



Histological Investigations of Healthy Tissues of *Catharanthus Roseus* to Localize Fungal Endophytes

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ABSTRACT

Catharanthus roseus is an important medicinal plant known for its potential therapeutic terpenoid indole alkaloids. The aerial and underground parts of the plant are transcriptionally distinct and known to produce distinct spectrum of terpenoid indole alkaloids. Production of a plant-based natural drug is not always up to the desired level. Considering the limitations associated with the productivity, microorganisms serve as the ultimate and inexhaustible source of novel structures bearing pharmaceutical potential. Endophytes, the microorganisms residing inside the healthy tissues of the host plant are relatively unstudied and offer potential sources of novel natural products for exploitation in medicine, agriculture and the pharmaceutical industry. The present study was carried out to examine the localization and distribution of endophytic fungi present inside the healthy plants tissues of *C. roseus* (purple and white) variety. The endophytic fungi were detected by incubating the sterilized explants of *C. roseus* in PDA plates to promote the growth of mycelia. To locate the distribution, fungal endophytes were stained with Lactophenol cotton blue. Dense fungal colony and hyphae were observed in the xylem, phloem, and pith region of both leaf and stem tissues. Dense colonies of endophytes were also observed in cortex of root tissue. Rich localization was observed for *C. roseus* white variety in leaf and stem tissues. Further studies are required for isolation and identification of potential endophytes and exploring their biosynthetic potential.

Keywords: *Catharanthus roseus*, Fungal Endophytes, Lactophenol cotton blue.

INTRODUCTION

Endophytes are group of microorganisms that colonize living internal tissues of plants without causing any immediate, negative effects¹. Many recent studies have revealed the ubiquity of these fungi, with an estimate of at least 1 million species of endophytic fungi residing in plants² and even lichens³. Endophytic fungi represent an important and quantifiable component of fungal biodiversity, and are known to affect plant community diversity and structure⁴. Plant endophytes are more subtle, rarely causing problems, coexisting with their hosts under most circumstances. They are generally nonpathogenic in nature, but may produce secondary metabolites that enable them to survive in the competitive world of plant interstitial space. Endophyte includes all organisms which colonize the living internal tissues of their hosts without producing symptoms of disease⁵. An overview of recent literature indicated that 51% of bioactive substances isolated from endophytic fungi were previously unknown, compared to 38% from soil fungi⁶.

Microorganisms have long served mankind by virtue of the myriad enzymes and secondary compounds that they produce⁷. The diversity of microbial life is enormous and the niches in which microbes live are truly amazing, ranging from deep ocean sediments to the Earth's thermal pools. One specialized and unique biological niche that supports the growth of microbes is the intracellular space between cells of higher plants. It turns out that each plant supports a suite of microorganisms known as endophytes⁸. Plants appear to be a reservoir of

untold numbers of endophytic organisms⁹. Common endophytes include a variety of bacteria, fungi and actinomycetes. The ubiquity of these symbiotic microorganisms is clear, but diversity, host-range, and geographical distributions are unknown¹⁰. Endophytic bacteria and filamentous fungi have been isolated from surface-sterilized seeds, roots, stems, leaves, needles, twigs and barks of various symptomless plant species¹¹.

Mankind in ancient times have predominantly dependent on remedies derived from plants (especially medicinal plants) in stabilizing incidence of pathogens causing diseases in human, animals and plants. Although synthetic chemicals have long been used in curing diseases, it is indistinct that they are not only costly but also potentially harmful for health, ecosystem and environment and induce resistance in pathogens against chemicals/drugs. To overcome the negative impact of chemical control it is necessary to find alternate sources to prevent diseases. Endophytes derived natural compounds/ products/drugs may compete with therapeutic agents derived from plants and synthetic chemicals.

Endophytic fungi that grow within their plant hosts without causing apparent disease symptoms^{9, 12} are relatively unexplored. These endophytes protect plants against diseases¹³, increasing tolerance against abiotic stress¹⁴. Therefore, they are viewed as potential source of bioactive compounds¹⁵. They produce unique bioactive metabolites possessing anti-bacterial, anti-viral, anti-fungal and anticancer activities¹⁶. Probably endophytic

fungi present in *C.roseus* could be an alternative source for production of vinca alkaloid.

