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Evaluation of bacterial endophytes against major fungal pathogens of groundnut under *in vitro* condition

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Abstract
A total of thirty five bacterial endophytes were isolated from apparently healthy groundnut leaves (7), stem (16) and root (12) and these endophytes were evaluated against major fungal pathogens of groundnut *viz., Sclerotium rolfsii* and *Rhizoctonia solani* by dual culture technique and against *Puccinia arachidis* by spore germination technique. Among them, SBDwSo-9, RBBeJa-3 and RBHaBn-11 showed the maximum inhibition of *S. rolfsii* (50.59 %), *R. solani* (32.55 %) and *P. arachidis* (69.61 %).

Keywords: Bacterial endophytes, Groundnut, *In vitro*, *Puccinia arachidis*, *Rhizoctonia solani*

Introduction
Groundnut (*Arachis hypogaea* L.) is considered to be one of the most important oilseed crops in the world. It is one of the most important food and cash crops of our country. In India the production and productivity of groundnut is very less as compared to major groundnut growing countries. This may be attributed to the rainfed nature of cultivation of this crop coupled with attack by a variety of biotic and abiotic stresses and more than 55 pathogens have been reported to affect groundnut (Subrahmaniyam *et al.,* 1985) [7]. The chemical insecticides which have been used in crops since the 1940's have, in many cases, proved to be efficient at controlling insect pests, although they could also affect non-target organisms (Smith and Stratton, 1986) [6]. The same is true for fungicides and herbicides meant to control phytopathogenic fungi and weeds respectively, but which also have the capacity to inhibit the growth and multiplication of beneficial microorganisms, including endophytic bacteria and fungi, which perform crucial environmental roles.
Endophytic microorganisms live within host plants without causing any noticeable symptoms of disease. It is hypothesized that the endophytes, in contrast to known pathogens, generally have far greater phenotypic plasticity and thus more options to interact with their host than pathogens. Since the 1970´s several reports have shown that these endophytes have important roles in protecting their host against pests and diseases (Shultz and Boyle, 2005) [4]. In recent decades, use of endophytes for plant protection is receiving much attention of researchers as they promote plant growth as well as protect plants from biotic and abiotic stresses (Shirasangi and Hegde, 2018) [5]. Already such studies are made in several countries and hence with a view of exploiting the role of endophytes in suppression of major fungal pathogens of groundnut, the present investigation was undertaken.

Material and Methods
Isolation of bacterial endophytes
A roving survey was conducted during 2016 and 2017 to isolate bacterial endophytes from groundnut plants. Apparently healthy leaves, stems and root samples were collected from the fields of Bagalakot, Belagavi, Dharwad and Haveri districts of northern Karnataka. Groundnut leaves, stem and root samples were washed in running tap water to remove dirt and split into longitudinal sections. After this, surface sterilization was done with ethanol (70 %) for a minute followed by sodium hypochlorite (1 %) for 3 minutes. Subsequently the sections were rinsed with sterile distilled water. Then the sections were rinsed with 0.02 M potassium phosphate buffer 3 times (0.1 ml aliquot was taken and transferred to 9.9 ml of nutrient broth which served as sterilization check). One gram of plant parts were macerated with 9 ml of potassium phosphate buffer in pestle and mortar and serial dilution was made up to 10^{-2} and 500 µl of this dilution was plated on Nutrient Agar (NA) medium. The plates were incubated at 28 ± 2°C for 72 hr for observing colonies developed on them and isolated colonies were picked up and streaked again on fresh NA plates and incubated.

Final pure cultures were transferred on NA slants and stored in refrigerator at 4°C for further studies.

Evaluation of bacterial endophytes against *S. rolfsii* and *R. solani* under *in vitro* condition

Bacterial endophytes were evaluated by dual culture technique, for this 20 ml of sterilized and cooled PDA was poured into sterilized Petri plates. The mycelial disc of pathogen was inoculated at one side and bacterial endophyte was streaked at other side of the Petri plate. For this, actively growing cultures were used and three replications were maintained for each treatment. After required period of incubation *i.e.*, after growth of colony in control plate reached 90 mm diameter, the radial growth of pathogen in treated plate was measured. Per cent inhibition over control was worked out according to formula given by Vincent (1947) [8].

