Original Research Article

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In vitro Evaluation of Fungal Endophytes against Major Fungal Pathogens of Groundnut

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ABSTRACT

Twenty eight fungal endophytes were isolated from leaves, 33 from stem and 21 from root, resulting in a total of 82 fungal endophytes from apparently healthy groundnut plant parts and evaluated these endophytes against major fungal pathogens viz., Sclerotium rolfsii and Rhizoctonia solani by dual culture technique and against Puccinia arachidis by spore germination technique. Among the leaf endophytes, LFDwAC-7 has shown maximum inhibition of mycelial inhibition of S. rolfsii (46.30 %), R. solani (48.63 %) and uredospore germination of P. arachidis (52.88 %). Among the stem endophytes, SFBeBu-18 and SFDwAC-8 have inhibited the maximum mycelial growth of S. rolfsii (62.59 %) and R. solani (49.63 %) respectively. Against P. arachidis SFBeBu-18 (56.91 %) has shown maximum inhibition of and uredospore germination. Among the root endophytes RFDwSo-34 has inhibited the maximum mycelial growth of S. rolfsii (43.70 %), RFDwSo-33 recorded the maximum mycelial inhibition and uredospore germination of R. solani (49.80 %) and P. arachidis (47.84 %).

Keywords
Fungal endophytes, Groundnut, In vitro, Puccinia arachidis, Rhizoctonia solani and Sclerotium rolfsii

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Introduction

Groundnut (Arachis hypogaea L.) an annual herbaceous plant is considered to be one of the most important oilseed crops in the world. In India, the average yield of rabi/summer groundnut is around 1600 kg/ha, whereas kharif groundnut is around 1000 kg/ha which is lower than 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). Among the diseases, stem rot, collar rot, root rot, leaf spots (early and late) and rust have been recognized as economically important diseases.

To manage such diseases, farmers presently use different fungicides with spray schedules utilizing two or more different fungicide groups or fungicide formulations containing two different chemical groups, which have resulted in several undesirable effects like pesticide pollution, fungicide resistance, elimination of beneficial fauna, environmental pollution and human health hazards (Ghewande, 2008). So integrated disease
management where biological control as one practice is becoming a key consideration for disease management. Use of endophytes as biocontrol agent may open up new area of research in plant protection in the recent decades under various agro-climatic situations. The term “endophyte” is derived from the Greek word “endon” means within and “phyte” means plant so the term includes all organisms that, during a variable period of their life, symptomlessly colonize the living internal tissues of their hosts (Stone et al., 2000). Fungal endophytes live in intercellular space or inside cells of host plant causing no apparent damage (Saikkonen et al., 1998). Fungi belonging to this group are ubiquitous and plant species not associated to fungal endophytes are not known. However, endophytic fungi, which colonize and grow asymptptomatically within healthy plant tissues may evolve from plant pathogenic fungi and become non-pathogenic. They are found in almost all kinds of plants, including trees, grass, algae and herbaceous plants. They will produce plant-growth-regulatory, antimicrobial, antiviral or insecticidal substances to enhance the growth and competitiveness of the host in nature (Carroll, 1988). With this background, in the present study an attempt was made to isolate fungal endophytes and evaluate them under in vitro condition against soilborne and airborne fungal pathogens.