Catharanthus roseus has been in use from ancient times for the treatment of blood pressure, diabetes mellitus, etc. in Indian system of medicine as well as in folk-lore medicinal practice¹⁷. The extract of this plant has been found to contain alkaloids having anti mitotic and anti microtubule activities. The vinca alkaloids include vinblastine (Fig.1), vincristine (Fig.2), vindesine and vinorelbine. They are dimeric compounds in which indole and dihydroindole nuclei are joined with other complex ring systems⁷. They are used in acute leukemia, Hodgkin's disease, non Hodgkin's lymphoma, rhabdomyosarcoma, neuroblastoma, Swing's sarcoma and Wilms tumor¹⁸.

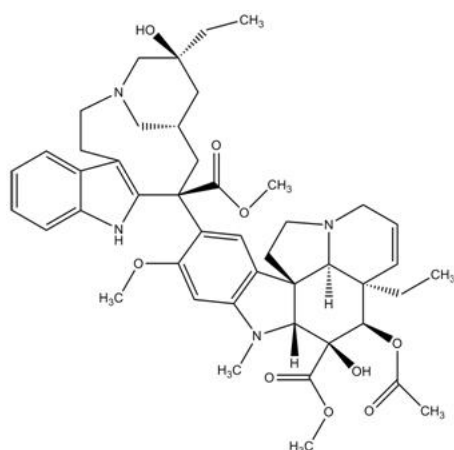
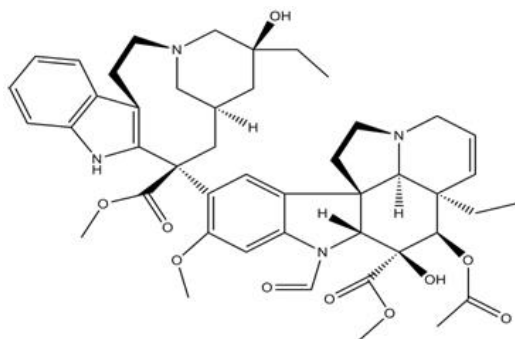


Figure 1: Vinblastine



2. Vincristine

Figure 2: Vincristine

Therefore, this study is conducted to report on the histology of endophytic fungi in *Catharanthus roseus*. The histological studies of *C.roseus* leaf stem and root tissues were carried out to access the location and diversity of fungal mycelia and spores within the tissues.

MATERIALS AND METHODS

The fungal endophyte distribution and localization was studied with the help of microscope (Olympus CH20 Japan) under the magnification of 100x and 1000x, attached with a digital camera (Nikon Coolpix 10megapixels). The fungal colonies and hyphae were observed as blue and light brown after staining it with Lactophenol Cotton blue, staining solution for fungi.

Plant material and collection site

Catharanthus roseus (L.) Don, plants under study was selected from the campus premises of Birla Institute of Technology, Mesra, Ranchi. Two plants each from purple and white variety of 4–5 months old samples were randomly selected and cut from a sharp sterilized blade. The plants were uprooted from the soil and the roots were washed under tap water before its packaging in sterile paper bags for its transportation to the laboratory before processing. Samples were processed for their histological investigation within 6 hr of collection.

Kingdom	: Plantae
Phylum	: Magnoliophyta
Class	: Magnoliopsida
Order	: Gentianales
Family	: Apocynaceae
Genus	: <i>Catharanthus</i>
Species	: <i>Catharanthus roseus</i>

Time of collection: 8:30am

Place of collection: Birla Institute of Technology, Mesra, Ranchi

Date of collection: 10th sept, 2012.

Explant preparation

The plant were taken out from the sterile bag and washed under running tap water. The leaves stem and roots were then separately cut and washed with distilled water again. The stem and primary root, usually the matured part of plant was cut into thin sections using the sterile blade. The leaves were cut by keeping the potato as its base.

