity***

Inorganic phosphate solubilizing activity was observedby plates assay as described by Pikovaskaya (Pikovskaya, 1948) on Pikovaskaya's Agar Medium (PVK).Tricalcium phosphate $Ca_3(PO_4)_2$ was used in Pikovaskaya's Agar Medium to determine phosphate solubilization activity by endophyticisolates. The culture plates were incubated at 25±2°Cfor 4 days. A halo zone was formed around the fungal colony confirming phosphate solubilization.

***Indole Acetic Acid (IAA) Production***

Auxin production by endophytic fungal isolates was determined in terms of IAA equivalents with L-tryptophan (Bric*et al.*1991). Fungal isolates were cultured in PD broth amended with 5 mM tryptophan and incubated at 25±2 °C for 1 week. Cell free supernatant collected from 1ml culture filtrate after centrifugation (10,000 x g for 10 min) was added in 2 ml Salkowski reagentand incubated for 1 h at room temperature under dark conditions. Development of pink color indicated positive sign for IAA production.

***Amylase activity***

The endophytic fungal isolates were spot inoculated on starch agar medium containing per liter of distilled water, beef extract 3 g, peptone 5 g, soluble starch 20 g and agar 15 g. Plates were incubated at 25 ±2°C for 4 days. At the end of incubation period, the plates were flooded with iodine solution, kept for a minute and then poured off. After few minutes blue color faded rapidly and a hollow zone appeared around the periphery of fungal growth. The colorless zone surrounding the colonies indicated the production of amylase (Collin *et al.* 1995).

**RESULTS AND DISCUSSION**

Endophytic fungi were isolated from healthy, asymptomatic plant tissue (medicinal part) segments of 12 different medicinal plants collected from the Achanakmar-Amarkantak Biosphere Reserve. These medicinal plants were selected based on the survey of site and ethnomedicinal property suggested by the local tribal people of that region (Table 1). A total of 39 endophytic fungal isolates were recovered belonging to 17 genera dominated by *Alysidium* sp., *Taeniolella* sp., *Aspergillus* sp. and *Grallomyces* sp. (Table 2, Fig 1). Most of the medicinal plants selected in the present study were not investigated earlier for their endophytic fungi. Five isolates belonging to *Taeniolella* were recovered from leaf of *Nyctanthes arbor-tristis* while it was not found in same host in a previous study conductedby Gond *et al.*(2012). Most of the researchers in India and abroad still depend on microscopic identification of fungi with the help of standard manuals (Gond *et al.*2012; Kharwar *et al.* 2011a; Guo *et al.* 2000). However, molecular approaches based on DNA sequences are now being applied to identify morphologically closely related species ofendophytic fungi, or to those non spore forming fungi (Naik *et al.* 2009).

The 21 days old fermented cell free broths of endophytic fungal isolates were active against human pathogens and shown significant antimicrobial activity (Table 3, Fig 2). The broad spectrum antimicrobial activity was shown by *Scytalidium* sp., *Taeniolella* sp., *Aspergillus* sp., and *Diplococcium* sp. against sets of human pathogens. In present study most of the fungal endophytic isolates were amylase positive and none of them were able to solubilize inorganic phosphate on Pikovaskaya's agar. Some of these endophytic fungal isolates were able to produce indole acetic acid by utilizing tryptophan. The isolate number AC-5/2 (*Fusarium* sp.) was observed to produce significantly good amount of IAA as intensity of pink color was greater than other isolates (Table 4). The fungal endophytes isolated from tall fescue have also been reported to produce IAA.

The endophytic fungi from five plants i.e *Finlaysonia obovata, Cassine glauca, Ougeinia dalbergioides, Nyctanthes arbor-tristis* and *Buchanania lanzan* have greater inhibitory potential against most of the human pathogens. *Taeniolella* sp. (AC- 9/5) isolated from *Nyctanthes arbor-tristis* has displayed antibacterial activity

