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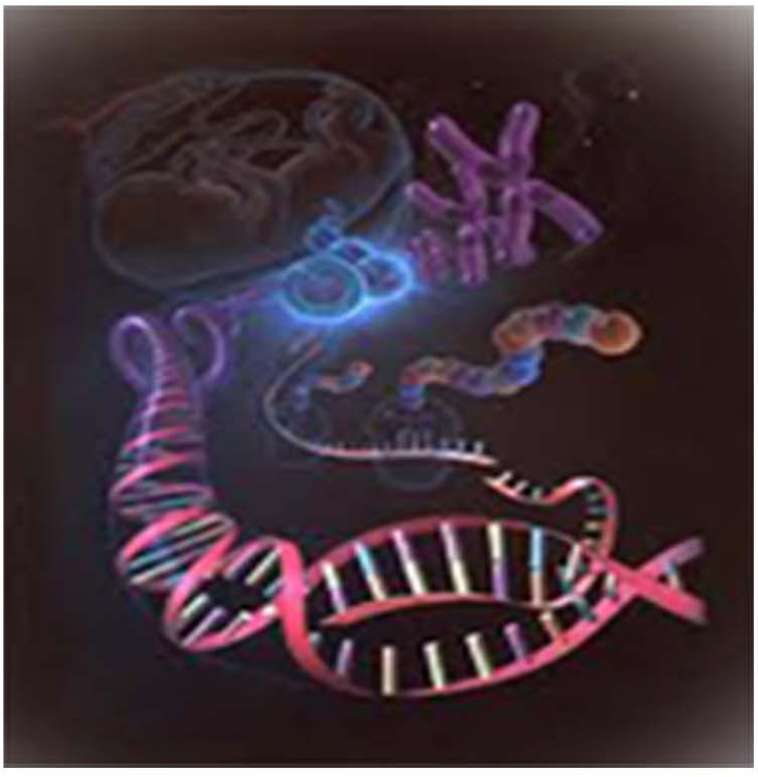

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Research Paper

ISOLATION, FREQUENCY DISTRIBUTION AND DIVERSITY OF NOVEL FUNGAL ENDOPHYTES INHABITING LEAVES OF *CATHARANTHUS ROSEUS*

Prerna Momsia[1] and Teena Momsia[1]

*Corresponding Author:**Prerna Momsia,** ✉ momsiaprerna875@gmail.com

A total of eight plant samples of *Catharanthus roseus* (Madagascar periwinkle) were collected from specific locations of Kukas (Jaipur), Rajasthan for isolation of endophytic fungi. A maximum frequency of *Alternaria alternata* (15.09 %) were recorded which are morphologically similar but ecologically variant. The present paper discusses the diversity of *Alternaria alternata* within the *Catharanthus roseus* plant samples on the basis of frequency distribution and occurrence.

Keywords: *Catharanthus roseus*, Endophytes, Potato Dextrose Agar, Tetracycline

INTRODUCTION

Catharanthus roseus, a popular ornamental plant, belonging family *Apocyanaceae,* is known to attain medicinal properties (Tembhurne *et al.,* 2012). Medicinal plants are also important for pharmacological research and drug development and is a rich source of alkaloids, which are distributed in all parts of the plant. The alkaloid content of *C. roseus* varies considerably in various parts; the maximum being in the root bark which ranges from 0.15 to 1.34% and even up to 1.79 in some strains (Singh and Jagdev, 1996). *C. roseus* harbour endophytic myco-flora. It have been studied for their endophyte biodiversity and their potential to produce bioactive secondary metabolites. Two of the dimeric alkaloids vinblastine and vincristine mainly present in the aerial parts, have found extensive application in the treatment of human neoplasma (Aslam *et al.,* 2010). There is a need to understand the biodiversity of endophytic fungi and their potential of producing novel compounds of medicinal importance.

All plants in natural ecosystems appear to be symbiotic with fungal endophytes. This highly diverse group of fungi can have profound impacts on plant communities through increasing fitness by conferring abiotic and biotic stress tolerance, increasing biomass

[1] Fungal Biotechnology Laboratory, Jaipur National University, Jaipur, Rajasthan, India-302025.

and decreasing water consumption, or decreasing fitness by altering resource allocation (Rodriguez *et al.,* 2009). Interest on fungal endophytes has recently surged, which has led to a considerable amount of research regarding the role of these fungi in host plants (Ananda and Sridhar, 2002).

Rajasthan is unexplored region for the study of endophytic fungi from *C. roseus*. Therefore the present study was carried out to determine fungal endophytes.

MATERIALS AND METHODS

Collection of Plant Samples

Healthy, fresh and mature leaves of *Catharanthus roseus* L. (Apocyanaceae) were collected from a healthy plant grown in a garden in Kukas region, Jaipur, Rajasthan. The plant material was brought to the laboratory in sterile bags and processed within a few hours after sampling. Fresh plant materials were used for isolation work to reduce the chance of contamination.