**Materials and Methods**

**Isolation of fungal endophytes**

A roving survey was conducted during 2016 and 2017 to isolate fungal endophytes in groundnut. Apparently healthy leaves, stems and root samples were collected from the fields of Bagalkot, Belagavi, Dharwad and Haveri districts of northern Karnataka. Collected plant samples were washed in running tap water to remove soil dirt and debris and cut into 1 cm sections. After this, surface sterilization was done with 70 per cent ethanol for a minute followed by 1 per cent sodium hypochlorite for 3 minutes. Subsequently the sections were rinsed with sterile distilled water and placed on 9 cm Petri plates containing potato dextrose agar (PDA) medium amended with streptomycin (250 mg/l) to slow down the bacterial growth. Sterilized tissue segments were pressed onto the surface of PDA medium to check the efficacy of surface sterilization procedure and to confirm endophytic isolations only from internal tissues of the plant segments. The absence of growth of any fungi on the medium confirmed that the surface sterilization procedure was effective in removing the surface fungi (Schulz et al., 1993). All plates were incubated at 25±1°C and observed for fungal growth at daily interval up to 7-10 days. Fungi growing out from the plant tissues were transferred on to fresh PDA medium. After purifying the isolates for several times, final pure cultures were transferred on to PDA slants and stored in refrigerator at 4°C for further studies.

*In vitro* evaluation of fungal endophytes against *S. rolfsii* and *R. solani* by dual culture method

Dual culture technique was adopted for antagonistic activity of isolated endophytes against *S. rolfsii*, *R. solani* and *F. solani* on PDA plates (Deepa and Sally, 2015). In dual culture technique 20 ml of sterilized and cooled PDA was poured into sterilized Petri plates. Fungal endophytes were evaluated by inoculating the pathogen at one side of Petri plate and the fungal endophyte inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap. For this, actively growing cultures were used with three replications. 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).

\[
\text{C - T} \\
\text{I} = \frac{\text{C}}{\text{T}} \times 100
\]

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

**In vitro evaluation of fungal 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 scraping rust pustule. The cavity slides were kept in the moist chamber and were incubated at 20°C. 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).

\[
\text{C - T} \\
\text{I} = \frac{\text{C}}{\text{T}} \times 100
\]

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**

A total of 82 (28 from leaf, 33 from stem and 21 from root) fungal endophytes were isolated from apparently healthy groundnut plant parts and evaluated these endophytes against major fungal pathogens viz., S. rolfsii, R. solani by dual culture technique and against P. arachidis by spore germination technique.

Among 28 leaf endophytes, the maximum mycelial inhibition against S. rolfsii was observed by the leaf endophyte LFDwAC-7 (46.30 %) which was significantly superior to other endophytes. This was followed by LFDwAC-6 (36.30 %), LFBaBa-26 (34.81 %) and LFBaCh-28 (34.81 %) which were on par with each other. The isolates LFBBeBu-15 (1.11 %), LFBBePa-14 (1.85 %) and LFDwHe-22 (1.85 %) were ineffective with least mycelial inhibition. Against R. solani, the endophyte LFDwAC-7 (48.63 %) showed the maximum mycelial inhibition which was on par with LFDwAC-9 (46.67 %), LFBaBa-26 (46.67 %) and LFBBeAV-21 (45.88 %). The endophyte LFDwAC-4 (3.14 %) showed the least mycelial inhibition. Against P. arachidis, the isolate LFDwAC-7 (52.88 %), LFBaBa-26 (52.67 %) and LFBBeAV-21 (51.95 %) showed maximum inhibition of uredospore germination. The isolate LFBaCh-27 (13.10 %) and LFDwBi-25 (16.57 %) were less effective with minimum inhibition of uredospore germination as compared to other endophytes and the results are depicted in Table 1 and Plate 1.

Among 33 stem endophytes, the maximum mycelial inhibition of S. rolfsii was observed in the stem endophyte SFBBeBu-18 (62.59 %) which was on par with SFDwAC-11 (54.44 %) and SFBBePa-17 (51.48 %). The endophyte SFDwAC-10 was less effective with least mycelial inhibition (29.26 %). Against R. solani, the endophyte SFDwAC-8 showed the maximum mycelial inhibition of 49.63 per cent which was on par with SFDwAC-7 (48.15 %) and SFDwBi-33 (47.78 %). The endophyte SFDwAC-15 was less effective with the least mycelial inhibition (22.22 %) followed by SFBBeKh-24 (25.56 %).
Table 1: *In vitro* evaluation of groundnut leaf fungal endophytes against *Sclerotium rolfsii*, *Rhizoctonia solani* and *Puccinia arachidis*