**Table 1:** Selected medicinal plants found in AchanakmarAmarkantak Biosphere Reserve and their uses as suggested by local tribes

| *Botanical Name* | Family | Local name | Used by tribe | Parts | Ethno-medical Uses |
|---|---|---|---|---|---|
| *Careya arborea* | Lecythidaceae | Kumbhi | Baiga, Gond | Leaf | Used in snakebite antidote (fruit and bark), in treating body pain, fever, cold and cough,. |
| *Dioscorea bulbifera* | Dioscoreaceae | kadukanda | Baiga, Gond | Leaf | Used in diarrhea, and dysentery, among other ailments. |
| *Buchanania lanzan* | Anacardiaceae | Chiraunji | Baiga | Leaf | Used in blood disorders, skin disease, bleeding disorder and nasal bleeding. |
| *Alstonia scholaris* | Apocynaceae | Koraya | Baiga, Gond | Leaf | Used in malaria and asthma. |
| *Abrus precatorius* | Fabaceae | Ratti | Baiga, Gond | Leaf | Traditionally used to treat tetanus, rabies. |
| *Antidesma acidum* | Phyllanthaceae | KhattaMitha | Baiga, Gond | Leaf | Used in the treatment of dysentery and bile complaints. |
| *Nyctanthes arbortristis* | Oleaceae | Harchingar | Baiga | Leaf | Bone and heart disease. |
| *Grewia hirsute* | Tiliaceae | Gursari | Baiga, Gond | Leaf | Useful as brain tonic, demulcent, anti-acidic expectorant, antipyretic, diuretic, aphrodisiac, carminative and cardiac tonic. |
| *Desmodiumo ojeinense* | Fabaceae | Tinsa | Baiga, Gond | Bark | Used in bloody stool |
| *Tinospora cordifolia* | Menispermaceae | Padhin | Baiga, Gond | Root | Used in stomachache, vomiting, dysentery, fever, snake bite, headache. |
| *Cassine glauca* | Celastraceae | Jamrashi | Baiga | Leaf | The fresh extract of stem bark and leaves is applied on cuts and wounds. |
| *Finlaysonia obovata* | Apocynaceae | Dudhilata | Gond | Leaf | Used in cuts and wounds.. |

against *Shigella boydii*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Escherichia coli,* and *Enterococcus faecalis*. The maximum inhibition (1.6 cm) observed in case of *Shigella boydii* and was also active against *T. mentagrophytes.* The *Scytalidium* sp. (AC – 5/1) isolated from *Buchanania lanzan* was active against *Shigella boydii*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Enterococcus faecalis*.The endophytic fungal isolates (*Taeniolella* sp. and *Diplococcium* sp.) of *Cassine glauca* has shown both antibacterial and anti-fungal activity against human pathogens (Table 3). More than 75% endophytic fungal isolatesfrom *Nythanthus arbortristis* had displayed antibacterial activity against several human pathogenic bacteria (Gond *et al.* 2012). Endophytic fungi have been the unexplored source of bioactive natural compounds that may be subjected to explore unparallel chemical and structural diversity with potent biological activity (Guo *et al.* 2000).

Most of the endophytic isolates have shown greater potential against *Enterococcus faecalis*. The endophytic *Humicola* sp. isolated from *Abrus precatorius* has shown antifungal activity against *M. gypseum* and *C. albicans. Humicola* sp. was also reported in rice plant having antagonistic activity (Naik *et al.* 2009). *Aspergillus* sp. isolated from *Desmodium oojeinensis*has shown both antibacterial and antifungal activity. Antimicrobial compounds have been isolated from endophytic *Aspergillus* isolated from *Mirabilis jalapa* showing significant antimicrobial potential against *Bacillus subtilis*, followed by *Micrococccus luteus* and *Staphylococcus aureus* (Mishra *et al.*2017). Some of the endophytic fungi like *Chaetomium* spp., *Aspergillus fumigatus*, *A. niger*, *Alternaria* spp., *Botryodiplodia* spp., *Cladosporium* spp., *Fusarium* spp., *Penicillium* spp., *Pestalotia* spp. and *Phomopsis* spp. isolated from different hosts have been characterized to produce numerous effective antimicrobial and cytotoxic compounds (Strobel

