



International Journal of Research in Agronomy

E-ISSN: 2618-0618
P-ISSN: 2618-060X
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NAAS Rating (2025): 5.20
www.agronomyjournals.com
2025; 8(11): 20-22
Received: 26-08-2025
Accepted: 29-09-2025

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A comparative study of rhizosphere and endophytic fungi from *Ruta graveolens* and its antibacterial study

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DOI: <https://www.doi.org/10.33545/2618060X.2025.v8.i11a.4136>

Abstract

The study of Endophytic fungi in the medicinal plants showed quite a variation. In total five fungi *Trichoderma viridae*, *Chalaropsis radicola*, *Penicillium pseudostromaticum*, *Colletotrichum* sp. and *Fusarium solani* were isolated from rhizosphere while four fungi *Bispora* sp., *Chalaropsis radicola*, *Penicillium pseudostromaticum* and *Fusarium solani* were isolated as endophytes from the plant *Ruta graveolens*. Two types of rhizosphere fungi *Trichoderma viridae* and *Colletotrichum* sp. were found to be 33.33% of the population of total fungi isolated while the only endophyte fungi *Bispora* sp. was 16.66%. Three isolates *Chalaropsis radicola*, *Penicillium pseudostromaticum* and *Fusarium solani* were common both as rhizospheric and endophytic being 50% of the total population of the isolates.

The fungal isolate *Chalaropsis radicola* from the plant *Ruta graveolens*, showed inhibition of gram negative bacteria *K. pneumoniae*. The dominant endophytic fungi *Fusarium solani* showed inhibition of both of the gram negative bacteria *K. pneumoniae* and *E. coli*. The dominant endophyte *Penicillium* sp., showed inhibition of only gram positive bacteria *S. aureus*. The endophyte *Bispora* sp., did not show any antibacterial activity.

Keywords: *Ruta graveolens*, rhizosphere fungi, endophytic fungi, *Chalaropsis radicola*, *Fusarium solani*, *Penicillium* sp.

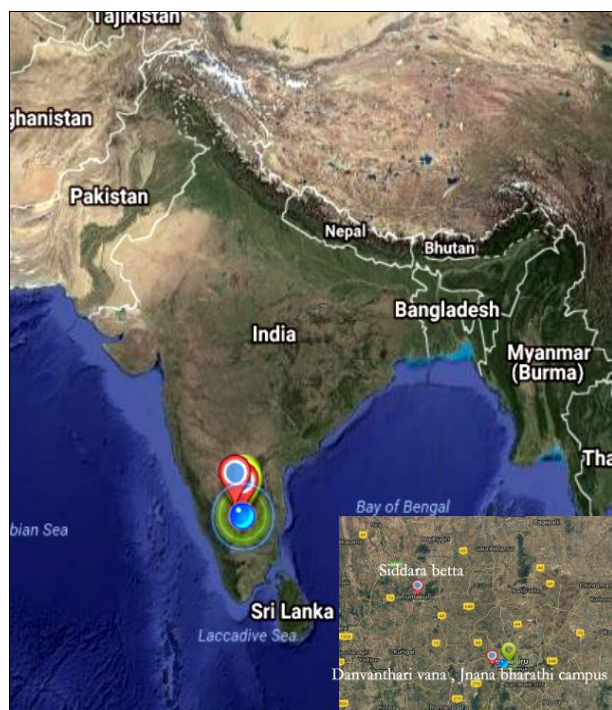
1. Introduction

Plants may serve as a repository of innumerable number of organisms known as endophytes. Tropical endophytes have been actively studied since 2000 to explore these diverse groups of endophytes with its greater biodiversity. They form inconspicuous infections within tissues of healthy plants for all or nearly all their life cycle. endophytic biodiversity, biochemical activities of endophytic metabolites and the relationships between endophytes and host plants. On the other hand, differences of endophytic fungal assemblage in different tissue types have been found in the same plant species or even in different tissues of an individual plant, which is a reflection of tissue specificity. based on their good antimicrobial activity more attention has been given to the potential of exploiting endophytic fungi for novel antibiotics (Debbab *et al.*, 2012) [4].

Sampling site: The study site chosen for collection of endophytic fungal samples was Dhanvantri Vana is located at Jnana Bharathi, Department of forestry, Government of Karnataka, Bengaluru, Karnataka, India.

