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DIVERSITY OF ENDOPHYTIC FUNGI IN MEDICINAL PLANTS FROM AMRAVATI

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Abstract:
Endophytic fungi spend the whole or parts of its life cycle colonizing inter or intracellularly inside the healthy tissues of the host plant. Some species of endophytic fungi have been identified as sources of anticancer, antidiabetic, insecticidal, immunosuppressive and biocontrol compounds. A large number of secondary metabolites have been isolated and characterized from endophytic fungi . Medicinal plants selected i.e. *Adhatoda vasica and Rauwalfia serpentina* for the study. The samples were surface sterilized by modified method of Dobranic *et al.* (1995) , fungi isolated and Identified were used for study.

Introduction

Endophytic fungi are a group of fungi that colonize living, internal tissue of plants causing any immediate, overt negative effects (Hirsch and Braun, 1992). The word endophyte means in the plant and refers to all microorganisms that live in the intercellular spaces of stems, petioles, roots and leaves of plants causing no apparent symptoms of disease (Petrini, 1991). Many recent studies have revealed the ubiquity of these fungi, with an estimate of at least one million species of endophytic fungi residing in plants (Dreyfuss and Chapela, 1994) and even lichens (Li *et al*., 2007). Endophytic fungi represent an important and quantifiable component of fungi biodiversity, and are known to affect plant community diversity and structure (Sanders, 2004; Gonthier *et al*.,2006; Krings *et al*.,2007). To date, only 80,000-100,000 fungal species have been described (Hawksworth and Rossman, 1987), out of a conservative estimate of 1.5 million (Hawksworth, 1991). Recent studies of endophytic fungi from tropical and temperate forest support the high estimates of species diversity (Kumar and Hyde, 2004; Santamaria and Diez, 2005; Sanchez Marquez *et al.*, 2007). These estimate do not include several additional sources of fungal diversity (Ganley and Newcomb, 2006).

Natural Products, derived from microorganisms and plants, and have been used for the treatment of various diseases and a large number of modern drugs have been developed from them.

Endophytic fungi spend the whole or parts of its life cycle colonizing inter or intracellularly inside the healthy tissues of the host plant. Some species of endophytic fungi have been identified as sources of anticancer, antidiabetic, insecticidal, immunosuppressive and biocontrol compounds. A large number of secondary metabolites have been isolated and characterized from endophytic fungi since the discovery of endophytes in Daren, Germany in 1904 (Strobel et al., 2004). The discovery of penicillin from *Penicillium notatum* inspired scientists to work with fungi. Cryptocandin, isolated from *Cryptosporiopsis quercina*, are currently being considered for use against a number of fungal diseases of the skin and nails (Strobel et al., 2004, Strobel et al, 1999). Taxol, an anticancer agent, was originally isolated from the bark of the Pacifica Yew tree *Taxus brevifolia* . Later, it was discovered that fungi (*Taxomyces andreanae* and *Pestalotiopsis microspora*) associated with this yew tree also produce taxol (Strobel et al. 1996). As part of our ongoing research on endophytic fungi of Bangladeshi medicinal plants several secondary metabolites were isolated from different endophytes. *Aquilaria malaccensis* Lamk (locally known as Agor) is one of the medicinal plants of Bangladesh. In this paper, we report the isolation of endophytic fungi from *A. malaccensis* and study of the fungal strain AL-2 for chemical and biological properties.

It is believed that medicinal plants and their endophytic flora produce similar pharmaceutical products. The use of endophytic fungus for the production of pharmacologically active metabolites has been on rise. Taxol, a potent anticancer drug, is one such example, produced by *Taxus brevifolia* plant and endophytic fungi, including *Taxomyces andreanae* and several other fungi (Strobel *et al*., 1996; Strobel, 2003; Gangadevi & Muthumary, 2007).

Endophytes are mostly unexplored group of microorganisms, but a few studies show them as a huge source of medicinal compounds. Dreyfuss & Chapela (1994) estimated that there may be one million species of endophytic fungi alone. There is a lack of information about endophytic diversity in this region. There was an immense need to understand the biodiversity of endophytic fungi in this semiarid region where the climatic conditions remain extreme to high and annual rain-fall is less than 15 mm, most of the time in a year. The present study was conducted to determine the diversity of

endophytic mycoflora of *Withania somnifera* in this geographical region.

Review Of Literature

Gangadevi and Muthumary (2008) reported that, endophytic taxol prodcing fungus from the leaves of a medicinal plant, *Justicia gendarussa*. Taxol is a potent anticancer drug used widely in the treatment of a variety of cancers. An endophytic fungus *Colletotrichum gloeosporioides* (strain JGC-9) was isolated from *Justicia gendarussa*, a medicinal plant and screened for taxol production.

Chuan-chao Dai et. al. (2008) reported that, the endophytic fungus are responsible for the growth of *Euphorbia pekinensis.* and they give the strategy to use the endophytic fungi as the growth promoter in the medicinal plants, *Euphorbia pekinensis.* The method used in this study could be applicable to similar studies on the relationship between endophytic fungi and their host plants.

Jalgaonwala et. al. (2010) reported that, the evolution of endophytes for their antimicrobial activity from indigenous medicinal plants belonging to North Maharashtra region of India. A total of seventy eight bacterial endophytes and 142 fungal endophytes were isolated from the aerial and underground parts of selected medicinal plants. 15 positive endophytic bacterial isolates and 14 positive endophytic fungal isolates possess antifungal and antibacterial activity respectively.

