Isolation of Rhizospheric and endophytic fungi from the roots of *Rutagraveolens* and the study of fungal metabolites for Auxins- its effect on plant growth of *Alternantherasessilis* and *Bacopamonnieri*

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Abstract:
Soil is a source of natural media with macro nutrients and micro nutrients for the growth of micro-organisms which is supported by the root exudates of plants. The symbiotic associations of fungi, rhizosphere and influence of root exudates plays an important role in understanding about the metabolites and their influence on microbes and plant growth. The present study of fungal endophytes for growth promoters were isolated from medicinal plants of *Rutagraveolens* reported rhizosphere fungi *Trichodermaviridae, Chalaropsisradicicola, Penicilliumpseudostromaticum, Colletotrichum*sp. and *Fusariumsolani*. The endophytic fungi included *Bispora*sp. *Chalaropsisradicicola, Penicilliumpseudostromaticum,* and *Fusariumsolani.* The study of growth promoters- auxins produced by *Bispora*sp. and *Fusariumsolani* on *Alternantherasessilis* showed a significant increase in plant growth.
Key words :Endophytic fungi, *Rutagraveolens* , Growth promoters, Auxins, *Fusariumsolani.*

Introduction:

The word endophyte -'endo' means within and 'phyte' means plant, in Greek (Caroll, 1988).Fungal Endophytes are ubiquitous in nature the with at least few million species in the plant symbiotic association. According to Bacon and White, 2000; Strobel., 2002, plants canserve as a reposition of innumerable types of microorganisms known as endophytes. The studies done by Heywood(1995); Staley *et al*., (1997), indicates that the microbial populations being endophytes are enormous but the data revealed and characterized is only 1% of bacteria and 5% of fungi and there is a huge population of microbes to be explored for the benefit of human welfare. The Endophytes produce a wide variety of unique bioactive compounds, such as alkaloids,phenolic acidsbenzopyranones,, terpenoids, chinones, flavonoids, , quinones, tetralones,xanthones, and few others , according to the studies done by Tan and Zou (2001).

The studies done by Muhammad Waqas*et al*., (2012) the plant growth substances support the fungal association and would benefit the host-plants, even during the environmental stress conditions. The metabolites containing gibberellins and auxins have been reported to play a major role in plant growth and their response to various environmental conditions. According to the studiestryptophan has been identified as one of the main precursor for the biosynthesis of Indole acetic acid (Karthikeyan and Suryanarayanan*et al*., 2010).

Rational of the study:

Plants produce various growth hormones like auxins and gibberellins constitutively for their growth. The main objective of the study is to understand about the endophytic fungal metabolites producing growth hormones like auxins. The study is to rationalize the study of fungal metabolites containing growth hormones to support plant growth as an endophyte in association.

Objectives:

1. Isolation of rhizosphere and endophytic fungi from the roots of the medicinal plant *Ruta graveolens.*

2. Screening of growth hormones -auxins from the endophytic fungal isolates.

3. To study the effect of endophytic growth promoters on plant growth of *Alternantherasessilis*

and *Bacopamonnieri*.

1. Materials and methods:

1.1.Sampling site: The study site for the isolation of rhizosphere and endophytic fungal samples was from Dhanvantrivana , located at JnanaBharathicampus- Bangalore University, Department of forestry, Government of Karnataka, Bengaluru, Karnataka, India.

Fig: 1.1 Geographical location of sample collection

1.2 Isolation of endophytic fungi:

The collected soil samples and root bits from *Rutagraveolens* were processed for the isolation of fungi**.** The soil sample was serially diluted and subjected to pour plate technique to isolate Rhizosphere fungi**.** The collected root samples from *Rutagraveolens* were gently washed, cut into small bits using a sterilized scalpel aseptically. The root bits were surface sterilized using 75% alcohol for 1 minute, it was followed by 5% of sodium hypochlorite solution for 8 minutes. The surface sterilized root bits were further immersed in 75% alcohol for 30 seconds and later processed in sterile water to remove the traces of disinfectant. Finally the root bits were blot dried in sterilized filter paper (Guo*et al*., 2008; Wang *et al*., 2008; Samaga*et al*., 2014). The root bits were placed on sterilized Potato Dextrose Agar (PDA) medium containing streptomycin. The plates were incubated at 28°C for 21 days and observed for the growth of fungus.

1.3 Identification of the fungal isolates: The isolated fungal colonies were studied for the colony characteristics like growth characteristics on PDA medium, pigmentation and their morphological characters were identified using lactophenol cotton blue.

