Investigation of endophytic fungi associated with *Bixa orellana* L., a medicinal plant collected from western Ghats of Sathyamangalam - a first report

**Abstract**

Mycoendophytes are microorganisms that reside internal tissues of living plants without causing any immediate overt negative effects, have been found in every plant species examined to date and recognized as the potential sources of novel natural products for exploitation in medicine, and agriculture with more bioactive natural products isolated from the microorganisms. In the past two decades, many valuable bioactive compounds with antimicrobial, insecticidal, cytotoxic, and anticancer activities have been successfully discovered from mycoendophytes. In the present investigation, endophytic fungi were screened from a commercial scale using microbes.6,7 In the past two decades, many valuable bioactive compounds with antimicrobial, insecticidal, cytotoxic, and anticancer activities have been successfully discovered from mycoendophytes. In the present investigation, endophytic fungi were screened from stem, twig and leaves of *Bixa orellana* L. collected form Sathyamangalam reserve forest. Predominant endophytic fungi isolated from the medicinal plant *Phyllosticta* sp., *Pestalotiopsis* sp., and *Phomopsis* sp. Statistical analysis of Shannon Diversity Index, Jaccard Similarity Coefficient, and Endophytic Infection Rate were performed to understand the nature of mycoendophytes in the selected medicinal plant. This is the first report on endophytic fungi documented from this medicinal plant from Sathyamangalam forest of Tamil Nadu, India.

**Keywords:** mycoendophytes, jaccard similarity coefficient, endophytic infection rate, shannon diversity index

**Introduction**

Endophytes are present in the internal tissues of living plants occur in almost every plant on earth from the arctic to tropics, and they are rich sources for bioactive natural products.1–4 Endophytic fungi are one of the major potential sources for new useful metabolites.5 Screening of diverse the endophytic fungal study may produce valuable medicinal plant products for obtaining the traditional Chinese medicine from plants on a commercial scale using microbes.6,7 In the present investigation, endophytic fungi were screened from stem, twig, flower and leaves of *Bixa orellana* collected from Sathyamangalam reserve forest. Predominant endophytic fungi isolated from the medicinal plant are *Phyllosticta* sp., *Pestalotiopsis* sp., and *Phomopsis* sp. Statistical analysis of Shannon Diversity Index, Jaccard Similarity Coefficient, and Endophytic Infection Rate were performed to understand the biodiversity of mycoendophytes in the selected medicinal plant. This is the first report on endophytic fungi documented from this medicinal plant from Sathyamangalam forest.

**Materials and methods**

**Collection of the plant materials**

Healthy plant sample of *Bixa Orellana* L. growing at various sites in the Sathyamangalam forest situated in Erode district were collected. They were brought in sterile polythene bags to the laboratory and the isolation of the fungal endophytes were commenced within 24 hours of collection (Figure 1).

**Isolation of endophytes**

**Surface Sterilization of the plant material:** The method most frequently utilized to detect and quantify endophytes involves isolation from surface-sterilized host plant tissues. Endophytic isolation was carried out under aseptic conditions. Different symptomless parts of the selected medicinal plants such as stem cuttings, leaves, and roots were used for the isolation of endophytes. The collected medicinal plant samples should be healthy and cleaned thoroughly in gentle running tap water in order to remove dirt and debris. The whole process of endophytic fungi be carried out aseptic condition. The 50 segment of stem and leaf from each plant sample was cut approximately 1 cm by modified surface sterilization method, the leaves sample from each plant were dipped in 70% ethanol for 5 seconds, then it is immersed in 4% sodium hypochlorite for 90 seconds and finally rinsed in sterile distilled water for 10 seconds. The surface sterilized leaf samples were placed in sterile plate to remove excess moisture. For the surface sterilization of stems of each plant carried out with modified method, in which stem segments were immersed first in 75% ethanol for 60 seconds, followed by 4% sodium hypochlorite for 180 seconds and then again in 75% ethanol for 30 seconds. 200 g of potato were weighed, peeled and boiled in distilled water. The filtrate of boiled potato extracts were made up to 1000 ml with distilled water. 1000 ml of potato extract were mixed with 20 g of dextrose and 20 g of agar. The medium is then sterilized in an autoclave at 121°C for 15 minutes.

**Figure 1** Habitat of *Bixa orellana* and *Bixa orellana* seeds.
Inoculation of plant segments: Surface sterilized 50 segment of both leaf and stem tissues were placed in petri dish containing Potato Dextrose Agar (PDA) medium. All the plates were incubated at 28°C±1°C to promote the growth of endophytes and were regularly monitored for any microbial growth. On observing the microbial growth, sub culturing was done. Each endophytic culture was checked for purity and transferred to freshly prepared PDA plate. To preserve as a pure culture, the endophytic fungi was inoculated in PDA slants.

Maintenance of endophytes: The purified endophytic isolates were transferred separately to PDA slants and accessioned accordingly depending upon the plant and plant parts from which they have been isolated. Finally all the purified endophytes were maintained at 4°C till further used.

