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Isolation and identification of endophytic fungi from *Osimum sanctum* Linn.

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Abstract

Thirty-two isolates of endophytic fungi were collected from the leaves of *Osimum sanctum* Linn. All isolates were identified based on colony morphology and examination of spores and fruiting bodies using stereo and light microscopes. Twenty isolates belonging to 05 genera were recorded, viz. *Cladosporium* sp., *Trichoderma* sp., *Monilia* sp., *Fusarium* sp., *Penicillium* sp,while 07 strains were unidentified. The dominant genera found were *Monilia* sp. and *Fusarium* sp. Thus, there seemed to be a significant difference in the genera of endophytic fungi from *Osimum sanctum* Linn.

Keywords: *Osimum sanctum Linn* endophytic fungi.

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Introduction

Endophytic fungi have been described as fungi that asymptomatically colonize healthy plant tissues, even though they may, after incubation or a latency period, cause diseases (Petrini 1991; Stone et al. 2000). Diverse associations with host plants have been reported, ranging from mutualistic relationships (Schulz et al. 2002) and cryptic commensalism (Deckert et al. 2001) to latent and quiescent pathogens (Sinclair and Cerkauskas 1996). The stability or the variability of the asymptomatic interaction depends on numerous factors such as environmental stress, senescence of the hosts, virulence of the endophytes and the host defense response (Schulz and Boyle 2005). Endophytes may be beneficial to the hosts in conferring resistance to insects and herbivores (Clay 1988), drought tolerance (West 1994), protection against pathogens (White and Cole 1985) and enhanced vegetative growth (Porter et al. 1979).

Endophytic fungi have been encountered from any plant ever investigated. In contrast, 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 (Li et al. 2001). recently, endophytic fungi isolated from a Brazilian medicinal plant were shown to produce guignardic acid (Rodrigues et al. 2001). Likewise, new bioactive metabolites were produced by a *Colletotrichum sp*., an endophytic fungus in *Artemisia annua*, a traditional Chinese medicinal herb (Lu et al. 2000) , so present investigation is an attempt to identify the fungal endophytes of *Osimum sanctum.*

MATERIALS AND METHODS

Isolation of Endophytic Fungi

Leaf samples of *O. sanctum* were collected from different parts of Udgir. Endophytic fungi isolation was carried out under aseptic condition. The leaf samples were detached with a sterilized sharp blade, cleaned by washing with running tap water several times and soaked in 70% (v/v) ethanol for 10-20 min. It was then washed several times with sterilized water, dipped into 0.1% $HgCl_2$ for 1-2 min, again washed with sterilized water 3-5 times and then put into a beaker of sterilized distilled water. The leaf samples were then cut into small pieces, each piece put on a plate of potato dextrose agar (PDA) medium supplemented with chloramphenicol (30 µg/ml) and streptomycin (30 µg/ml), and the plate cultivated at 30°C to promote fungal growth and sporulation. Individual hyphal tips of the fungus were then picked up from each plate, inoculated onto another PDA medium plate, and incubated at 30°C for at least 1 week. Each fungal culture was checked for purity and transferred to another agar plate using the hyphal tips. The purified fungal isolates were numbered, transferred separately to PDA slants, and kept at 4^0C.

Identification of Endophytic Fungi

For characterization of the morphology of fungal isolates, slides prepared from cultures were stained with bromothymol blue reagent and examined with a bright-field microscope. Identification was based on morphological characteristics such as growth pattern, hyphae, colour of colony and medium, surface texture, margin character, aerial mycelium, mechanism of spore production and characteristics of the spore.

Result

Thirty-two isolates of endophytic fungi were collected from 20 samples (Table 2). All endophytic fungi could be cultivated 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).

All isolates were identified as belonging to 05 genera, namely *Cladosporium* sp. *Trichoderma* sp., *Monilia* sp., *Fusarium*., *Penicillium* .*Trichoderma* sp. was the dominant genus found in the south of 4 isolates.

Some fungi, viz. 07 isolates 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 *O. sanctum leaves.* Huang et al reported that endophytic fungi can produce antitumour or antifungal activities.

Table 1. Genera of isolated endophytic fungi

Genus	Number of endophytes isolates
Cladosporium sp.	06
Trichoderma sp.	09
Monilia sp.	10
Fusarium sp.	07
Penicillium sp.	06
unidentified strains	06

Conclusion

Endophytic fungi from the *O. sanctum* were isolated and identified by their morphology and spore characteristics. From twenty samples from Udgir were obtained 32 isolates, which were identified as belonging to 05 genera with some unidentified strains.

these results should be considered as those of an initial study. Further investigation, e.g. 18S rDNA sequence comparisons (molecular techniques), is required to confirm the classification of these isolates.

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