Materials and Methods

For the study of Endophytic Fungi medicinal plants selected i.e. *Adhatoda vasica, Rauwalfia serpentina*. For the deep study of endophytic fungi there is need of basic equipment in the laboratory. For microscopic examination, isolation or culturing and identification of a microorganism as well as to study its structure, function and application. For isolation of endophytic fungi two types of media were used, Potato Dextrose Agar and Water Agar medium.

The freshly collected samples were rinsed gently in running tap water to remove dusts and debris. The stem leaves (lateral and midrib) and Root were cut into segments (0.5 – 1cm). The samples were surface sterilized by modified method of Dobranic *et al.* (1995). The samples were immersed in 70% ethanol for 5 s, followed by 4% sodium hypochlorite for 90 s and then rinsed in sterile distilled water for 10 s. The surface sterilized segments were placed in Petridishes containing PDA and WA medium. The Petridishes were sealed using parafilm and incubated at 26 + 1°C at 12-h light/dark cycle. Keep this Petriplates for 4 to 6 weeks for the growth of fungi; the Petridishes were monitored every day to check the growth of endophytic fungal colonies from the segments. The procedure were carried under the highly aseptic condition. Isolated fungi were identified and classified on the basis of observing culture growth with morphological and cultural characteristic, such as shape, color and size.Observation were tabulated in table .

Table 1: Diversity of endophytic fungi from Medicinal Plant. (*Adhatoda vasica*).

Sr. No.	Name. of Endophytes	Root, Stem, Leaf	
		No. of Endophytes	% Abundance
1	*Alternaria alternata*	11	18
2	*Bipolaris nodulosa*	2	14
3	*Bipolaris specifer*	6	15
4	*Cladosporium herbera*	3	18
5	*Cladosporium cladosporiodes*	4	14
6	*Drechslera australiensis*	2	11
7	*Fusarium semitectum*	3	12
8	*Pestolopsis hypodermia*	2	16

Table 2: Diversity of endophytic fungi from Medicinal Plant. (*Rauwalfia serpentina*).

Sr. No.	Name. of Endophytes	Root, Stem, Leaf	
		No. of Endophytes	% Abundance
1	*Alternaria alternata*	18	15
2	*Bipolaris nodulosa.*	4	19
3	*Cladosporium atrum.*	5	17
4	*Cladosporium chlorosephalum*	4	10
5	*Cladosporium herbera*	6	12
6	*Fusarium moniliforme*	3	11
7	*Fusarium poae*	4	22
8	*Fusarium semitectum*	4	13
9	*Myrothecium roridum*	4	12
10	*Penicillium corylophilum*	6	14
11	*Sterile Mycelia*	4	19
12	*Verticillium albo-atrum*	3	17

Result and Discussion

The study of Endophytic fungi on medicinal plant from the Amravati showed that there were 10 genera and 22 species of fungi which is may be have beneficial or harmful activity to the plant. The most common and most important genera is found to be *Alternaria, Cladosporium* , and the most common species are found to be *Alternaria alternata, , Cladosporium cladosporiodes and Fusarium oxysporum.*

Endophytic organisms have received considerable attention as they are found to protect their host against pest, pathogens and even domestic herbivorous (Weber, 1981). Only a few plants have been investigated for their endophytic flora and their potential to produce bioactive compounds. Some studies have been conducted about the endophytic biodiversity, taxonomy, reproduction, host ecology and their effects on host (Faeth *et al.*, 2004; Petrini, 1986; Dayle *et al*., 2001; Redman *et al.*, 2002; Clay & Schardl, 2002). Currently, endophytes are considered as unexplored source of bioactive natural compounds. They have been found to play a crucial role in the production of beneficial chemical compounds. Taxol, an antifungal and anticancer compound, is one such example. It was found to be a product of endophytic fungi that colonizes on *Taxus brevifolia* and other plants (Strobel *et al.*, 1996; Strobel, 2003; Gangadevi & Muthumary, 2007).

There is a need to study the biodiversity of endophytic fungi in the semiarid regions where the climatic conditions remain extreme to high and annual rain-fall is less than 15 mm, the present work were initiated to find out endophytic fungal population in widely used medicinal plant, *Adhatoda vasica, Rauwalfia serpentina.* Diverse endophytic population was detected to colonize this plant. Thirty-six different species were isolated. The endophytic fungal communities found at three different sites were root, Stem and Leaf different. Almost all the isolates were recovered from older plant samples than younger ones. The highest species richness, as well as frequency of colonization was found in stems. In this study, most dominant endophyte was found to be *Cladosporium and Fusarium*. Overall colonization frequency was determined as 14.15% in surface sterilized tissues. In most of the cases, Ascomycetes, Deuteromycetes and Basidiomycetes are reported as endophytic fungi (Petrini, 1986; Dayle *et al*., 2001). A large number of genera and species of fungi, belonging to first two classes, are able to live endophytically in plants.

Finally the present study concluded that there were diversity in occurrence of endophytic fungi in the medicinal plants that are taken for the study. Furthermore it is also concluded that the diversity may be due to host specificity and host-preference. The endophytic fungi may increase the growth response in inoculated host plants through nutrient cycling, by secreting plant growth promoting substances and which can offer resistance to the host plants under different harsh environmental conditions.

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