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Isolation and Identification of Endophytic Fungi from *Semecarpus anacardium*

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ARTICLE DETAILS

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ABSTRACT

Endophytic fungi are those living inside the host plant without causing any apparent negative effect on host plant. Endophytic fungi from Semecarpus anacardium in Botany Research Centre, Maharashtra Mahavidyalaya, Nilanga were isolated and identified by morphology. 82 isolates were isolated from roots, stem and leaves of *Semecarpus anacardium* species. Among them, 01 unidentified isolate was identified by using molecular level i.e., *Eupenicillium ochrasalmoneum*. Other 81 isolates were identified as species belong to *Curvalaria lunata, Trichoderma viridae, Fusarium equiseti, Chaetomium globosum, Alternaria alternata, Fusarium oxysporum oxysporum, Aspergillus niger, Aspergillus terrus, Aspergillus versicolor, Phomopsis, Trichoderma harzianum, Aspergillus flavus and Eupenicillium ochrasalmoneum* by morphological characters. This study is an important step to isolate and identification of endophytic fungi and to explore the novel bioactive compounds from *Semecarpus anacardium*.

1. Introduction

Endophytes are live within plants for at least a part of their life cycle without causing any visible manifestation of disease (Bacon and white, 2000). A wide range of plants have now been examined for endophytes, and endophytes have been found in nearly all of them, including trees, grass, algae and herbaceous plants. Evidence of plant-associated microorganisms found in the fossilized tissues of stems and leaves has revealed that endophyte-plant associations may have evolved from the time higher plants first appeared on the earth (Redecker et al., 2000). Hawksworth and Rossman estimated that nearly one million species of endophytes may exist in the unexplored plants (Strobel and Daisy, 2003; Arnold, 2005). Endophytes microorganisms were discovered including fungi, bacteria, and actinomycetes. And fungal endophytes are the most frequently encountered endophytes (Staniek et al., 2008). It have great promise with diverse potential for exploitation (Li et al., 2012; Staniek et al., 2008). An enormous number of different fungi can be isolated from plants growing in their native habitat. The aim of this research focused on isolate endophytic fungi from roots, stem and leaves of *Semecarpus anacardium* and identified by morphological characters.

2. Materials and methods

2.1 Plant Sample Collection

Semecarpus anacardium was collected from Mahur forest Dist. Nanded. All the samples like root, stem and leaves were collected from of *Semecarpus anacardium.*

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2.2 Isolation of Endophytic Fungi

Samples of *Semecarpus anacardium* like root, stem and leaves were washed thoroughly in running tap water. Surface of the plant tissues was treated by following the methodology of (Murali et al.2007). Plant tissues were immersed in 75% ethanol for 1 minute and in an aqueous solution of sodium hypochlorite (2.5% available chlorine) for 3 minute, followed by washing with 70% ethanol for 5 seconds. The tissues were then rinsed in sterile distilled water and allowed to surface dry under sterile conditions. The surface-sterilized samples were placed on Petri dishes containing Potato Dextrose Agar (PDA) (supplemented with streptomycin (100 µg ml−1) to inhibit bacterial growth) and incubated at temperature at around 28°C.

2.3 Identification of Endophytic Fungi

The isolates of endophytic fungi were identified by the morphology of the fungal culture, including colony and medium color, Colony characters, Spore characters, Mycelium characters, Fruiting structures by following the standard mycological manuals (Barnett and Hunter, 1987; Domsch and Games, 1993; Sutton, 1980; Nag Raj, 1993). The sterile isolates were grown on PDA with decoction of host leaves medium to observe sporulation. For tentative identification, microscopic slides of each endophytic fungus were prepared and examined under binocular compound microscope for morphological identification.

3. Results and Discussion

From roots, stem and leaves of *Semecarpus anacardium,* in this study yielded 82 isolates (Table. 1). More isolates were obtained from stem of *Semecarpus anacardium* than roots and leaves.

Table. 1 Number of isolates from root stems and leaves of *Semecarpus anacardium*

Host Plant	Root	Stem	Leaves	Total
Semecarpus anacardium	18	33	31	82

1. *Curvalaria lunata*

Colonies effuse hairy and black color on PDA medium. Mycelium immersed in natural substrates. Conidia with hilum scarcely, remaining smooth-walled, dark brown color. Conidia predominantly 3-septate, the middle septum below the centre and the third cell strongly curved, tapering gradually towards the base.