Locality	Latitude	Longitude	Habitat/ Forest type
Dhanvantri Vana, Bengaluru	77.498159° W	12.942061° S	Cultivated

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2. Materials and Methods

1. Isolation of Rhizosphere fungi

The Rhizosphere soil collected from the region of plant roots was processed within 24 hours by Serial dilution technique and Pour plate technique.

Serial dilution Technique: The soil samples were suspended in 90ml saline and homogenized to mix the soil sample uniformly. The sample was diluted in a series of 9ml saline tubes to obtain different dilutions of sample from lower to higher dilutions like 10^{-1} upto 10^{-7} respectively. The dilution tube technique helps in reducing the number of organisms to obtain easy isolation of colonies for characterization.

Pour plate Technique: 1ml of the selected dilution tube was transferred into sterilized Petri plates. 20ml of Potato dextrose agar medium was added and mixed by rotating the media plate clockwise and anticlockwise direction. This helps in uniform distribution of fungi in the plate for growth. These inoculated plates were incubated at 28 °C for 1week to observe for the growth of fungi and mounted with lactophenol cotton blue to observe for the key characters of the fungi.

2. Isolation of endophytic fungi

The collected root samples from *Ruta graveolens*, were washed and cut into small bits and subjected to surface sterilization. The processed root bits were disinfected in 75% alcohol for one minute followed by immersion in 5% of sodium hypochlorite for 8 minutes. The sterilized root bits were again exposed to 75% alcohol for 30 seconds and later suspended in sterile distilled water to remove the traces of sterilants on the surface of root bits. Finally the root bits were blot dried in sterile blotting paper a modified method.

The processed root bits were placed on sterilized Potato Dextrose Agar (PDA) medium containing streptomycin and incubated at 28 °C for 21 days and observed for growth of fungus.

3. Identification of fungal isolates

The isolated fungal colonies were studied for their colony

characteristics like growth characteristics, pigmentation and the morphological characters using lactophenol cotton blue.

4. Study of Antibacterial Activity of fungal isolates:

Three clinically important bacterial strains were used as test organisms for *in vitro* antibacterial screening of fungi. The bacterial strains include - gram positive *Staphylococcus aureus* (NCIM5345) and gram negative *Klebsiella pneumonia* (NCIM2957) and *Escherichia coli* (NCIM 2068).

The antibacterial study was done by two methods

- Turbidity method
- Agar diffusion technique

Two mycelial agar discs (5 mm diam.) of the fungal culture incubated for 21 days were inoculated in 100 ml potato dextrose broth in 250 ml Erlenmeyer flasks. After 21 days of incubation at 28 ± 2 °C, culture broths were filtered with sterilized cheese cloth. The cell free culture filtrates were used to study the antibacterial activity by Turbidity method.

The 1ml of sterile nutrient broth was taken and added with the 1ml of crude fungal broth followed by 100 µl of bacterial culture. The tubes were then incubated at 37°C for 48 hrs. The tubes were observed for the presence or absence of turbidity.

A plate of nutrient agar medium was inoculated with the test organism by spread plate technique. Circular sterile paper discs soaked in fungal extract is placed on the agar medium. The fungal broth extracted with ethyl acetate and dissolved in DMSO was used for the study of antibacterial property. The plates were then incubated at 37 °C for 36-48 hours and observed for the zone of inhibition around the paper disc. The diameter of the inhibition zone around the discs is measured in millimeter (mm). Agar disc diffusion method was followed by the method given by (Kirby- Baurer).

3. Results and Discussion

Studies in the plant *Ruta graveolens*

A. Studies of Rhizospheric and endophytic fungi isolated from *Ruta graveolens*.

In total five fungi *Trichoderma viridae*, *Chalaropsis radicola*, *Penicillium pseudostromaticum*, *Colletotrichum* sp. and *Fusarium solani* were isolated from rhizosphere while four fungi *Bispora* sp., *Chalaropsis radicola*, *Penicillium pseudostromaticum* and *Fusarium solani* were isolated as endophytes from the plant *Ruta graveolens*.

Two types of rhizosphere fungi *Trichoderma viridae* and *Colletotrichum* sp. were found to be 33.33% of the population of total fungi isolated while the only endophyte fungi *Bispora* sp. was 16.66%. Three isolates *Chalaropsis radicola*, *Penicillium pseudostromaticum* and *Fusarium solani* were common both as rhizospheric and endophytic being 50% of the total population of the isolates.