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent inhibition of mycelial growth, C = Radial growth in control (mm) and T = Radial growth in treatment (mm).

Evaluation of bacterial endophytes against *P. arachidis* by spore germination method

25 per cent concentrated culture filtrate of each endophytic isolate was prepared and it was used for uredospore germination study in cavity slides. In a cavity slide, 25 µl of above mentioned concentration of culture filtrate was separately taken and around hundred uredospores were added per cavity by scrapping rust pustule. The cavity slides were kept in the moist chamber and were incubated at 20^0C. Three replications were maintained for each treatment. Uredospore germination was observed at 24 hrs after incubation at 100X magnification. Later per cent inhibition over control was calculated by using formula given by Vincent (1947) [8].

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent inhibition of spore germination, C = Number of spores germinated in control and T = Number of spores germinated in treatment.

Results and Discussion

From the Table 1 it is noticed that, among the 7 groundnut leaf bacterial endophytes tested against *S. rolfsii*, the maximum mycelial inhibition was observed by the leaf endophyte LBBePa-1 (38.43 %) which was significantly superior to other endophytes and this was followed by LBBeKh-3 (20.00 %). The endophyte LBBeBu-2 (1.57 %) recorded the least mycelial inhibition and was less effective as compared to other endophytes. Against *R. solani*, the endophyte LBBePa-1 (29.41 %) recorded the maximum mycelial inhibition and this was on par with LBDwAC-4 (26.27 %). The endophyte LBDwUn-6 (13.33 %) recorded the least mycelial inhibition followed by LBHaSh-7 (20.00 %) (Plate 1). Against *P. arachidis*, the endophyte LBBePa-1 (56.51 %) recorded the maximum inhibition of uredospore germination, which was significantly superior to all other endophytes. This was followed by LBBeKh-3 (49.73 %). The endophyte LBHaSh-7 (14.96 %) was less effective, which showed the least inhibition of uredospore germination followed by LBDwUn-6 (17.16 %).

Among the 16 groundnut stem bacterial endophytes tested against *S. rolfsii*, the maximum mycelial inhibition was observed in SBDwSo-9 (50.59 %) endophyte, which was significantly superior to other endophytes, this was followed by SBDwBi-11 (32.94 %). The endophyte SBBeBu-2 and SBBeSi-3 (1.57 % each) were less effective with the minimum mycelial inhibition. Against *R. solani*, the endophyte SBBeBu-2 (26.27 %) recorded the maximum mycelial inhibition, which was on par with SBDwAC-7 (25.88 %), SBBeKh-4 (25.10 %), SBBeJa-5 (24.31 %). The endophyte SBDwHe-8 (1.96 %) was less effective with the minimum mycelial inhibition followed by SBBeAv-6 (2.35 %) and SBHaSh-14 (3.14 %), which were on par with each other. Against *P. arachidis*, the isolate SBDwSo-9 (63.62 %) recorded the maximum inhibition of uredospore germination, which was significantly superior over SBDwBi-11 (55.93 %) and SBBeAv-6 (54.47 %). The endophyte SBHaSh-14 (9.20 %) was less effective with the minimum inhibition of uredospore germination (Table 2 and Plate 2).

The higher mycelial inhibition against *S. rolfsii* was observed by the root endophyte RBBeJa-3 (28.63 %), which was significantly superior to other endophytes, this was followed by RBDwSo-5 (24.31 %). The endophyte RBBePa-1 (1.96 %) recorded the least mycelial inhibition followed by RBBeBu-2 (4.31 %). Against *R. solani*, the endophyte RBBeJa-3 (32.55 %) recorded the maximum mycelial inhibition, this was on par with GRBE-11 (29.80 %). Whereas, the endophyte RBBePa-1 (4.71 %) recorded the least mycelial inhibition followed by RBHaKa-10 (9.41 %). Against *P. arachidis*, the endophyte RBHaBn-11 (69.61 %) recorded the maximum inhibition of uredospore germination, which was significantly superior over all the endophytes. This was followed by RBBePa-1 (65.58 %), the endophyte RBDwSo-5 (11.69 %) was less effective with the minimum inhibition of uredospore germination (Table 3 and Plate 3).