**Table 2:** Colony morphology and Microscopiccharacteristics of fungal endophytes isolated from different medicinal plants

| | | | Colony Characteristics | | | Mycelium Characteristics | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Host | Isolate no. | Host tissue type | Colony Colour | Margin of colony | Base colour of colony | Septat-ion | Colour | Branching | Spores | Morphologically identified as |
| *Careya arborea* | AC – 2/1 | Leaf | Off white | Colourless | Greenish | Septate | Colourless | Branched | branched chain, spherical, oval limoniform | *Alysidium* sp. |
| | AC – 2/2 | Leaf | White | Colourless | Off white | Septate | Colourless | Branched | Dark brown, round and cylindrical, single or arrange in chain | *Taeniolella* sp. |
| *Dioscorea bulbifera* | AC – 4/1 | Leaf | Black | White | Off white | Septate | Colourless | Branched | Asexual spore present | *Aspergillus* sp. |
| | AC – 4/2 | Leaf | Off white | Colourless | Greenish | Septate | Colourless | Branched | branched chain, spherical, oval limoniform | *Alysidium* sp. |
| | AC – 4/3 | Leaf | White | Colourless | Off white | Aseptate | Colourless | Unbranched | Dark brown, round and cylindrical, single or arrange in chain | *Taeniolella* sp. |
| *Buchanania lanzan* | AC – 5/1 | Leaf | Off white | White | Grey | Septate | Colourless | Highly Branched | Conidia, Brown multicelled | *Scytalidium* sp. |
| | AC – 5/2 | Leaf | White | Colourless | White | Septate | Colourless | Branched | colourless spores (conidia), which are canoe-shaped | *Fusarium* sp. |
| | AC – 5/3 | Leaf | Off white | White | Grey | Septate | Dark grey | Branched | Spores occur individually with three or more transverse division or septa. | *Curvularia* sp. |
| *Alstonia scholaris* | AC – 6/1 | Leaf | Off white | Colourless | Greenish | Septate | Colourless | Branched | branched chain, spherical, oval limoniform | *Alysidium* sp. |

(*Contd. part table 2*)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AC – 6/2 | Leaf | White | Colourless | Off white | Septate | Colourless | Branched Branched | Dark brown, round and cylindrical, single or arrange in chain | *Taeniolella* sp. |
| | AC – 6/3 | Leaf | White | Colourless | Off white | Septate | Colourless | Branched | Dark brown, round and cylindrical, single or arrange in chain | *Taeniolella* sp. |
| | AC – 6/4 | Leaf | White | Colourless | Off white | Septate | Colourless | Branched | Dark brown, round and cylindrical, single or arrange in chain | *Taeniolella* sp. |
| *Abrus precatorius* | AC – 7/1 | Leaf | White | Colourless | Off white | Septate | Colourless | Branched | Golden brown, end to end arranged | *Humicola* sp. |
| | AC – 7/2 | Leaf | Pale yellow | Pale yellow | Yellow | Septate | Colourless | Branched | Brown or reddish brown, sporodochia, usually unbranched, straight or flexus | *Wallemia* sp. |
| | AC – 7/3 | Leaf | Off white | White | Grey | Septate | Colourless | Branched | Conidia, Brown multicelled | *Scytalidium* sp. |
| *Antidesmaacidum* | AC – 8/1 | Leaf | White | White | Brown-green | Septate | Colourless | Branched | dark setae and curved conidia | *Colletotrichum* sp. |
| | AC – 8/2 | Leaf | White | Grey | Dark grey | Multi-septate | Colourless | Branched | Olivaceous brown | *Grallomyces* sp. |
| | AC – 8/3 | Leaf | White | Grey | Dark grey | Multi-septate | Colourless | Branched | Olivaceous brown | *Grallomyces* sp. |
| | AC – 8/4 | Leaf | White | Grey | Dark grey | Multi-septate | Colourless | Branched | Olivaceous brown | *Grallomyces* sp. |
| | AC – 8/5 | Leaf | White | Grey | Dark grey | Multi-septate | Colourless | Branched | Olivaceous brown | *Grallomyces* sp. |
| | AC – 8/6 | Leaf | Off white | White | Grey | Septate | Colourless | Branched | Dark brown cells swollen | *Centrospora* sp. |
| *Nyctanthes arbor-tristis* | AC – 9/1 | Leaf | White | Colourless | Off white | Septate | Colourless | Branched | Dark brown, round and cylindrical, single or arrange in chain | *Taeniolella* sp. |