The results are presented in Table 1 and Figure 1

Table 1: Rhizospheric and endophytic fungi isolated from *Ruta graveolens*

Sl No	Fungal isolates	Rhizosphere fungi	Endophytic fungi
1	<i>Trichoderma viridae</i>	+	-
2	<i>Bispora</i> sp.	-	+
3	<i>Chalaropsis radicola</i>	+	+
4	<i>Penicillium pseudostromaticum</i>	+	+
5	<i>Colletotrichum</i> sp.	+	-
6	<i>Fusarium solani</i>	+	+

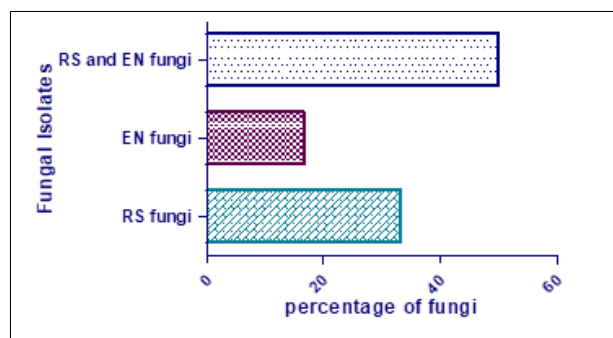


Fig 1: Percentage of rhizospheric (RS), endophytic (EN) fungi and total fungal isolates from *Ruta graveolens*

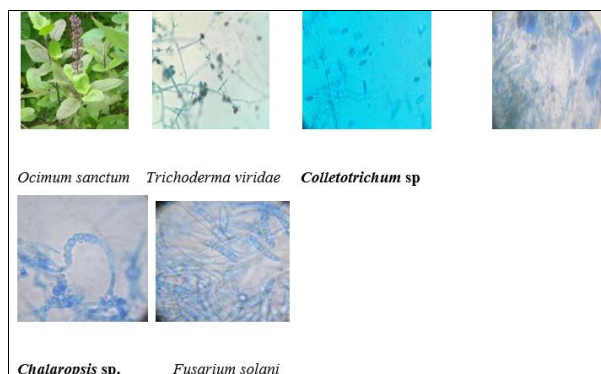


Fig 2: Fungal isolates of rhizospheric (RS), endophytic (EN) fungi from *Ruta graveolens*

4. Study of Antibacterial activity of Rhizospheric and Endophytic Fungal broth

The rhizospheric fungi *Colletotrichum* sp. isolated from *Ruta graveolens* showed inhibition of all the three bacteria *E.coli* and *K. pneumoniae* and *S.aureus*.

The endophytic fungal isolate *Chalaropsis radicola* from the plant *Ruta graveolens*, showed inhibition of gram negative bacteria *K. pneumoniae*. The dominant endophytic fungi *Fusarium solani* showed inhibition of both of the gram negative bacteria *K. pneumoniae* and *E. coli*. The dominant endophyte *Penicillium* sp., showed inhibition of only gram positive bacteria *S. aureus*. The endophyte *Bispora* sp., did not show any antibacterial activity.

Medicinal plant		<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>
<i>Ruta graveolens</i> Endophytic fungi	<i>Bispora</i> sp.	-	-	-
	<i>Chalaropsis radicola</i>	-	+	-
	<i>Fusarium solani</i>	+	+	-
	<i>Penicillium pseudostromaticum</i>	-	-	+
<i>Ruta graveolens</i> Rhizosphere fungi	<i>Trichoderma viridae</i>	-	-	-
	<i>Colletotrichum</i> sp.	-	+	-

5. Discussion

A comparative study of Rhizosphere and Endophytic fungal study was done for the medicinal plant species- *Ruta graveolens* showed varied fungal microflora in both Rhizosphere and endophytes. The endophyte *Bispora* did not show any antibacterial activity. The dominant endophytic fungi *Fusarium solani* showed inhibition of both of the gram negative bacteria *K. pneumoniae* and *E.coli*. The fungi *Colletotrichum* showed inhibition of *Klebsiella pneumoniae* species. These endophytes can be cultivated in large mass for the preparation of

antibacterial properties.

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