| Endophyte | Per cent inhibition of |                       |                  |
|-----------|------------------------|-----------------------|------------------|
|           |                        | *S. rolfsii* | *R. solani* | *P. arachidis* |
| LFDwAC-1  | 8.52 (16.96)*          | 39.61 (38.98)*       | 34.82 (36.15)*   |
| LFDwAC-2  | 5.93 (14.07)           | 37.65 (37.80)        | 42.31 (40.56)    |
| LFDwAC-3  | 5.56 (13.63)           | 14.51 (22.31)        | 38.83 (38.53)    |
| LFDwAC-4  | 11.48 (19.79)          | 3.14 (10.16)         | 44.54 (41.85)    |
| LFDwAC-5  | 29.26 (32.73)          | 33.73 (35.46)        | 25.98 (30.62)    |
| LFDwAC-6  | 36.30 (37.03)          | 41.57 (40.13)        | 25.53 (30.33)    |
| LFDwAC-7  | 46.30 (42.86)          | 48.63 (44.19)        | 52.88 (46.63)    |
| LFDwAC-8  | 21.85 (27.86)          | 40.00 (39.19)        | 37.33 (37.65)    |
| LFDwAC-9  | 2.59 (9.22)            | 46.67 (43.07)        | 29.72 (33.02)    |
| LFDwAC-10 | 18.52 (25.48)          | 41.57 (40.13)        | 36.15 (36.94)    |
| LFDwAC-11 | 2.59 (9.22)            | 15.69 (23.30)        | 33.42 (35.30)    |
| LFBePa-12 | 14.81 (22.63)          | 36.86 (37.36)        | 42.72 (40.79)    |
| LFBePa-13 | 28.15 (32.03)          | 41.96 (40.35)        | 36.66 (37.24)    |
| LFBePa-14 | 1.85 (7.73)            | 38.82 (38.53)        | 28.29 (32.11)    |
| LFBeBu-15 | 1.11 (6.05)            | 30.59 (33.54)        | 31.70 (34.25)    |
| LFBeBu-16 | 27.04 (31.32)          | 40.78 (39.66)        | 29.21 (32.70)    |
| LFBeSi-17 | 2.59 (9.22)            | 40.78 (39.67)        | 16.99 (24.32)    |
| LFBeKh-18 | 3.70 (11.07)           | 38.43 (38.29)        | 29.66 (32.98)    |
| LFBeJa-19 | 4.07 (11.62)           | 40.78 (39.67)        | 28.44 (32.20)    |
| LFBeAv-20 | 2.96 (9.87)            | 41.96 (40.36)        | 28.40 (32.19)    |
| LFBeAV-21 | 2.96 (9.87)            | 45.88 (42.62)        | 51.95 (46.10)    |
| LFDwHe-22 | 1.85 (7.73)            | 40.39 (39.44)        | 32.51 (34.74)    |
| LFDwSo-23 | 5.93 (14.07)           | 39.61 (38.99)        | 22.22 (28.08)    |
| LFDwSo-24 | 2.59 (9.22)            | 38.43 (38.30)        | 20.10 (26.62)    |
| LFDwBi-25 | 2.22 (8.57)            | 39.61 (38.98)        | 16.57 (24.01)    |
| LFBaBa-26 | 34.81 (36.14)          | 46.67 (43.07)        | 52.67 (46.51)    |
| LFBaCh-27 | 12.96 (21.09)          | 33.73 (35.48)        | 13.10 (21.20)    |
| LFBaCh-28 | 34.81 (36.14)          | 34.12 (35.72)        | 24.82 (29.85)    |
| S.Em. ±   | 0.46                   | 0.96                 | 0.62             |
| C.D. (1%)  | 1.73                   | 3.61                 | 2.33             |
| C.V.       | 4.17                   | 4.49                 | 3.11             |