Among the 35 bacterial endophytes, 4 endophytes showed better inhibition of all the three pathogens as compared to remaining endophytes in dual culture method. Among them, SBDwSo-9, RBBeJa-3 and RBHaBn-11 showed the maximum inhibition of *S. rolfsii* (50.59 %), *R. solani* (32.55 %) and *P. arachidis* (69.61 %). The findings of present study are in agreement with the work of Deepthi (2013), who isolated total of 45 (5 fungi and 40 bacteria) endophyte species from groundnut root and evaluated these endophytes against *S. rolfsii* by following the dual culture technique, Dey *et al*. (2013) tested the efficacy of *Bacillus subtilis* and *Pseudomonas fluorescens* against *Puccinia sorghi* at 20 per cent concentration and their results revealed that *B. subtilis* (33.85 %) and *P. fluorescens* (46.20 %) recorded the maximum inhibition of uredospore germination.

Endophytes could become better biocontrol agents as compared with rhizosphere micro flora because they do not compete for nutrition and/or niche in apoplast. Endophytic microorganisms may increase the plant fitness by improving the tolerance to heavy metals and drought could promote plant growth and reduce the herbivory or phytopathogen settling (Rubini *et al.*, 2005) [3]. Results of present *in vitro* studies on efficacy of endophytes against three pathogens like *S. rolfsii*, *R. solani* and *P. arachidis* revealed that there is a significant inhibition of pathogens.

Table 1: Evaluation of groundnut leaf bacterial endophytes against *Sclerotium rolfsii*, *Rhizoctonia solani* and *Puccinia arachidis* under *in vitro* condition

Endophyte	Per cent inhibition		
	Mycelial growth		Uredospore germination
	S. rolfsii	***R. solani***	***P. arachidis***
LBBePa-1	38.43 (38.30)*	29.41 (32.83)	56.51 (48.73)
LBBeBu-2	1.57 (7.09)	22.35 (28.20)	39.26 (38.78)
LBBeKh-3	20.00 (26.55)	22.35 (28.20)	49.73 (44.83)
LBDwAC-4	10.98 (19.34)	26.27 (30.82)	31.54 (34.15)
LBDwHe-5	7.84 (16.22)	23.92 (29.25)	27.47 (31.58)
LBDwUn-6	12.94 (21.08)	13.33 (21.35)	17.16 (24.46)
LBHaSh-7	11.76 (20.04)	20.00 (26.55)	14.96 (22.74)
Range	1.57-38.43	13.33-29.41	14.96-56.51
S.Em. ±	0.56	0.71	0.67
C.D. (1%)	2.36	2.99	2.81
C.V.	4.58	4.36	3.30

*Arc sine values

Table 2: Evaluation of groundnut stem bacterial endophytes against *Sclerotium rolfsii*, *Rhizoctonia solani* and *Puccinia arachidis* under *in vitro* condition

Endophyte	Per cent inhibition		
	Mycelial growth		Uredospore germination
	S. rolfsii	***R. solani***	***P. arachidis***
SBBePa-1	2.75 (9.49)*	7.06 (15.37)	46.47 (42.96)
SBBeBu-2	1.57 (7.09)	26.27 (30.82)	43.87 (41.46)
SBBeSi-3	1.57 (7.09)	9.80 (18.21)	46.07 (42.73)
SBBeKh-4	24.71 (29.79)	25.10 (30.05)	47.13 (43.34)
SBBeJa-5	26.27 (30.81)	24.31 (29.53)	53.31 (46.88)
SBBeAv-6	25.10 (30.05)	2.35 (8.62)	54.47 (47.55)
SBDwAC-7	23.14 (28.74)	25.88 (30.56)	55.70 (48.26)
SBDwHe-8	32.55 (34.77)	1.96 (7.95)	41.93 (40.34)
SBDwSo-9	50.59 (45.32)	19.61 (26.27)	63.62 (52.99)
SBDwUn-10	31.37 (34.05)	22.75 (28.47)	28.24 (32.09)
SBDwBi-11	32.94 (35.01)	23.14 (28.73)	55.93 (48.39)
SBBaBa-12	13.73 (21.69)	18.82 (25.70)	36.62 (37.22)
SBBaCh-13	4.31 (11.96)	16.86 (24.22)	24.08 (29.32)
SBHaSh-14	5.49 (13.53)	3.14 (10.16)	9.20 (17.64)
SBHaKa-15	13.33 (21.40)	20.00 (26.55)	42.33 (40.57)
SBHaBn-16	5.88 (14.03)	12.16 (20.39)	49.63 (44.77)
Range	1.57-50.59	1.96-26.27	9.20-63.62
S.Em. ±	0.59	0.63	0.98
C.D. (1%)	2.29	2.45	3.80
C.V.	4.38	4.85	4.14