Slide preparation

The sections were then stained with Lactophenol cotton blue for 2 minutes and washed with distilled water till no more blue colored water was observed. The sections were then carefully transferred in clean glass slides; a cover slip was placed above the section and few drops of immersion oil. The sections were then observed under the microscope (Olympus CH20 Japan), attached with a digital camera (Nikon Coolpix 10megapixels).

Drugs and Chemicals

Lactophenol cotton blue (HiMedia Laboratories Pvt. Ltd., Mumbai)

RESULTS AND DISCUSSION

The localization and abundance of endophytic fungi in the tissue of *Catharanthus roseus* was examined. The microscopic examination revealed that the white flowered variety of *C. roseus* harbor dense colonies of endophytic fungi than purple flowered variety in both transverse section and longitudinal section at low magnification 100x and high magnification 1000x

respectively. The sections were stained with Lactophenol cotton blue¹⁹. Colonies observed were spherical and oval in shape. The density of fungal colony increased from leaf to roots.

The following observations were made for white flower variety of *Catharanthus roseus* (CRW)

Transverse section of leaf explants CRWL at lower magnification, showed dense colonies of endophytes observed in the mid rib, hyp-a & hyp-b hyphae in fig. 3a. At higher magnification, septate hyphae strand myc-a & branching of hyphae strands myc-b along with stomata guard cell (stg) were observed in fig.3b.

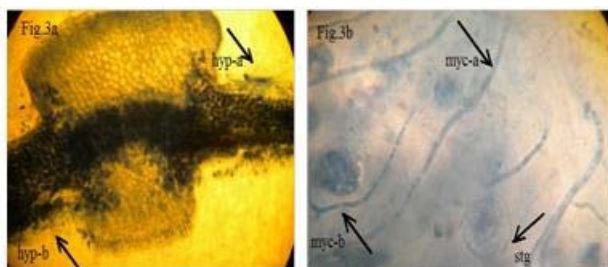


Figure 3: Leaf explants: (a) Dense colonies of endophytes observed in the mid rib, (hyp-a & hyp-b) hyphae clusters present in the epidermis region CRWL, transverse section at lower magnification. (b) Septate unbranched hyphae (myc-a) and branched hyphae (myc-b) in the leaf epidermis region, stomata guard cell (stg) of CRWL, transverse section at higher magnification.

Transverse section of stem explants CRWS at lower magnification, showed dense blue endophytic colonies scattered around the epidermis, endodermis, cortex and pith region in fig.4a. At higher magnification intracellular and extracellular endophyte were observed in fig.4b.

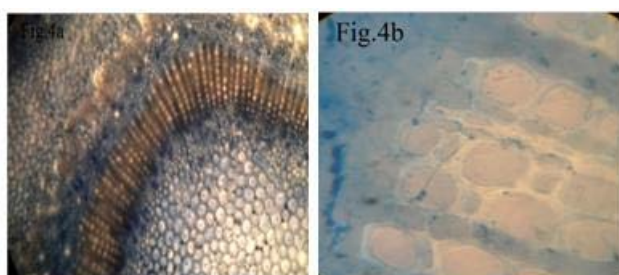


Figure 4: Stem explants: (a) Dense blue endophytic fungal colony in CRWS, transverse section at lower magnification. (b) Blue intracellular and extracellular chains of endophyte in CRWS, transverse section at higher magnification.

Longitudinal section of stem explants at lower magnification, showed dense endophyte colonies in vascular bundle and unbranched hyphae (hyp) was observed in fig.5a. At higher magnification, unbranched intracellular hyphae (hyp) and intracellular endophytic fungi were observed fig.5b.

Transverse section of root explants CRWR at lower magnification, showed dense blue fungal colonies were scattered in fig.6a. At higher magnification, intracellular and extracellular dense fungal colonies fig.6b was observed.