*Arc sine values
Table 2 *In vitro* evaluation of groundnut stem fungal endophytes against *Sclerotium rolfsii*, *Rhizoctonia solani* and *Puccinia arachidis*

| Endophyte  | Mycelial growth | Spore germination |
|------------|-----------------|-------------------|
|            | *S. rolfsii*    | *R. solani*       | *P. arachidis*       |
|            | Per cent inhibition |                |                    |
| SFDwAC-2  | 39.26 (38.70)*  | 43.33 (41.15)    | 28.74 (32.40)*      |
| SFDwAC-3  | 33.33 (35.24)   | 41.48 (40.07)    | 3.94 (10.48)        |
| SFDwAC-4  | 48.15 (43.88)   | 35.93 (36.81)    | 36.32 (37.04)       |
| SFDwAC-5  | 39.63 (38.97)   | 41.48 (40.08)    | 30.42 (33.45)       |
| SFDwAC-7  | 31.85 (34.34)   | 48.15 (43.92)    | 49.40 (44.64)       |
| SFDwAC-8  | 41.11 (39.81)   | 49.63 (44.77)    | 47.15 (43.34)       |
| SFDwAC-9  | 37.04 (37.46)   | 37.41 (37.69)    | 28.61 (32.31)       |
| SFDwAC-10 | 29.26 (32.73)   | 37.04 (37.47)    | 25.81 (30.52)       |
| SFDwAC-11 | 54.44 (47.55)   | 37.41 (37.69)    | 55.18 (47.96)       |
| SFDwAC-12 | 39.63 (38.91)   | 38.15 (38.13)    | 35.36 (36.47)       |
| SFDwAC-13 | 41.48 (40.06)   | 39.63 (39.00)    | 40.37 (39.43)       |
| SFDwAC-14 | 36.67 (37.25)   | 32.22 (34.57)    | 14.89 (22.64)       |
| SFDwAC-15 | 41.48 (40.04)   | 22.22 (28.11)    | 35.53 (36.56)       |
| SFBPa-16  | 43.70 (41.31)   | 35.93 (36.81)    | 31.88 (34.36)       |
| SFBPa-17  | 51.48 (45.83)   | 38.89 (38.55)    | 48.65 (44.21)       |
| SFBBu-18  | 62.59 (52.28)   | 35.65 (36.58)    | 56.91 (48.96)       |
| SFBBu-19  | 45.19 (42.18)   | 38.52 (38.35)    | 33.03 (35.04)       |
| SFBSi-20  | 39.26 (38.75)   | 44.44 (41.79)    | 36.74 (37.28)       |
| SFBSi-21  | 33.70 (35.47)   | 33.33 (35.25)    | 33.69 (35.46)       |
| SFBKh-22  | 34.44 (35.92)   | 27.04 (31.30)    | 45.75 (42.54)       |
| SFBKh-23  | 37.04 (37.47)   | 37.04 (37.47)    | 26.64 (31.05)       |
| SFBKh-24  | 35.19 (36.37)   | 25.56 (30.33)    | 40.17 (39.31)       |
| SFBJa-25  | 41.11 (39.86)   | 36.30 (37.03)    | 13.67 (21.62)       |
| SFBAv-26  | 35.93 (36.80)   | 30.37 (33.42)    | 42.55 (40.69)       |
| SFBAv-27  | 33.70 (35.47)   | 27.04 (31.30)    | 20.15 (26.66)       |
| SFBAv-28  | 33.33 (35.25)   | 33.33 (35.25)    | 44.81 (42.00)       |
| SFdSo-29  | 40.37 (39.42)   | 39.63 (39.00)    | 33.12 (35.12)       |
| SFdSo-30  | 34.81 (36.14)   | 43.33 (41.15)    | 36.36 (37.05)       |
| SFdKa-31  | 35.93 (36.81)   | 28.15 (32.01)    | 30.18 (33.30)       |
| SFdBi-32  | 35.93 (36.80)   | 36.30 (37.03)    | 32.55 (34.77)       |
| SFdBi-33  | 37.78 (37.89)   | 47.78 (43.71)    | 51.84 (46.04)       |
| SFdUn-34  | 39.63 (39.00)   | 38.89 (38.56)    | 37.88 (37.97)       |
| SFBaCh-35 | 40.37 (39.43)   | 44.07 (41.57)    | 31.54 (34.14)       |
| S.Em. ±   | 1.81            | 0.70             | 1.02               |
| C.D. (1%) | 6.78            | 2.62             | 3.83               |
| C.V.      | 8.05            | 3.22             | 4.93               |