Identification of endophytic fungi: Based on the morphology of surface texture and spores at the hyphal tips which were used to identify the endophytic fungi at species level using standard manual of. The fungal isolates mounted on the sterile slides then it was stained with lacto phenol cotton blue and then examined in confocal microscopy. Some endophytic fungi do not produce spores and it was grouped as a one species named sterile form.

Statistical analysis

The endophytic fungal isolates from each host plant tissue segment were analyzed based on the Percentage of density of colonization, Relative percentage occurrence of different groups of fungi and Percentage of Endophytic Infection Rate (EIR).

Colonization frequency

Number of species isolated

\[ CF(\%) = \frac{\text{Number of species isolated}}{\text{Number segments screened}} \times 100 \]

Relative percentage occurrence (RPO) of different groups of fungi

Relative Percentage Occurrence (RPO) of each group (viz., Ascomycetes, Hyphomycetes, Coelomycetes and Sterile forms) of fungal species in each plant species was calculated as follows:

Density of colonization of one group

\[ \text{RPO} = \frac{\text{Density of colonization of one group}}{\text{Total Density of colonization}} \times 100 \]

Endophytic infection rate

Number of infected segments

\[ \text{EIR}(\%) = \frac{\text{Number of infected segments}}{\text{Total number of segments screened}} \times 100 \]

Biodiversity indices

I. Shannon Diversity Index

\[ H_e = -\sum_j (p_j \ln p_j) \]

II. Shannon Evenness

\[ \text{E}_{\text{H}} = H / \ln S \]

III. Relative index for Shannon

\[ H_{\text{rel}} = H_e / H_{\text{max}} = H_e / \ln N \]

IV. Gleason index

\[ H_{\text{Gr}} = N_p - 1 / \ln N_i \]

V. Relative index for Gleason

\[ H_{\text{GR}} = H_{\text{Gr}} / H_{\text{Grmax}} = N_p - 1 / N_i - 1 \]

Results and discussion

Isolation of endophytic fungi from *Bixa orellana* L.

Altogether, 300 segments (approx. 0.5cm²) from each of the leaf and stem tissues of *Bixa Orellana* L., were sterilized and screened for the presence of endophytic fungi (Figure 2 & 3).

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Colonization frequency of *Bixa orellana* L

Colonization frequency of *Bixa orellana* in leaf were dominated by Pestalotiopsis Sp, followed by Phoma Sp, Phyllosticta and Nigrospora Sp. Whereas stem was dominated by Pestalotiopsis Sp and followed by Nigrospora sphaerica, ASV02 and Chaetomium Sp. Relative percentage occurrence of *Bixa orellana* in Stem were dominated by Sterile forms (40%) followed by Hyphomycetes (20%), Coelomycetes (20%) and Ascomycetes (20%). Whereas Leaf was dominated by Coelomycetes (56%), Hyphomycetes (22%), sterile forms (22%) and Ascomycetes were found nil. Endophytic Infection Rate of *Bixa orellana* leaf was found to be 100% and that of stem was 80 (Figures 4-10).

**Figure 4** Micrographs of endophytic fungi reported from selected medicinal plants.

**Figure 5** Colonization frequency of *Bixa orellana* Leaf.

**Figure 6** Colonization frequency of *Bixa orellana* Stem.

**Figure 7** Relative Percentage occurrence of *Bixa orellana* Stem.

**Figure 8** Relative Percentage occurrence of *Bixa orellana* Leaf.

**Figure 9** Endophytic Infection Rates of *Bixa orellana*.

**Figure 10** Biodiversity Indices of *Bixa orellana*.
Discussion

One of the most interesting features of endophytic fungi is their immense diversity. There are more than one million species of endophyte globally, many of which inhabit individual leaves or other parts of the host plant. Tropical and subtropical regions host the largest diversity of endophytic species, since these ecosystems are the richest in plant diversity.16

According to17 the surface-disinfection of plant tissues is the most important step in the isolation process and aims to eliminate the external (epiphytic) community of microorganisms, maintaining a viable internal (endophytic) community of plant samples. The process of isolation of endophytes from surface-disinfected plant samples, with cultivation on an appropriate culture medium, has been employed by other authors.18,22 The high isolation frequencies of endophytic fungi from E. azurea (87.86%) and E. crassipes (88.85%) are similar to the results reported22 where 90% of leaf samples from tropical rubber trees were colonization by endophytes. Aquatic macrophytes are higher than those obtained21 were the colonization frequencies of endophytic fungi in aquatic/riparian Chinese plants varied between 18 and 63%. The genera Alternaria and Bipolaris were the most prevalent in E. azurea and E. crassipes, respectively. The genus Alternaria comprises cosmopolitan fungus that can occur as pathogens, infecting and causing harm to several plants of economic importance, such as tangerines (Citrus reticulata), apples (Malus domestica), pear (Pyrus pyrifolia), tomatoes (Lycopersicon esculentum), and potatoes (Solanum tuberosum). In addition, Alternaria isolates have been found as endophytes in other tropical host plants.24–28

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Conflict of interest

The authors of the research article declared no conflict of interest exist.

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