2. *Trichoderma* species

Colony color ranged from white, yellowish-green and dark green upon sporulation. Conidia are mostly green, sometimes hyaline, with smooth or rough walls and are formed in slimy conidial heads (gloiospora) clustered at the tips of the phialides.

3. *Fusarium* species

The colonies fast growing with discrete sporodochia and white-ochraceous colour. Aerial mycelium floccose. Macro-conidia abundant and more-celled, slightly curved or bent at the pointed ends; central part straight, cylindrical, typically canoe-shaped. Phialides bearing micro-conidia very long.

4. *Alternaria alternata*

Colonies are fast growing, black to olivaceous-black or greyish, and are suede-like to floccose. Conidia are obclavate, obpyriform, sometimes ovoid or ellipsoidal, often with a short conical or cylindrical beak, pale brown, smooth-walled or verrucose.

5. *Aspergillus* species

Colonies growing rather slow on PDA with creamy-yellow color. Mycelium partly immersed, partly superficial. Stroma none; Setae and hyphopodia absent. Vesicles small, variable in shape. Conidial heads globose and bright yellow. Conidia globose to subglobose, smooth-walled, uninucleate, the chains sometimes sliming down.

6. *Phomopsis species*

Colonies grow fast on PDA with abundant floccose, whitish to olivaceous-grey aerial mycelium; reverse uncoloured. Pycnidia abundantly produced in the centre of the colonies, olivaceous-brown, dark around the ostiole. Pseudo sclerotia absent; conidia oblong, two-celled.

7. *Eupenicillium ochrasalmoneum*

Colonies show purple-red shade and get dark reddish brown. Clestothecia are spherical, orange brown in colour, 500µm in diameter. Clestothecia develops ascospores that are oval or oblong or spherical, 12-15µm in diameter. Conidiophores originate from branches of hyphae are hyaline to greenish and septate. Conidia are in chains and smooth walled.

Table 2. Numbers of isolates from samples of endophytic fungi

Endophytic fungi	Samples			Total
	Root	Stem	Leaves	
Alternaria alternata	2	5	3	10
Aspergillus flavus	3	1	4	08
Aspergillus niger	1	5	5	11
Aspergillus terrus	-	4	3	07
Aspergillus versicolor	-	3	3	06
Chaetomium globosum	-	1	1	02
Curvularia lunata	4	3	1	08
Eupenicillium ochrasalmonium	-	-	1	01
Fusarium equiseti	2	1	2	05
Fusarium oxysporum	1	3	3	07
Phomopsis	2	3	-	05
Trichoderma harzianum	1	2	3	06
Trichoderma viridae	2	2	2	06

In this study, *Aspergillus niger* had the highest relative frequency (Table.2). *Alternaria alternata* and *Aspergillus flavus* which are frequently identified as endophytes (Masroor Qadri et al., 2013) was the second most frequent endophytic group. Followed by *Curvularia lunata*, *Aspergillus terrus, Aspergillus versicolor, Chaetomium globosum, Eupenicillium ochrasalmonium, Fusarium equiseti, Fusarium oxysporum, Phomopsis, Trichoderma harzianum* and *Trichoderma viridae* . These were the dominant genera or order of endophytic fungi found in this study, similar to the findings reported previously for many tropical endophytic fungi (Luiz H. Rosa et al., 2012; Nur Amin et al., 2014; Jane Frohlich, 1999).

4. Conclusion

Eighty two species of endophytic fungi were isolated and identified from roots, stem and leaves of *Semecarpus anacardium* which belong to *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Aspergillus terrus, Aspergillus versicolor, Chaetomium globosum, Curvularia lunata, , Fusarium equiseti, Fusarium oxysporum, Phomopsis, Trichoderma harzianum, Trichoderma viridae, 01* unidentified isolates was *Eupenicillium ochrasalmonium*, which was identified by molecular method. *Aspergillus niger* had the highest relative frequency in this study and *Alternaria alternata* was the second most frequent endophytic group, followed by *Aspergillus terrus* and *Fusarium oxysporum.*

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