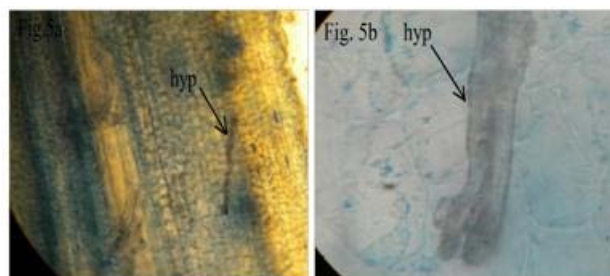


Figure 5: Stem explants: (a) Dense endophyte colonies in vascular bundle and unbranched hyphae (hyp) in CRWS, longitudinal section at lower magnification. (b) Unbranched intracellular hyphae (hyp) and intracellular endophytic fungi in CRWS, longitudinal section at higher magnification.

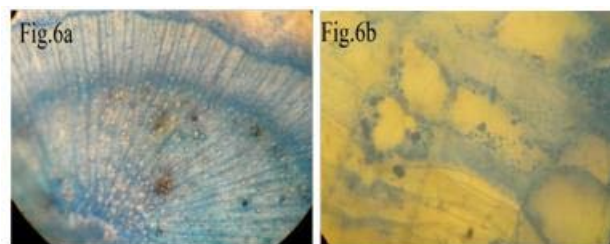


Figure 6: Root explants: (a) Dense blue fungal colonies in CRWR, transverse section at lower magnification. (b) Intracellular and extracellular dense fungal colonies in CRWR, transverse section at higher magnification.

Longitudinal section of root explants at lower magnification, showed fungal colonies scattered all over the section of root fig.7a. At higher magnification, intracellular and extracellular fungal colonies were observed in fig.7b.

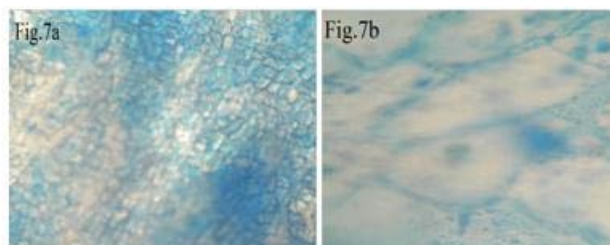


Figure 7: Root explants: (a) Fungal colonies scattered all over the root section CRWR, longitudinal section at lower magnification. (b) Intracellular and extracellular fungal colonies in root section CRWR, longitudinal section at higher magnification.

The following observations were made for purple flower variety of *Catharanthus roseus* (CRP)

Transverse section of leaf explants CRPL at lower magnification, showed fungal colonies scattered around the vascular bundle (Vas cam) in fig.8a. At higher magnification, intracellular endophytic fungi with spore (sp) were observed fig.8b.

Transverse section of stem explants CRPS at lower magnification, showed dense blue endophytic colonies scattered around the metaxylem, pith and phloem region fig.9a. At higher magnification, blue fungal colonies were observed fig.9b.

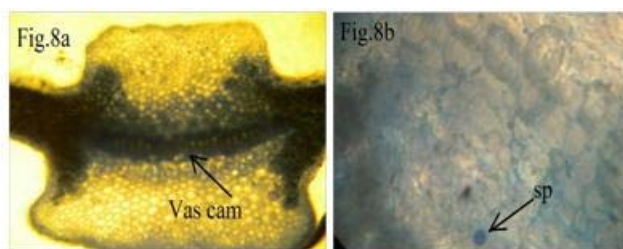


Figure 8: Leaf explants: (a) Fungal colonies scattered around the vascular bundle (Vas cam) of leaf section CRPL, transverse section at lower magnification. (b) Intracellular endophytic fungi with spore (sp) for leaf section CRPL, transverse section at higher magnification.

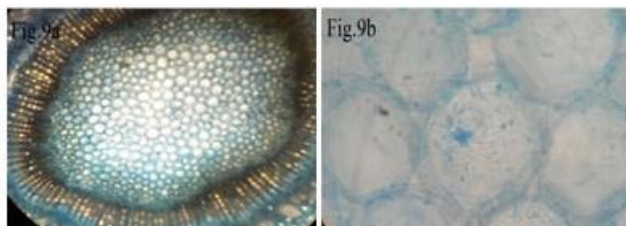


Figure 9: Stem explants: (a) Dense blue endophytic colonies scattered around the metaxylem, pith and phloem of stem explants CRPS, transverse section at lower magnification. (b) Blue fungal colonies of stem explant CRPS, transverse section at higher magnification.