*Arc sine values

Table 3: Evaluation of groundnut root bacterial endophytes against *Sclerotium rolfsii*, *Rhizoctonia solani* and *Puccinia arachidis* under *in vitro* condition

Endophyte	Per cent inhibition		
	Mycelial growth		Uredospore germination
	S. rolfsii	***R. solani***	***P. arachidis***
RBBePa-1	1.96 (7.95)*	4.71 (12.46)	65.38 (53.94)
RBBeBu-2	4.31 (11.96)	21.18 (27.38)	22.65 (28.40)
RBBeJa-3	28.63 (32.33)	32.55 (34.76)	27.10 (31.35)
RBDwAc-4	14.90 (22.70)	22.35 (28.20)	26.78 (31.14)
RBDwSo-5	24.31 (29.53)	25.88 (30.56)	11.69 (19.96)
RBDwBi-6	11.76 (20.05)	20.00 (26.55)	17.28 (24.52)
RBBaBa-7	20.00 (26.55)	20.78 (27.10)	23.58 (29.03)
RBBaCh-8	12.94 (21.06)	20.78 (27.10)	28.99 (32.56)
RBHaSh-9	8.24 (16.64)	21.57 (27.66)	34.88 (36.18)
RBHaKa-10	7.84 (16.22)	9.41 (17.77)	52.49 (46.41)
RBHaBn-11	7.45 (15.79)	29.80 (33.06)	69.61 (56.52)
RBHaBd-12	17.65 (24.83)	17.25 (24.52)	26.52 (30.98)
Range	1.96-28.63	4.71-32.55	11.69-69.61
S.Em. ±	0.56	0.74	0.67
C.D. (1%)	2.23	2.93	2.66
C.V.	4.78	4.85	3.32

*Arc sine values

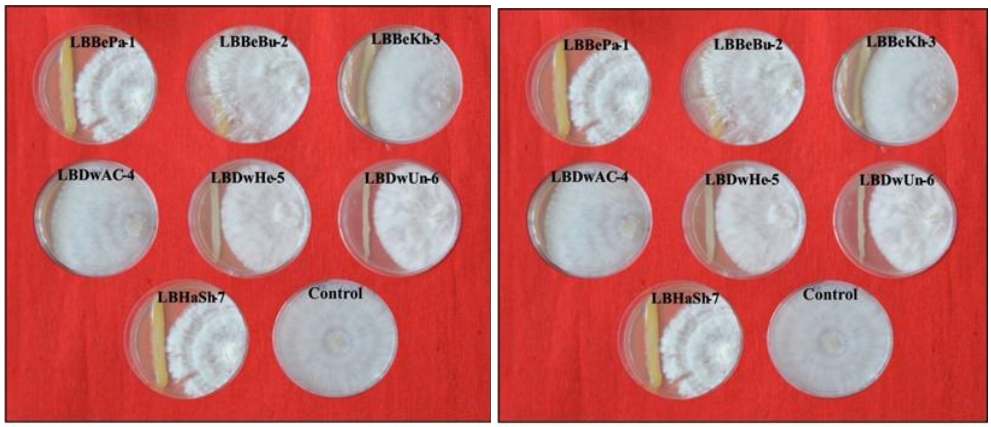

Plate 1: Evaluation of groundnut leaf bacterial endophytes against *S. rolfsii* and *R. solani* by dual culture method under *in vitro* condition