1.4 Screening for Growth promoters from fungi.

The fungal isolates obtained from *Rutagraveolens* were studied for production of Auxins.

a) **Production of Auxins:**The Potato Dextrose Broth containing Tryptophan 100 µg/ml concentration as a precursor, was inoculated with the specific fungal culture and incubated at 28°C for 21 days in dark condition. The broth containing metabolites was quantified for presence of auxins (Brick *et al*., 1991)
b) **Detection of Indole acetic acid by Salkowski's method:** The fungal culture broth containing the metabolites was centrifuged and the supernatant was used for the assay. 1ml of the supernatant taken in a test tube is treated with 2ml of Salkowski's reagent and incubated for 20 mins in dark at room temperature. The development of pink colour in the test indicates the presence of auxins; Brick *et al*., (1991); Khamna*et al*., (2009).
c) **Study of the effect of growth promoters on plants by pot trial method:**

In this method**,** the desired plant for the study is applied with the culture metabolite as an external supplement. The test plant is studied for certain criteria such as height of the plant in cm, number of branches, number of leaves produced and size of the leaf blade. The selected test plants for the study were *Alternantherasessilis* and *Bacopamonnieri.*

2. Analysis and Data analysis:

2.1 Rhizospheric and endophytic fungi isolated from *Rutagraveolens*

In total five fungi *Trichodermaviridae,Chalaropsisradicicola*, *Penicilliumpseudostromaticum*, *Colletotrichum*sp. and *Fusariumsolani*were isolated from rhizosphere while four fungi *Bispora*sp., *Chalaropsisradicicola*, *Penicilliumpseudostromaticum*and *Fusariumsolani*were isolated as endophytes from the plant *Rutagraveolens.*

The isolated rhizosphere fungi include *Trichodermaviridae* and *Colletotrichum*sp. were found to be 33.33% of the total fungi isolated, while the only endophyte fungal isolate include *Bispora*sp. being16.66 %. The three isolates *Chalaropsisradicicola*, *Penicillumpseudostromaticum* and *Fusariumsolani* were common both as rhizospheric and endophytic being 50% of the total fungal isolates. The results are presented in Table 2.1 and Fig 2.1.

Table 2.1: Rhizospheric and endophytic fungi isolated from *Rutagraveolens*

Sl No	Fungal isolates	Rhizosphere fungi	Endophytic fungi
1	*Trichodermaviridae*	+	-
2	*Bispora*sp.	-	+
3	*Chalaropsisradicicola*	+	+
4	*Penicillium pseudostromaticum*	+	+
5	*Colletotrichum*sp.	+	-
6	*Fusariumsolani*	+	+

(+) Present (-) Absent

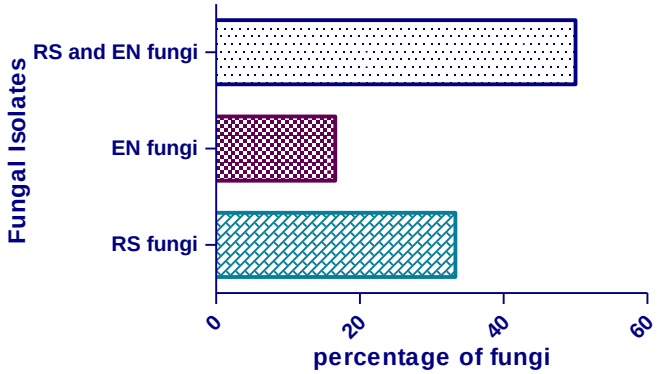

Figure 2.1: Percentage of rhizospheric (RS), endophytic (EN) fungi and total fungal isolates from *Rutagraveolens*

2.2 Identification of Rhizospheric and Endophytic fungi isolated from the plant *Rutagraveolens*

*RutagraveolensFungal isolatesChalaropsis*sp..*Fusariumsolani*

Bispora sp.*Penicilliumpseudostromaticus*

2.2 : Fungal isolates from plant roots of *Rutagraveolens* sampled at Dhanavantrivana

2.3 Detection of Indole acetic acid by Salkowski's method:

Table 2.3: The fungal isolates studied for the presence of Indole acetic acid:

SL. No	Name of thePlant	Endophytic fungi	Result
1	*Rutagraveolens*	*Bispora*sp.	Positive
2		*Chalaropsisradicicola*	Negative
3		*Penicillium pseudostromaticum*	Negative
4		*Fusariumsolani*	Positive

The source of fungal isolates from *Rutagreveolens* which showed the presence of auxins were *Bispora*sp and *Fusariumsolani*

2.4 Effect of crude auxins produced by fungi on seed germination.

The fungal crude auxins showed varied growth of coleoptiles where the control was compared with the lengths of other test samples. The control showed 90% germination while *Fusariumsolani* showed 100% germination while *Bispora*sp. showed 93.3% germination. The length of the coleoptile was also measured and results are given in table (table 2.4).

Table: 2.4- Effect of fungal isolate on percent germination and coleoptiles length (cm). Data given as mean ±SEM, (n=15)

	Percentage of Germination (%)	Mean of coleoptiles length (cm)
Control	90	1.993±0.3407
*Bispora*sp.	93.3%	2.167 ± 0.3731
Fusariumsolani	100%	2.147 ± 0.3158

The average length of coleoptile for control (1.993±0.3407 cm) , *Bispora* sp. (1.847 ±0.2960 cm) and *Fusariumsolani*(2.147 ± 0.3158 cm.) which clearly shows that the isolates of these fungi increases the radical length, with 0.46 cm higher than that of the control showing a difference with that of control value.