Longitudinal section of stem explants at lower magnification, showed dense endophytic colonies in the vascular bundle and unbranched hyphae (hyp) fig.10a. At higher magnification, unbranched intracellular hyphae (hyp) were observed fig.10b.

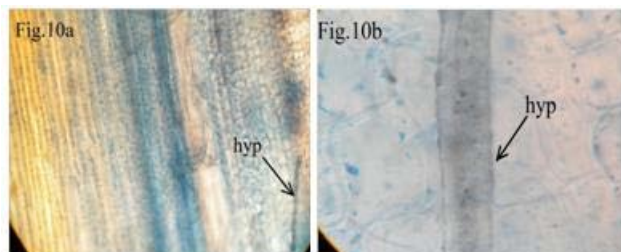


Figure 10: Stem explants: (a) dense endophytic colonies in the vascular bundle and unbranched hyphae in stem explant CRPS, longitudinal section at lower magnification. (b) unbranched intracellular hypha (hyp-p) in stem explants CRPS, longitudinal section at higher magnification.

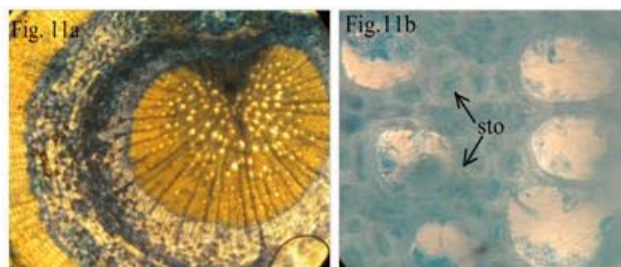


Figure 11: Root explants: (a) Dense blue fungal colonies scattered in metaxylem region of root explants CRPR, transverse section at lower magnification. (b) Stomata guard cells (sto) were scattered around the vascular bundle of root explants CRPR, transverse section at higher magnification.

Transverse section of root explants CRPR at lower magnification, showed dense blue fungal colonies scattered in metaxylem region, except for the pith fig.11a. At higher magnification, stomata guard cells (sto) were scattered around the vascular bundle fig.11b.

Longitudinal section of root explants at lower magnification, showed blue fungal colonies in the epidermis region fig.12a. At higher magnification, intracellular and extracellular fungal colonies were observed fig.12b.

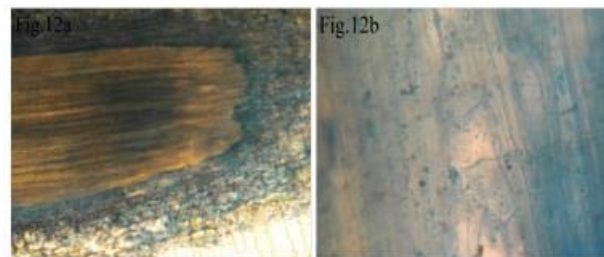


Figure 12: Root explants: (a) Blue fungal colonies in the epidermis region of root explant CRPR, longitudinal section at lower magnification. (b) Intracellular and extracellular fungal colonies of root explant CRPR, longitudinal section at higher magnification.

CONCLUSION

The present study helps us to determine the localization and distribution of fungal endophytes in *C. roseus*. The histological investigations revealed that the dominance of fungal endophytes in *C. roseus* was observed in the aerial parts of the plant system. Dense colonies of endophytes were observed in white flowering plant CRW compared to the purple flowering plant CRP of *C.roseus*. Leaf explants of CRW followed by the stem explants of white flowering variety showed maximum occurrence of fungal endophytic colonies. The root section of CRW showed fewer colonies of fungal endophytes. The least number of fungal endophytes were accounted in the root explant, for white flowering variety. In case of purple flowering variety CRP, maximum number of fungal endophytes was observed in leaf and stem and least in the root section. The septate and branched hyphae were observed in leaf tissues of both purple and white flowering variety. Aseptate and unbranched hyphae were observed in stem tissues of both purple and white flowering variety. No hyphae were observed in root tissue of both the varieties.

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