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**RESEARCH ARTICLE**

**Isolation of fungal Endophytes from *Murraya koenigii* and *Costus pictus* valuable medicinal plants**

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**Abstract**

A total 11 endophytic fungi were isolated from two plants collected from Amravati district of Maharashtra region for obtaining biological diversity in endophytic fungi. Total 5 five endophytic fungi were isolated from *Costus pictus*. All the endophytic fungi were belong to deuteromycetia. Total six endophytic fungi were isolated from *Murraya koenigii* two fungi separated were belonged to zygomycetes where as the remaining belong to Deuteromycetes. In the present study rich diversity of endophytic fungi were isolated from stems leaves, and fruits tissue of the two plant species total endophytic fungi isolates were obtained from different tissue fragment of *Murraya koenigii* and *Costus spp.*

**Introduction**

Medicinal plants have been playing important role for treatment of various human ailments since time immemorial. Mittal *et al.*, (2014) described the prominence of a medicinal plant and their uses. According to the world health organization, over 80% of the world's population or 4.3 billion people rely upon such traditional plant based systems of medicine to provide them with primary health care. Anitha *et al.*, (2013) isolated and characterized endophytic fungi from medicinal plant and reported that they were

exploited for endophytes as a possible source of bioactive secondary metabolites. Nevertheless indiscriminate exploitation of this plant resource has rapidly declined their population making some of them critically endangered the medicinal plants harbour some distinct fungal endophyte that are believed to be associated with the production of pharmaceutical products. The leaves of *Murraya koenigii* are used extensively for seasoning and flavoring dishes curry leaf is exported as curry leaf as curry leaf oil from India. The oil extracted from the leaves and extracts of different parts of plants. Possessed have micrological activities in the present investigation an attempt was made for isolation. Identification and screening of fungi endophytes from aerial and non aerial parts of first time for identification and screening of all available fungal endophytes from the aerial and non aerial parts of the plant. *Costus pictus*, commonly known as fiery costus, step ladder or spiral flag or Insulin plant, is native to South and Central America. This is a recent introduction to India from America as an herbal cure for diabetes and hence commonly called as 'insulin plant.' It is widely grown in gardens as ornamental plant in South India and also run wild in many places. It is used in India to control diabetes, and it is known that diabetic people eat one leaf daily to keep their blood glucose low. Leaves of *Costus igneus* were one among the plants known to be

effectively used for treating diabetes by the tribal people of Kolli hills of Namakkal district, Tamilnadu. Endophytic fungi that live inside the tissues of living plants are under explored group of microorganisms estimated that there may be at least one million sp of endophytic fungi alone. The nature and biological role of endophytic fungi with their plant host is variable. Endophytic fungi are known to have mutualistic relations to their hosts, often protecting plants against herbivorous insect attack or tissue invading pathogens and in some instance the endophyte may survive as a latent pathogen, causing or quiescent infections for a long period and symptoms only when physiological ecological condition favours virulence in Malaysia extract from many types of local plants are used in traditional manner for treatment of various ailments. The question is whether they are produced by the plant itself or as a consequence of a mutualistic relationship with beneficial organisms in their tissue. Selvi and Balagengatharathilakam (2014) studied fifty one fungal endophytic belonging to twenty one genera were isolated from medicinal plants in virudhu district. The crude metabolite of endophytic fungus, *Cladosporium spp.* displayed a significant antimicrobial activity against all test pathogens. Many reports showed that in a microbe plant relationship endophytic contribute substances that possess various types of bioactivity, such as antifungal and antibacterial. Thus in this study we focus on the isolation of endophytic fungi and their characteristics.

### **Materials and methods**

In the present study two plants viz., *Murraya koenigii* and *Costus pictus* were collected from Brijlal Biyani Science College botanical garden Amravati, Maharashtra, India from different plant samples viz., root inner bark, leaves flower stem etc. were collected from each nature healthy plant and were immediately processed.

Plant samples were collected randomly from two healthy individual plants during winter season. The sample was collected in plastic bags immediately brought to the laboratory stored at 4°C until isolation procedure was accomplished. The mean temperature during the study period was 32 °C. The plant species were identified authenticated and maintained in our laboratory. The sample were rinsed gently in running water to remove dust and debris. After proper washing stem sample were cut into long 0.5-1cm pieces where as leaves were cut into 3.4 mm x 0.5-1 cm pieces under condition. surface sterilization was done by 1-13% sodium hypochloride (NaCl) according to the type of tissues. Each set of plant material was treated with 75% ethanol, distilled water, blotting paper. The sterilized stem leaf explants were cultured in Petri dish containing PDA medium supplemented with 100 µg/ml of streptomycin Petri dishes were sealed with par film and incubated at 27 °C for 15 days under dark conditions and monitored every day Fungi growing out of the plant explants were sub cultured on separate PDA plated at room temperature and identified in their sporulation state by staining with lacto phenol blue. The fungi which failed to sporulate were designated as mycelia sterile for colony character the mycelia were transferred in to PDA media. The plates were checked every day for two weeks for the growth of endophytic fungi. The hyphal tip of endophytic fungus growing out of the plant tissues were immediately transferred to a sterile PDA plants and maintained at 40°C. The fungal isolates were identified based on their morphological and reproductive character using the standard identification. The fungal cultures that failed to sporulate were categorized as sterile mycelia.