2.5 Effect of fungal crude phytohormones on plant growth by pot trial method.

a). **Study of the fungal phytohormones on the plant *Alternantherasessilis***

The plant *Alternantherasessilis* showed a considerable difference with that of control. The height of the plantwith 14.33±1.20 cm and control of13.80±0.98cm. The number of branches with1.33±0.33 and control with 2.00±0.00.The length of the branch in the control plant showed 2.50±0.28 cm, while the plant influenced by broth of *Fusariumsolani* showed a length of 4.33±0.33 cm.

Table: 2.5 Effect of fungal phytohormones on the growth of plant *Alternantherasessilis*

	Height(cm)	**Number of branches**	**Length of the branches (cm)**
Control	13.80±0.98	2.00±0.00	2.50±0.28
*Bispora*sp.	15.33±0.88	1.00±0.00	3.66±1.20
Fusariumsolani	14.33±1.20	1.33±0.33	4.33±0.33

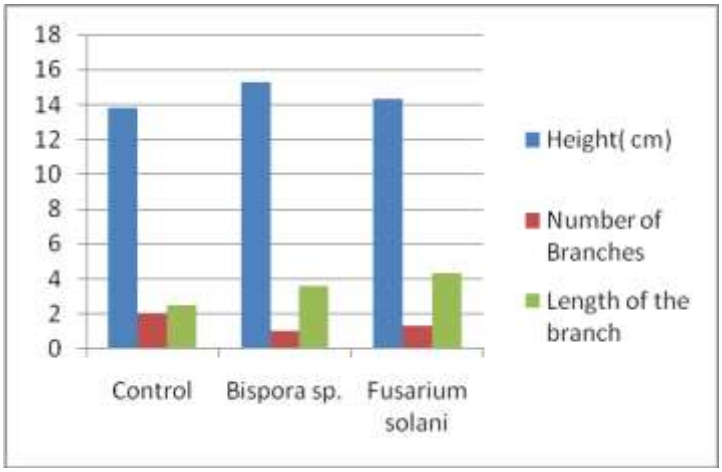

Table: 2.5 Graphical representation of the effect of fungal auxinson the growth of plant *Alternantherasessilis*

b) **Study of the fungal phytohormones on the plant *Bacopamonnieri:***

The plant *Bacopamonnieri*showed considerable differences with that of control. The height of the plant with 7.36±0.18 cm and control with 6.56±0.34 cm.The number of leaves with (8.33±0.33) and control with (5.33±0.66). The size of the leaf blade for the control plant showed 1.20±0.20 cm,while the plant influenced by the fungal broth of *Fusariumsolani* showed 1.60±0.20 cm as the leaf expansion indicating the influence of fungal auxins.

Table: 2.5 (b) Effect of fungalphytohormones on the growth of the plant *Bacopamonnieri*

	Height(cm)	**Number of Leaves**	**Size of leaf blade(cm)**
Control	6.56±0.34	5.33±0.66	1.20±0.20
*Bispora*sp.	12.66±1.45	9.33±0.88	2.00±0.28
Fusariumsolani	7.36±0.18	8.33±0.33	1.60±0.20

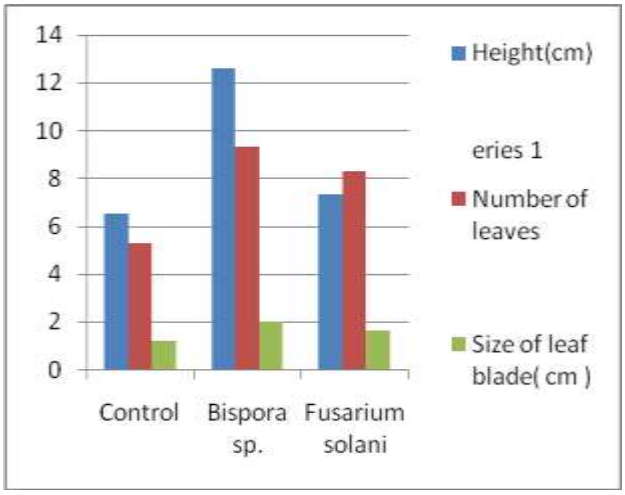

Table: 2.5 (b) Graphical representation of the Effect of fungal phytohormones on the growth of plant *Bacopamonnieri*

Conclusions: The isolated fungal endophytes promote the growth of plants by producing phytohormones like auxins and hence the fungi *Fusariumsolani*showed moderately significant growth enhancement in the experimental plants *Alternantherasessilis*and *Bacopamonnieri.*The fungal culture and the metabolites can be used as a bioinoculant in soil to enhance plant growth.

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