(*Contd. part table 2*)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AC – 9/2 | Leaf | White | Colourless | Off white | Septate | Colourless | Branched | Dark brown, round and cylindrical, single or arrange in chain | *Taeniolella* sp. |
| | AC – 9/3 | Leaf | White | Colourless | Off white | Septate | Colourless | Branched | Dark brown, round and cylindrical, single or arrange in chain | *Taeniolella* sp. |
| | AC – 9/4 | Leaf | White | Colourless | Off white | Septate | Colourless | Branched | Dark brown, round and cylindrical, single or arrange in chain | *Taeniolella* sp. |
| | AC – 9/5 | Leaf | White | Colourless | Off white | Septate | Colourless | Branched | Dark brown, round and cylindrical, single or arrange in chain | *Taeniolella* sp. |
| *Grewia hirsuta* | AC – 11/1 | Leaf | White | Colourless | Off white | Septate | Colourless | Branched | Dark brown, round and cylindrical, single or arrange in chain | *Taeniolella* sp. |
| | AC – 11/2 | Leaf | Off white | Colourless | Greenish | Septate | Colourless | Branched | branched chain, spherical, oval limoniform | *Alysidium* sp. |
| *Desmodium oojeinense* | AC – 12/2 | Bark | Off white | Colourless | Greenish | Septate | Colourless | Branched | branched chain, spherical, oval limoniform | *Alysidium* sp. |
| | AC – 12/3 | Bark | Off white | Colourless | Greenish | Septate | Colourless | Branched | branched chain, spherical, oval limoniform | *Alysidium* sp. |
| | AC – 12/4 | Bark | Black | White | Off white | Septate | Colourless | Branched | Spherical spores, cleistothecia present | *Aspergillus* sp. |

(*Contd. part table* 2)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AC – 12/5 | Bark | Off white | Colourless | Greenish | Septate | Colourless | Branched | branched chain, spherical, oval limoniform | *Alysidium* sp. |
| *Tinospora cordifolia* | AC – 14/1 | Root | White | Colourless | Off white | Septate | Colourless | Branched | Pale brown, smooth and O-septate | *Hansfordia* sp. |
| | AC – 14/2 | Root | Dark green | Off white | Grey | Septate | Colourless | Highly branched | Conidiophores are branched, septate, and dark | *Cladosporium* sp. |
| *Cassine glauca* | AC – 15/1 | Leaf | White | Colourless | Off white | Aseptate | Colourless | Branched | Dark brown, round and cylindrical, single or arrange in chain | *Taeniolella* sp. |
| | AC – 15/2 | Leaf | White | None | Off white | Septate | Colourless | Branched | Oval, single or bicelled spore formed between mycelium | *Pendulispora* sp. |
| | AC – 15/3 | Leaf | Dark grey | White | Yellow | Septate | Colourless | Branched | Spherical spores arranged in clusters. | *Diplococcium* sp. |
| *Finlaysonia obovata* | AC – 16/2 | Leaf | White | Colourless | Off white | Septate | Colourless | Branched | Dark brown, round and cylindrical, single or arrange in chain | *Taeniolella* sp. |
| | AC – 16/3 | Leaf | Dark grey | White | Yellow | Septate | Colourless | Branched | Spherical spores arranged in clusters. | *Diplococcium* sp. |

and Daisy 2003; Schulz *et al.*2002; Gond *et al.* 2012; Kharwar *et al.* 2011b).

**CONCLUSION**

The present study is very important because the tribal people of Central India are known to use plants as folk remedies for treating a variety of ailments and this has caused the exploitation of ethnomedicinal plants from their natural habitats.It is hypothesized that if endophytic microbes produce important bioactive compounds of their host origin, this could preserve the overe-xploitation of rare medicinal plantsand biodiversity. Isolate no. AC – 5/1 and AC – 9/5 are having good antimicrobial activity against a wide range of human pathogens. These isolates could be selected for characterization of antimicrobial and other bioactive compounds.