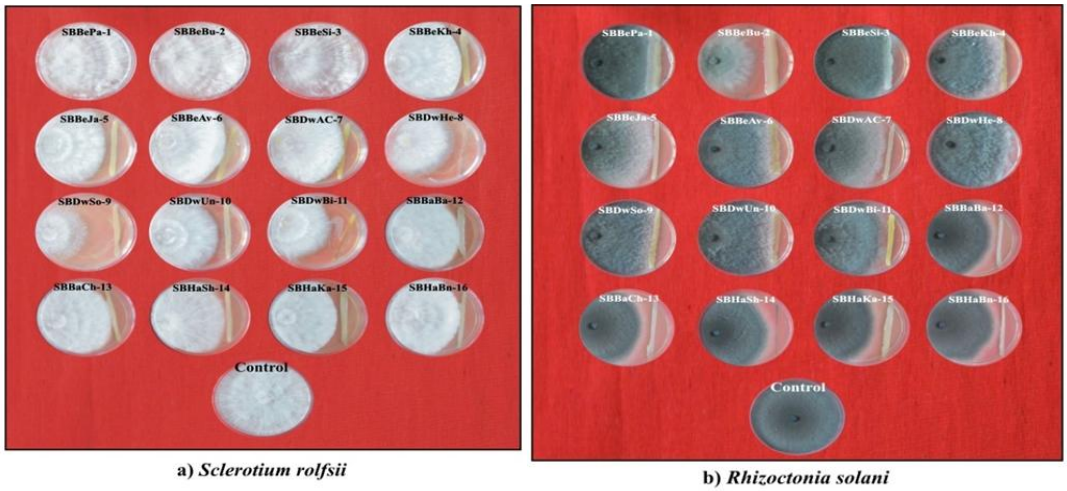

Plate 2: Evaluation of groundnut stem bacterial endophytes against *S. rolfsii* and *R. solani* by dual culture method under *in vitro* condition

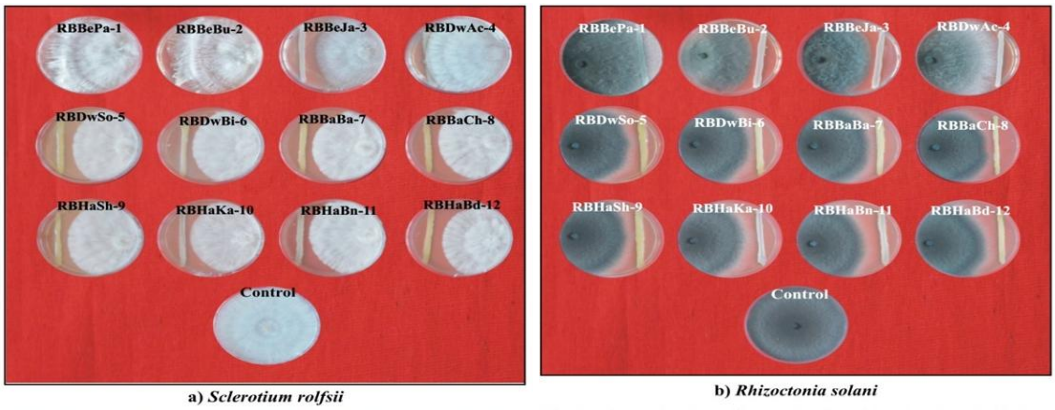

Plate 3: Evaluation of groundnut root bacterial endophytes against *S. rolfsii* and *R. solani* by dual culture method under *in vitro* condition

Conclusion

In dual culture method, the inhibition of *S. rolfsii* and *R. solani* ranged from 1.57 to 50.59 per cent and 1.96 to 32.55 per cent respectively. The inhibition of uredospore germination was ranged from 9.20 to 69.61. Among the bacterial endophytes, the maximum mycelial inhibition of *S. rolfsii* was recorded by SBDwSo-9 (50.59 %), against *R. solani* was recorded by RBBeJa-3 (32.55 %) and the maximum inhibition of uredospore germination of *P. arachidis* was recorded in RBHaBn-11 (69.61 %).

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