Isolation of Fungal Endophytes

For the isolation of fungal endophytes the fresh leaves and nodes were used as explants for isolation of fungal endophytes (Raviraja, 2005; Tiwari, 2012). The isolation of endophytic fungi was done according to the method described by Petrini (1986). Leaves were cut into 3-4 mm in diameter and 0.5-1.0 cm in length with and without midrib. All explants were surface-sterilized by dipping in 75% ethanol for 1 min, 4 % sodium hypochlorite for 5 min followed by rinsing three times in sterilized distill water. In each petri dish (9 cm diameter), a total of 27 processed segments were evenly spaced onto the surface of Potato Dextrose Agar (PDA) media supplemented with 200 µg /ml tetracycline incubated at 28°C and daily observation was recorded. The sporulating mycelia of fungi appeared on the plates were carefully isolated, subcultured and maintained the pure culture (Raviraja, 2005; Tiwari, 2012).

Identification of Fungal Endophytes

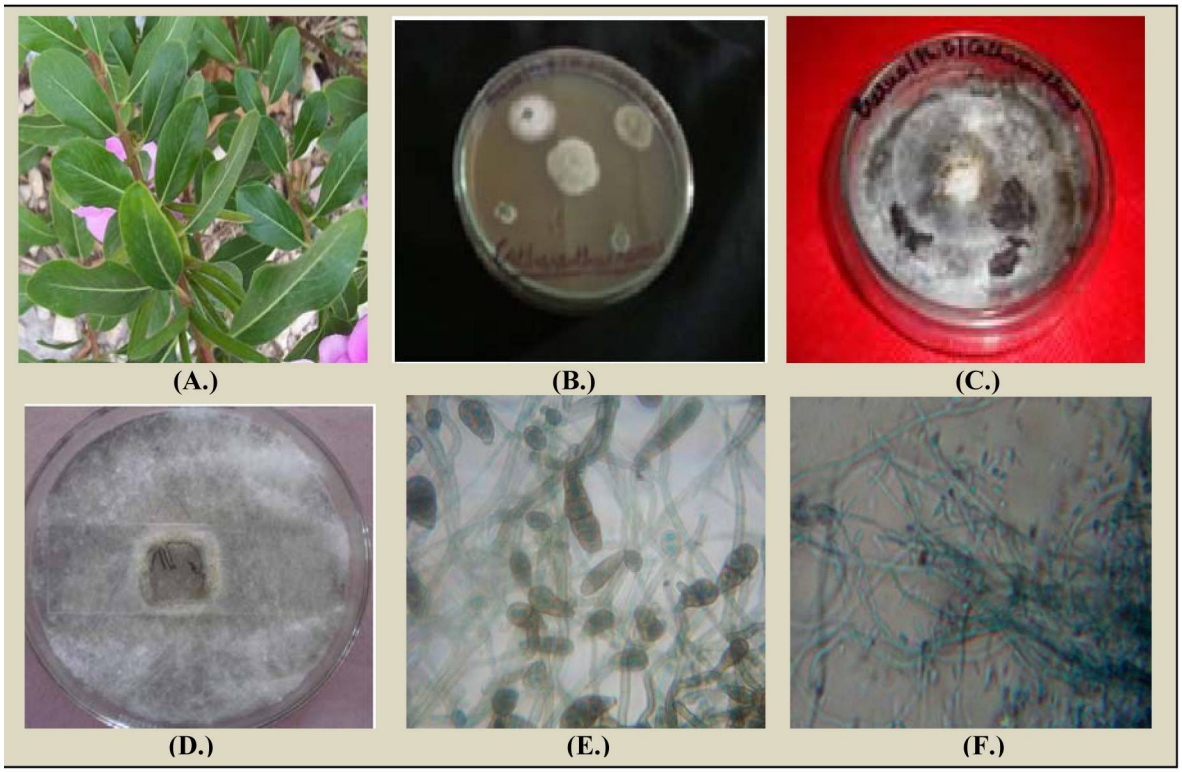
The isolated endophytic fungi have been described and identified on the basis of morphologically (microscopic and culture characteristics) features like colony characterization, growth of fungi (slow-growing or fast-growing), color of colony (front and reserve), conidial development, size and shape of conidia, shape of conidial head and attachment of conidia (Barnett and Hunter, 1998; Nagamani *et al*., 2005). Microscopic study is done by slide culture technique, using a small drop of growth media on slide culture by which the spores of the fungus remain undisturbed and attached to the sporophores thus facilitate the identification of desired fungi. This technique was performed for various stages of conidia formation and proper identification of the sporulating fungi (Anthony and Walkes, 1962). The microscopic identification of fungi was carried out by lacto phenol cotton blue staining method (Nagamani *et al*., 2005).

