Isolation of Host Specific Endophytic Fungus, Fusarium Equiseti, from *Nothopegia Bedomei, Wayanadica* Occurring in the Southern Parts of India

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**Abstract**

*Fusarium equiseti* was isolated from the leaves of the endangered forest tree, *Nothopegia bedomei*, wayanadica. *Fusarium* is a large genus of filamentous fungi which belongs to the class Sordariomycetes, distributed in soil and in association with plants, mostly symptomless. Cultural and morphological characterisation of the organism was done. True endophytes have been evolving with the host for millions of years. This organism can have an important role in the survival and protection of the plant against harsh environment and adverse conditions.

**Keywords:** Endophytic fungi; *Nothopegia bedomei*, *Fusarium equiseti*; Western Ghats

**Introduction**

Endophytes are microorganisms that live inside the host plant tissues without causing symptoms of disease which [1]. Ubiquitous in nature, endophytes can contribute to their host plant by producing a plethora of substances that provide protection and ultimately survival value to the plant. The compounds have potential for use in modern medicine, agriculture, and industry [2]. Novel antibiotics, antifungicides, immunosuppressants, and anticancer compounds are only a few examples of what has been found after the isolation and culturing of individual endophytes followed by purification and characterization of some of their natural products.

*Nothopegia bedomei* Gamble var wynaadica belongs to the Anacardiaceae family [3]. It is endemic to Southern Western Ghats. This species is known only from a single site in Wayanad near the border with Karnataka. The tree is found in low and medium elevations in semi-evergreen and evergreen forests.

**Materials and Methods**

**Collection of samples:** Leaves were collected from at random, during the months of April (pre-monsoon), May (monsoon) and October (post-monsoon) 2013-2014. Sixty healthy leaves were sampled in each sampling time. The latitude and longitude of collection site in each sampling time. The latitude and longitude of collection site were October (post-monsoon) 2013-2014. Sixty healthy leaves were sampled during the months of April (pre-monsoon), May (monsoon) and October (post-monsoon) 2013-2014.

**Isolation of endophytes:** Endophytic fungi was isolated by the method of Muthukumar [4]. The samples were processed within 24 hr of collection. The samples were washed thoroughly in running water, surface sterilized in 70% ethanol for 3 min and washed in sterile water for 20s. Sixty segments of leaf (0.5 cm diameter), were placed on Oat Meal Agar (OMA) amended with streptopenicillin. The cultures were incubated at room temperature (26 ± 2°C) in 12 h light and 12 dark cycles of 28 days. The tissue segments were observed daily for fungal growth and the isolates were scored and transferred to OMA slants for preservation. From among the isolates, a white fungus with pigmentation showed host-specificity. Cultural characteristics of all the fungi isolated were recorded.

**Colonies:** 15 different colonies were obtained with colours ranging from gray, white and pink. Among the colonies obtained, one of the cultures was found to be frequently occurring in all the sampling, and was considered as host-specific. The host specific fungus was fast growing, with pink cottony mycelium. Macro and micro conidia was observed from slender phialides. Macroconidia were hyaline, two to several celled, fusiform to sickle shaped mostly with elongated apical cell and pedicellate basal cell. Microconidia were 1 to 2 celled hyaline, pyriform, fusiform to ovoid, straight and curved. The other 14 cultures were infrequent and were considered as casual endophytes.

**SEM studies:** Scanning electron microscopy was performed using the techniques described by Ezra et al [5]. After processing, the fungal material was dried, coated with gold by sputtering technique and examined under JEOL 6100 SEM (Figure 1).

**Results**

**Fungal endophytes:** 15 cultures were obtained. The tissue cultures constantly yielded one endophyte, which was identified as *Fusarium equiseti*.

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The fungus was recovered from during all seasons. Other isolates viz., *Aspergillus niger*, *Phomopsis sp*, *Rhizopus sp* and several other strains of white, gray sterile mycelium were infrequent and not isolated in all seasons (Table 1). Hence, *Fusarium equiseti* is apparently the only host-specific endophyte of the plant.

**Discussion**

*Fusarium equiseti*, a weak pathogen, is a soil-inhabiting fungal species that colonize plant roots, also a natural root endophyte [6] that can colonize plant roots and possess characters that makes it a promising candidate for biological control of root pathogens and nematodes. As a pathogen, reports are there for *Fusarium* as the causative agent of Bokanee disease with a complex of disease symptoms including seedling blight, root and crown rot, stunting, and the classic symptoms of etiolation and abnormal elongation induced by the fungal production of gibberellin hormones [7-9]. *Fusarium equiseti* is also reported to act against parasitic plants such as *Striga hermonthica* [10]. *Fusarium equiseti* is reported to inhibit gram-positive bacteria [11].

*F. equiseti* is capable of synthesising a vast range of phytotoxic and cytotoxic secondary metabolites. [12,13], like trichotheccenes: 4-acetylvalenal, nivalenol, scirpentriol and numerous other mycotoxins, such as zearalenone, beauvericin, fusarochromanone, equsetine and butenolidole [14]. There are reports for the production of a new enniatin antibiotic from the endophyte *Fusarium tricinctum Corda* [15]. *Fusarium* usually dominates root endophytic communities specialised for the colonisation of herbaceous plants [16] suggesting the importance of the roots as an endophytic niche for *Fusarium* species. Symptomless endophytic colonization of maize by *Fusarium moniliforme* has been reported by Bacon, [17].

*Nothopegia beddomei*, *Wynaadica* is an endangered tree endemic to Kerala part of Western Ghats. No reports are available about this species until now. We have isolated different compounds from *F. equiseti*, and the therapeutic studies of the compounds isolated are in progress.

**Table 1:** Fungal endophytes from *N. beddomei* *wynaadica*.

<table>
<thead>
<tr>
<th>Fungi/Location</th>
<th>Waynad</th>
<th>April</th>
<th>June</th>
<th>October</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fusarium equiseti</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Phomopsis sp.</em></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Rhizopus sp.</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Sterile white mycelium</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Sterile grey mycelium</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

*Contains a group of 32 morphotypes none of which were isolated constantly from any season.* +, present; -, absent.

References