### **Results and discussion**

Endophytic fungi are known to be antiquitous and every plant species examined to date have been found colonized with fungal endophytes.

Following the standard protocol of the endophytic fungi isolation with some required changes, a total 11 endophytic fungi were isolated from two plants collected from Amravati district of Maharashtra region for obtaining biological diversity in endophytic fungi. Total 5 five endophytic fungi were isolated from *Costus pictus*. All the endophytic fungi were belong to deuteromycetias. Total six endophytic fungi were isolated from *Murraya koenigii* two fungi separated were belonged to zygomycetes where as the remaining belong to Deuteromycetes. Endophytic fungi isolated from different parts of *Costus pictus* table 1. Jena and Tayung (2013) isolated a total of 458 endophytic isolate from leaf stem and fruit tissue of Solano rub-rum and. The dominant endophytic fungi belong to genera, *Aspergillus*, *Colletotrichum*, *Curvularia* and *Mycellastarilia*. Maximum endophytic were obtained from leaves. The study revealed that medicinal plants associated endophytic could be a rich source of antimicrobial agents. In the present study rich diversity of endophytic fungi were isolated from stems leaves, and fruits tissue of the two plant species total endophytic fungi isolates were obtained from different tissue fragment of *Murraya koenigii* and *Costus species*. Maximum endophyte isolated were obtained from on *Murraya koenigii* table 2. The colonization of endophytic fungi is more prevalent in stem and leaf than other tissue. This may be due to their anatomical structure supply of nutrient elements. In both the plants endophytic fungi class Hypomycetes were dominant. The dominant nature of class Hypomycetes may be attributed to their ability to colonize host rapidly produce large more a sexual spores. Diversity and species more high of endophytic fungi were studied in different tissue of two plants .the result indicated that both the plant spp. were higher in endophytic fungi. As endophytic in different tissue of both the plant germination of more number of spores of these fungi due to

favourable environmental condition the way for the symbiotic microbes to survive and explore.

**Table1: Endophytic fungi isolated from different parts of *Costus pictus***

Sr. No.	Species	Site of isolation	No. of sample	Fungi isolated
1	<i>Nigrospora spp.</i>	Stem	06	02
2	<i>Penicillium spp.</i>	Stem	06	01
3	<i>Alternaria sp.</i>	Leaf	04	01
4	<i>Mycelia starillia</i>	Leaf	05	01
5	<i>Curvularia spp.</i>	Stem	05	01

**Table 2: Endophytic fungi isolated from different parts of *Murraya koenigii***

Sr. No.	Species	Site of isolation	No. of sample	Fungi isolated
1	<i>Fusarium oxysporum</i>	Stem	08	02
2	<i>Cladosporium spp.</i>	Stem	08	01
3	<i>Rhizopus spp.</i>	Stem	06	01
4	<i>Candida spp.</i>	Stem	05	01
5	<i>Aspergillus flavus</i>	Leaf	05	01
6	<i>Mucar spp.</i>	Leaf	05	01

**The characterization of the isolated fungi was carried out based on observations recorded with simple microscope and are presented in fig 1 under following head.**

- 1) ***Fusarium oxysporum***: Growth moderate, white, peach, to salmon pink or violet conidiogeous hyaline, enterobiastic more or polyphialidic fusarium species produce several types of conidia, microconidia hyaline 0-1or septed small, macro conidia hyaline, curved, phragmospores with a foot cell bearing some kind of heel chlamyospores may absolute present borne terminally or intercalary or on the macro conidia. Micro conidia are oval to cylindrical or even and produced on simple short phial idesmicroconidia 3-5 saptate 27-60x 3-5 cm.
- 2) ***Mycelia sterilia***: Many fungi do not produce any recognizable sexual/asexual conidia state in culture. Such forms are frequently classified for convenience in the mycelia sterilia. This group is catchcall which may include a few well defined castly recognizable genera but mope

often is a depository for a large no. eg. nondescript mycelial isolates.

3) *Aspergillus flavus*: Colonies white or silvery white never yellow to orange brown or reddish brown. Conidial head columnar in size vesicles globes to ovate, modular fertile over entire vesicle, conidial heads splitting over age. Conidia smooth, globes, 2-3 in diameter.

4) *Penicillium spp* : Mycelium with grey color at the centre whitish the colony secretes reddish orange color into the media and concentric grey coloration each of the rings having different gradations of color oil droplets are formed on the fifth day of the inoculation the colony turned saffron yellow in color after six to seven day of inoculation the hyphae is separate, smooth walled the length of the phyllades was 11.15  $\mu\text{m}$  and the breath 1.81  $\mu\text{m}$ . The spores were circular in chains with length of each spore begin 3.27  $\mu\text{m}$  and breath 1.86  $\mu\text{m}$ .