RESULTS AND DISCUSSION

Statistical Analysis

The rate of colonization (%) of fungal endophytes was equal to the number of segments colonized by a single endophyte divided by the total number of segments observed × 100 (Figure 2). In *Catharanthus roseus* L., more endophytic fungi were isolated from the leaves (CR=64.11 %) than the nodes

Figure 1: (A) Healthy, mature plant of *Catharanthus roseus* L. collected from Kukas , Jaipur (Rajasthan); (B) Isolated fungal endophytes from medicinal plant leaves; (C) Pure culture of fungal endophyte maintained on PDA media; (D) Conidia of isolated fungal endophyte culture in the developmental stage by Slide culture technique; (E) Microscopic study of isolated fungal endophyte *Alternaria alternata* on 40X magnification; (F) Microscopic study of isolated fungal endophyte *Alternaria alternata* on 10X magnification



(CR=29.11 %) (Figure 3). A total of seven different endophytic fungal genera along with six different mycelia sterilia were found in its leaves and nodes. Among these *Alternaria alternata* showed the highest colonizing frequency (14.56 %), followed by *Aspergillus* sp. (12.60 %) and *Curvularia* sp. (5.21%). In comparison, *Penicillium* sp. (8.33%), *Trichoderma* sp. (3.26%) were isolated with low frequency of colonization. *Helminthosporium* sp. (3.26 %) , *Fusarium* sp., (2.17 %) and *Phoma* sp. (0.90 %) were isolated with very low frequency of colonization. *Curvularia* sp., *Phoma* sp., was found absent in site I sample

Figure 2: Colonization Rate (CR) of Fungal Endophytes in Different Sites of *Catharanthus roseus* L.

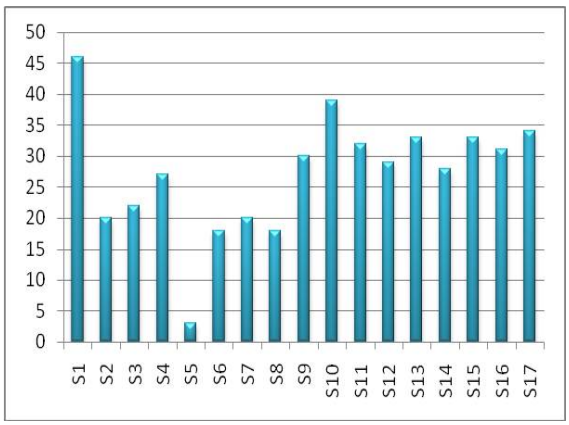
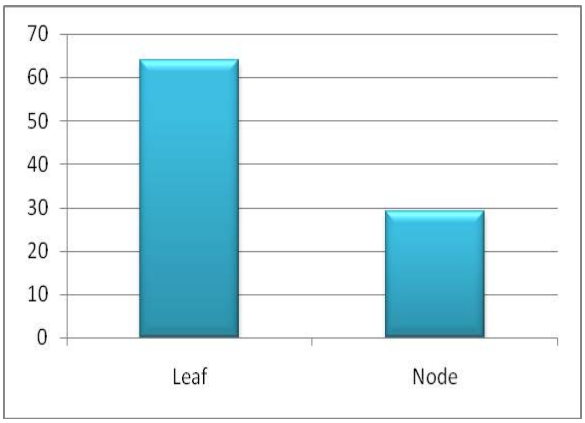


Figure 3: Colonization Rate (CR) of Endophytic Fungi in Different Explants of *Catharanthus rosues* L. (Leaf and Node)



of S1, S2, S3, S4 in comparison with the *Verticillium* sp., found absent in site II sample of S6, S7, S8, S9. similarly *Fusarium* sp. was found absent in site III, sample S 10, S11, S12, S13 where as *paeselomycese* sp., and *Verticillium* sp., was found absent in site IV, sample S14, S15, S16 and S17, respectively. The difference in endophyte assemblages from various tissues indicated that some individual dominant endophytic taxa have an affinity for different tissue types, and this might reflect their capacity for utilizing or surviving within a specific substrate in connection with the location of the plant sample.

CONCLUSION

Host plants and endophytic fungi are symbionts, in which hosts and endophytes benefit from each other. Plants provide nutrition and protection to their endophytes in return, endophytes excrete functional products and increase in their host resistance to biotic and abiotic stresses. The living aerial parts of all plants may have mutualistic internal endophytic fungi, which exhibit momentous part of fungal diversity. Endophytic fungi of the plant *Catharanthus roseus* of Kukas region exhibit high diversity. A total of 463 endophytic fungal isolates were obtained from seven different locations of Kukas region. These endophytic fungal isolates were classified into 10 fungal genera. Most of these fungi belonged to *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., and *Helminthosporium* species. The maximum colonization rate was observed in *Alternaria alternata*. Ecologically as a fungal endophyte *Alternaria alternata* diversified in most of the locations of this region in the plant *Catharanthus roseus*. However, these endophytic fungi exhibited various Colonization Rate (CR). The evidence of host preference, tissue specificity and spatial heterogeneity was found in endophyte distribution based on fungal community compositions and colonizing frequencies. Mycelia sterilia was a large group of fungi which failed to sporulate and was ubiquitous in plant endophytic isolation.

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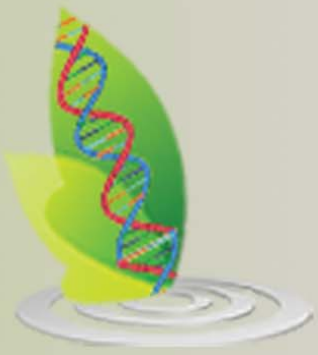

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