* *Arc sine values*
Table 3 In vitro evaluation of groundnut root fungal endophytes against *Sclerotium rolfsii*, *Rhizoctonia solani* and *Puccinia arachidis*

| Endophyte | Per cent inhibition of | Mycelial growth | Spore germination |
|-----------|------------------------|-----------------|-------------------|
|           |                        | *S. rolfsii*    | *R. solani*       | *P. arachidis* |
| RFDwAC-7  | 32.96 (35.02)*         | 44.71 (41.94)   | 6.35 (14.59)      |
| RFDwAC-8  | 34.44 (35.92)          | 48.24 (43.97)   | 17.71 (24.87)     |
| RFDwAC-9  | 30.37 (33.41)          | 46.27 (42.84)   | 20.17 (26.68)     |
| RFDwAC-15 | 31.48 (34.11)          | 35.69 (36.66)   | 28.59 (32.31)     |
| RFDwAC-16 | 35.93 (36.80)          | 42.75 (40.81)   | 32.90 (34.98)     |
| RFDwAC-17 | 32.59 (34.80)          | 41.96 (40.36)   | 47.07 (43.30)     |
| RFBePa-22 | 32.22 (34.57)          | 34.12 (35.73)   | 45.30 (42.28)     |
| RFBeJa-29 | 38.52 (38.35)          | 41.57 (40.13)   | 38.08 (38.09)     |
| RFBeJa-30 | 32.59 (34.80)          | 36.47 (37.13)   | 43.81 (41.42)     |
| RFBeAv-31 | 30.74 (33.66)          | 25.49 (30.30)   | 29.51 (32.89)     |
| RFBeAv-32 | 38.52 (38.35)          | 36.47 (37.12)   | 22.36 (28.20)     |
| RFDwSo-33 | 40.74 (39.64)          | 49.80 (44.87)   | 47.84 (43.74)     |
| RFDwSo-34 | 43.70 (41.37)          | 47.45 (43.52)   | 42.79 (40.78)     |
| RFDwSo-35 | 40.74 (39.65)          | 39.61 (38.99)   | 25.82 (30.50)     |
| RFDwUn-37 | 42.59 (40.72)          | 41.18 (39.90)   | 28.12 (30.83)     |
| RFBaCh-38 | 36.30 (37.03)          | 42.35 (40.58)   | 22.65 (28.41)     |
| RFBaCh-39 | 29.26 (32.73)          | 43.92 (41.49)   | 42.48 (40.66)     |
| RFHaBn-40 | 34.81 (36.14)          | 43.53 (41.27)   | 32.57 (34.71)     |
| RFHaBn-41 | 30.37 (33.43)          | 45.88 (42.62)   | 31.92 (34.39)     |
| RFHaBd-42 | 31.48 (34.12)          | 41.96 (40.35)   | 15.93 (23.50)     |
| RFHaBd-43 | 25.93 (30.59)          | 44.71 (41.94)   | 37.95 (38.01)     |
| S.Em. ±   | 0.50                   | 0.71            | 1.11              |
| C.D. (1%)  | 1.92                   | 2.72            | 4.23              |
| C.V.       | 2.43                   | 3.08            | 5.72              |

*Arc sine values
Plate 1 *In vitro* evaluation of groundnut leaf fungal endophytes against *S. rolfsii* and *R. solani* by dual culture method

