Isolation of Endophytic Fungi from *Tephrosia purpurea* L.

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Abstract

Endophytic fungi were isolated from the leaves of *Tephrosia purpurea L.*Linn. The isolated fungi were identified based on colony characters, dimensions of spores and fruiting bodies using stereo and light microscopes. Twenty one isolates belonging to 05 genera were recorded, viz. *Cladosporium* sp., *Trichoderma* sp., *Monilia* sp., *Fusarium* sp., *Penicillium* sp, while 09 strains were unidentified. The dominant genera found were *Monilia* sp. and *Fusarium* sp. Thus, there should be a significant corelation in the genera of endophytic fungi from *Tephrosia purpurea L.*from other plants.

Keywords: *Tephrosia purpurea L.*, endophytic fungi.

Introduction

Endophytic fungi are that live in plant tissues without any substantive harm or gaining benefit other than residency (Kadouri, 2003). Fungal endophytes can be isolated from surface-disinfected plant tissue or extracted from internal plant tissue (Hallman.et.al. 1997).

Endophytes enter plant tissue primarily through the root zone; however, aerial portions of plants, such as flowers, stems, and cotyledons, may also be used for entry. Specifically, the bacteria enter tissues via germinating radicles (Gagne et al., 1987), secondary roots (Agarwal and Shende, 1987) or as a result of foliar damage. Endophytes inside a plant may either become localized at the point of entry or spread throughout the plant (Hallmann et.al., 1997). These microorganisms can reside within cells (Jacobs et.al., 1985), in the intercellular spaces, or in the vascular system (Bell et al., 1995.). Significant variations in the populations of both indigenous and introduced endophytes have been reported. These variations are attributed to plant source, plant age, tissue type, time of sampling, and environment. Generally, fungal populations are larger in roots and decrease in the stems and leaves.

In some cases endophytes can also accelerate seedling emergence and promote plant establishment under adverse conditions (Chanway, 1997) and enhance plant growth and overall development The internal colonization strategies of endophytic fungi provide additional benefits as the internal parts of the plant tissue provide a more uniform and protective

environment for the them as compared to rhizosphere .

Endophytic fungi in medicinal plants, especially in tropical regions, are still poorly explored though they could represent a source of valuable new and bioactive compounds .Present studies focus on identification of fungal endophytes of *Tephrosia purpurea L.*

MATERIALS AND METHODS

Isolation and Identification of Endophytic:

Leaf samples of *Tephrosia purpurea L.*were collected from Udgir. Endophytic fungi isolation was carried out under aseptic condition. The leaf samples were detached with a sterilized scalpel blade, subsequently washing was done with running tap water several times and soaked in 70% (v/v) ethanol for 10-20 min. It was then washed several times with double distilled sterilized water, and given treatment of 0.1% $HgCl_2$ for 1-2 min, again washed with double distilled sterilized water 2-3 times and then put into a beaker of sterilized distilled water.

The leaves were cut into small pieces, each piece put on a plate of potato dextrose agar (PDA) medium added with chloramphenicol (30 µg/ml) and streptomycin (30 µg/ml), and the plate incubated at 25 -30°C to promote fungal growth and sporulation.

Fungal hypal growth from each plate, inoculated to another PDA medium plate, and incubated at 30°C for at least 1 week. Each fungal culture was assessed for purity and transferred to another agar plate using the hyphal tips. The pure culture of fungal isolates were numbered, transferred separately to PDA slants, and kept at 4^0 C.

Fungal mycelium and spores were stained with bromothymol blue reagent and examined with a bright-field microscope. Identification was based on morphological characteristics and spore dimensions.

Result

Twenty eight endophytic fungi were collected from 20 different samples (Table 2). All endophytic fungi cultured on artificial media and maintained as a pure culture. They exhibited characteristic colony and microscopic morphology that could be used to differentiate them. Most of them belonged to Ascomycetes and fungi imperfecti, as shown in Table (1).

The Endophytic fungi isolated belonging to 05 genera, namely *Cladosporium* sp. *Trichoderma* sp., *Monilia* sp., *Fusarium*., *Penicillium* .*Fusarium* sp. was the dominant genus found in all samples..Some fungi, viz. could not be identified due to lack of spore formation. These results indicated that there is significant difference in the genera of endophytic fungi from *Tephrosia purpurea L.leaves.*

Table 1. Genera of isolated endophytic fungi

Fungal genera	Number of endophytes isolates
Cladosporium sp.	11
Trichoderma sp.	12
Monilia sp.	09
Fusarium sp.	14
Penicillium sp.	09
unidentified strains	09

Conclusion

*Tephrosia purpurea L.*yielded the 05 different genera from twenty samples and some unidentified strains also were isolated and identified by their morphology and spore characteristics..

These results considered as those of an initial study. Further investigation, e.g. 16S rDNA sequence comparisons (molecular techniques), is required to confirm the classification of these fungal endophytes.

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