5) *Nigrospora spp.*: Colonies white later brown to black when speculation is abundant conidiophores micronematous, branched, flexors, colorless to brown, smooth, conidia, solitary, acregerous simple, spherical or broadly ellipsoidal compressed dorsiventrally, black shinning, smooth o septet 10-18  $\mu\text{m}$  dimmer.

6) *Alternaria spp.*: Conidiophores dark, septate, sometimes inconspicuous, simple or branched, bearing conidia at the apex spores solitary or more often produced in curopetalsucession to form simple or branched chains darkly pigmental, ovate to obclavate tapering abruptly or gradually towards the distal overall conidial dimension are 15-20  $\mu\text{m}$ .

7) *Rhizopus spp.*: Fungi are characterized by a body of branching mycelia composed of three types of hyphae: stolons, rhizoids, and usually unbranching sporangiophores. The black sporangia at the tips of the sporangiophores are rounded and produce numerous nonmotile multinucleate spores for asexual reproduction.

9) *Cladosporium spp.*: On leaves, forming pale to dark brown, dense patches. Colonies of

*Cladosporium* are epiphyllous, scattered, caespitose, pale brown to olivaceous-brown, floccose-villose. Mycelium external, superficial; hyphae branched, septate, at first 3-6  $\mu\text{m}$  wide, later distinctly swollen and often constricted at the septa, up to 10  $\mu\text{m}$  wide or even wider, almost subhyaline or pale olivaceous, later pale olivaceous to medium brown or somewhat reddish brown, smooth or almost so to irregularly rough-walled, rugose, walls slightly to distinctly thickened, sometimes even two-layered, protoplasm of the cells often aggregated at the septa, olivaceous-yellowish, refractive and somewhat granular, surrounding walls much paler. Stromata lacking, conidiophores solitary which arising from external, creeping hyphae, swollen hyphal cells or stromatic hyphal aggregations.

10) *Mucor spp.*: Typically exhibits rapid growth, producing globose sporangia on sporangiophores that are either solitary or branched. The sporangia contain the entire columella and spores that are mucus bound. The sporangial wall collapses irregularly, if at all. The sporangia may also be deliquescent.

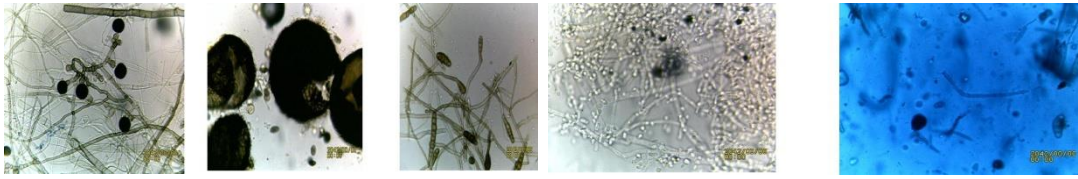
Fungal species isolated from *Murraya koenigii* was shown profuse growth during investigation. *Cladosporium spp.*, *Nigrospora spp.*, *Penicillium spp.* shows abundant growth during whole period this study. Microscopic observation of *Cladosporium spp.* was represented colony of whitish colour, Mycellia are regularly growing with flat growth and thick. The edge of mycelia shapes uneven and the color is white. colonies are fast growing while under microscope, mycelium not extensive, conidia hyaline, 1-celled, ovoid to fusoid, forming short chains by budding. Produce apically or laterally on mycelium. *Anitha et al.*, (2013) isolated and characterized endophytic fungi from medicinal plant were exploited for endophytes as a possible source of bioactive secondary metabolites. A total 14 fungal species were isolated and identified based on the

morphology of the fungal at culture which supports our study.

### **References**

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**Fig1: Isolated different fungi from two different plant species**



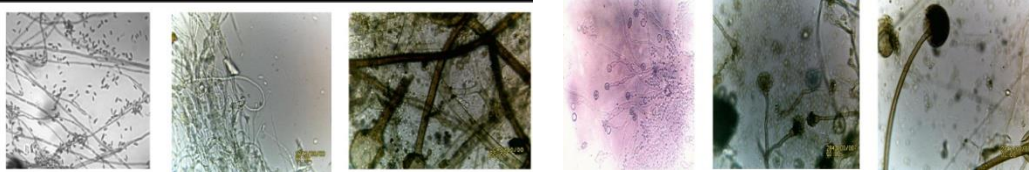
*Nigrospora* spp.

*Penicillium* spp.

*Alternaria* spp.

*Mycellia sterilia*

*Curvularia* spp



*Fusarium oxysporum*

*Cladosporium* spp.

*Rhizopus* spp.

*Candida* spp.

*Aspergillus flagus*

*Mucar* spp.