**a) Sclerotium rolfsii**

**b) Rhizoctonia solani**
Plate 2 *In vitro* evaluation of groundnut stem fungal endophytes against *S. rolfsii* and *R. solani* by dual culture method

**a) Sclerotium rolfsii**

**b) Rhizoctonia solani**
Plate.3 *In vitro* evaluation of groundnut root fungal endophytes against *S. rolfsii* and *R. solani* by dual culture method

**a) Sclerotium rolfsii**

**b) Rhizoctonia solani**
Plate 4 In vitro evaluation of groundnut fungal endophytes against *P. arachidis* by spore germination technique

a) LFDCwAC-7

b) SFBeBu-18

c) RFDwSo-34

d) Control
Against *P. arachidis*, the endophyte SFBeBu-18 (56.91 %) recorded the maximum inhibition of uredospore germination which was on par with SFDwAC-11 (55.18 %). The endophyte SFDwAC-3 (3.94 %) recorded the least inhibition of uredospore germination as compared to other endophytes (Table 2 and Plate 2).

Among 21 root endophytes, the maximum mycelial inhibition against *S. rolfsii* was observed by the root endophyte RFDwSo-34 (43.70 %) which was on par with RFDwUn-37 (42.59 %), RFDwSo-33 (40.74 %) and RFDwSo-35 (40.74 %). The endophyte RFHaBd-43 (25.93 %) was less effective with the least mycelial inhibition. Against *R. solani*, the endophyte RFDwSo-33 (49.80 %) showed the maximum mycelial inhibition which was on par with RFDwAC-8 (48.24 %), RFDwSo-34 (47.45 %) and RFDwAC-9 (46.27 %). The endophyte RFBeAv-31 (25.49 %) showed the least mycelial inhibition and it was less effective as compared to other endophytes. Against *P. arachidis*, the endophyte RFDwSo-33 (47.84 %) recorded the maximum inhibition of uredospore germination and this was on par with RFDwAC-17 (47.07 %), RFBePa-22 (45.30 %) and RFBeJa-30 (43.81 %). The endophyte RFDwAC-7 (6.35 %) was recorded the least inhibition of uredospore germination as compared to other endophytes evaluated and the results are presented in Table 3 and Plate 3.

Endophytes could become better biocontrol agents as compared with rhizosphere microflora 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). 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 from fungal endophytes in dual culture.

In dual culture method among the 82 fungal endophytes, 10 endophytes (LFDwAC-7, LFBaBa-26, SFDwAC-7, SFDwAC-8, SFDwAC-11, SFBePa-17, SFBeBu-18, SFDwBi-33, RFDwSo-33 and RFDwSo-34) have shown maximum inhibition of mycelium/uredospore germination of all three pathogens. These endophytes were fast growing and were more effective against one and the other pathogens.

All these endophytes showed the clear inhibition zone and inhibition of uredospore germination, which may be due to the production of antimicrobial compounds from the endophytes.

The findings of the present study are in agreement with Durga Prasada (2008), Seema and Devaki (2012) and Ghewande (2008) who evaluated fungal endophytes against *S. rolfsii*, *R. solani* and *P. arachidis* respectively by employing dual culture method and spore germination test.

In dual culture method, the extent of inhibition of *S. rolfsii* and *R. solani* by fungal endophytes ranged from 1.11 to 62.59 per cent and 3.14 to 53.73 per cent, respectively. The inhibition of uredospore germination by fungal endophytes was ranged from 3.94 to 68.28 per cent. Among the fungal endophytes, SFBeBu-18 (62.59 %) showed the maximum mycelial inhibition of *S. rolfsii* followed by TSFE-7 (54.12 %) and TSFE-4 (53.73 %) showed the maximum mycelial inhibition of *R. solani* followed by TLFE-4 (53.33 %) and against *P. arachidis* TSFE-7 (68.28 %) showed maximum inhibition of uredospore germination followed by TLFE-7 (65.97 %).
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