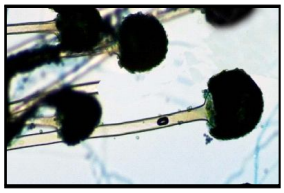
**Table 3:** Anti bacterial/ anti fungal activity of fermented cell free broth of endophytic fungal isolates against human pathogenic microbes

| Host plant | Endophytic Isolate | Inhibition zone against bacterial pathogens (cm.) | | | | | Inhibition against fungal pathogens | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Shigellaboydii* | *Staphylococcus aureus* | *Klebsiella pneumoniae* | *Escherichia coli* | *Enterococcus faecalis* | *Microsporum gypseum* | *Trichophyton mentagrophytes* | *Candida albicans* |
| *Careya arborea* | AC – 2/1 | – | – | – | – | – | – | – | – |
| | AC – 2/2 | – | – | – | – | – | – | – | – |
| *Dioscorea bulbifera* | AC – 4/1 | – | – | 1.0 | – | 1.0 | + | – | – |
| | AC – 4/2 | – | – | – | – | – | + | + | – |
| | AC – 4/3 | – | – | – | – | – | – | – | – |
| *Buchanania lanzan* | AC – 5/1 | 1.0 | 1.0 | 1.0 | – | 1.0 | – | + | – |
| | AC – 5/2 | – | – | – | – | 1.2 | + | – | – |
| | AC – 5/3 | – | 1.4 | 1.7 | – | – | – | – | – |
| | AC – 5/4 | – | – | – | – | 1.4 | + | – | – |
| *Alstonia scholaris* | AC – 6/1 | – | – | – | – | 1.2 | + | – | – |
| | AC – 6/2 | – | – | 1.3 | 1.2 | 1.3 | – | – | – |
| | AC – 6/3 | 1.0 | 1.0 | – | – | – | – | + | – |
| | AC – 6/4 | – | – | – | – | – | – | – | – |
| *Abrus precatorius* | AC – 7/1 | – | – | – | – | – | + | – | + |
| | AC – 7/2 | – | – | – | – | 1.7 | + | – | – |
| | AC – 7/3 | – | – | – | – | – | + | – | – |
| | AC – 7/4 | – | – | – | – | – | – | – | – |
| *Antidesma acidum* | AC – 8/1 | 1.0 | 1.5 | – | – | – | – | – | – |
| | AC – 8/2 | – | – | – | – | – | – | + | – |
| | AC – 8/3 | 1.5 | 1.5 | – | – | – | – | – | – |
| | AC – 8/4 | – | – | – | 1.1 | – | – | – | – |
| | AC – 8/5 | – | – | – | – | – | – | + | + |
| | AC – 8/6 | – | – | 1.2 | – | – | + | – | – |
| *Nyctanthes arbortristis* | AC – 9/1 | 1.4 | – | – | – | – | – | – | + |
| | AC – 9/2 | – | 1.2 | – | – | – | – | – | – |
| | AC – 9/3 | – | – | – | – | – | – | – | – |
| | AC – 9/4 | – | – | – | – | – | + | – | – |
| | AC – 9/5 | 1.6 | 1.3 | 1.4 | 1.1 | 1.2 | – | + | – |
| *Grewia hirsuta* | AC – 11/1 | – | 1.2 | – | – | – | – | – | – |
| | AC – 11/2 | – | – | – | – | – | – | – | + |
| | AC – 12/2 | 1.0 | – | – | – | – | + | + | – |
| | AC – 12/3 | – | – | 1.0 | – | 1.0 | – | – | – |

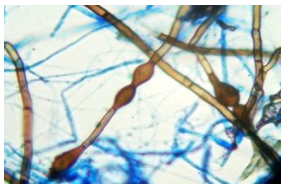
(*Contd. part table* 3)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Desmodium oojeinense* | AC – 12/4 | – | – | 1.0 | – | 1.0 | + | + | – |
| | AC – 12/5 | – | 1.0 | 1.0 | – | 1.0 | – | – | – |
| | AC – 12/6 | – | – | – | – | 1.2 | + | + | – |
| *Tinospora cordifolia* | AC – 14/1 | – | – | – | – | – | + | – | – |
| | AC – 14/2 | – | – | – | – | – | – | – | – |
| | AC – 15/1 | – | – | 1.0 | – | 1.0 | – | – | – |
| | AC – 15/2 | – | – | – | – | – | – | – | – |
| *Cassine glauca* | AC – 15/3 | – | – | – | – | – | – | – | – |
| | AC – 15/4 | 1.0 | – | – | – | – | – | – | – |
| | AC – 16/2 | – | – | 2.5 | – | – | – | + | – |
| | AC – 16/3 | 1.0 | 1.4 | – | – | 1.5 | + | – | + |
| Streptomycin sulphate(1µg/ml) | | 2.3 | 2.9 | 2.0 | 2.1 | 3.0 | – | – | – |
| Cycloheximide (1µg/ml) | | – | – | – | – | – | + | + | + |

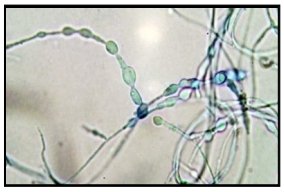
("+" = inhibition and "–" = no inhibition)



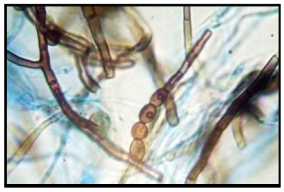
*Aspergillus* sp.



*Alysidium*sp.



*Scytalidium* sp.

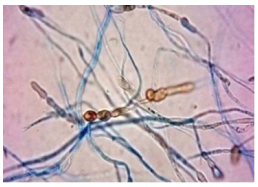


*Taeniolella* sp.

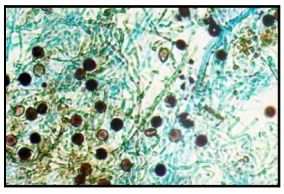
*Humicola* sp.

*Cladosporium*sp.

*Curvularia*sp.

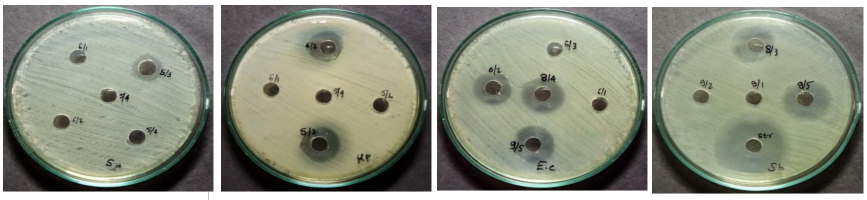


*Grallomyces*sp.



*Hansfordia*sp.

**Fig. 1 :** Microscopic observation of endophytic fungal isolates at 400X magnification



*Staphylococcus aureus* | *Klebsiellapneumoniae* | *Escherichia coli* | *Shigellaboydii*

**Fig. 2 :** Anti- microbial activity of endophytic fungal isolates

**Table 4:** Biochemical activity of endophytic fungal isolates

| Isolates | Phosphate solubilization | Amylase production | IAA production |
|---|---|---|---|
| AC – 2/1 | – | + | – |
| AC – 2/2 | – | + | – |
| AC – 4/1 | – | + | – |
| AC – 4/2 | – | + | – |
| AC – 4/3 | – | + | – |
| AC – 5/1 | – | + | – |
| AC – 5/2 | – | + | ++ |
| AC – 5/3 | – | + | – |
| AC – 6/1 | – | + | + |
| AC – 6/2 | – | + | – |
| AC – 6/3 | – | + | – |
| AC – 6/4 | – | + | – |
| AC – 7/1 | – | + | + |
| AC – 7/2 | – | + | – |
| AC – 7/3 | – | + | – |
| AC – 8/1 | – | + | – |
| AC – 8/2 | – | + | – |
| AC – 8/3 | – | + | – |
| AC – 8/4 | – | + | – |
| AC – 8/5 | – | + | – |
| AC – 8/6 | – | + | – |
| AC – 9/1 | – | + | – |
| AC – 9/2 | – | + | – |
| AC – 9/3 | – | + | – |
| AC – 9/4 | – | + | – |
| AC – 9/5 | – | + | – |
| AC – 11/1 | – | + | – |
| AC – 11/2 | – | + | + |
| AC – 12/2 | – | + | + |
| AC – 12/3 | – | + | + |
| AC – 12/4 | – | + | – |
| AC – 12/5 | – | + | + |
| AC – 14/1 | – | + | – |
| AC – 14/2 | – | + | – |
| AC – 15/1 | – | + | – |
| AC – 15/2 | – | – | + |
| AC – 15/3 | – | – | – |
| AC – 15/4 | – | + | – |
| AC – 16/2 | – | + | – |
| AC – 16/3 | – | – | – |

"+" = activity present, "++" =significantly greater activity present and "–" =activity absent

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