Brazilian Journal of Biological Sciences, 2015, v. 2, n. 4, p. 221-233.
ISSN 2358-2731

Plant growth promoter and biocontrol mechanism of endophytic fungi *Botrytis* sp

G. Senthilmurugan@viji* and S. Sekar

PG and Research Department of Botany, Thiagarajar College (Autonomous), Theppakulam-Madurai, Tamilnadu State, India. *Email: viji.m6@gmail.com.

Abstract. In this study, an endophytic fungus *Botrytis* sp isolated from healthy aerial root of *Ficus benghalensis* (Indian Banyan). It was tested for antagonistic and volatile activity against plant pathogens (*Fusarium* sp and *Diplocarpon rosae*). Moreover we have analyzed that endophytic fungal culture filtrate for herbicidal activity, seed germination test, pot culture experiment and estimation of chlorophyll and protein. Endophytic fungi *Botrytis* sp has showed significant inhibition in antagonistic and volatile activity against *Diplocarpon rosae* but slight antagonism and volatile activity against *Fusarium* sp however, endophytic fungi secondary metabolites showed no wilt symptom in herbicidal activity against *Parthenium hysterophorous* weed. The seed germination test analysis of endophytic fungi culture filtrate was found to be efficient enhancing germination of *Sorghum bicolor* and *Vigna unguiculata* seeds. In pot culture experiment also, *Sorghum bicolor* plants were treated with endophytic fungi culture filtrate promoted growth, when compared to control and also showed higher chlorophyll content at 20% treatment and protein was found to be higher in all concentrations.

Keywords: Aerial root, *Botrytis* sp, Endophytic fungi, *Ficus benghalensis*.

Received
June 25, 2015

Accepted
August 13, 2015

Released
December 31, 2015

Open Acess
Full Text Article

Introduction

Nearly one million endophytic species present ubiquitously in all plants (Amirta et al., 2012). The fungal communities live inside the healthy tissue of medicinal plants which increases the absorption of soil nutrients and also change in nutrient cycle occurs (Krishnamoorthy et al., 2008). Endophytic fungi are biotechnological interest due to their potential use as genetic vectors, metabolites and biological control agents (Jalgonwalal et al., 2011). The equilibrium between the host and the fungi seems to be controlled in part by chemical factors, for example herbicidal natural products produced by the fungi versus antifungal metabolites synthesized by the host plants (Haung et al., 2001). The colonization of the endophyte may lead to the production of special compounds within the host plant (Haung et al., 2008). Fungi have been widely known as a source of bioactive compounds. An excellent example for this is the anticancer drug taxol, which was previously supposed to occur only in the plant tissues (Strobel and Daisy, 2003). Endophytes and their role in ecology and the characterization of their secondary metabolites were studied by Saikkonen et al. (2004) and Arnold (2007). A very few endophytes are known to produce volatile antibiotics for the control of plant diseases. *Muscodar albus* a newly described endophytic fungus from *Cinnamum zeylanicum* effectively inhabits and kills fungal and bacterial pathogens by producing the mixture of volatile compounds (Worpong et al., 2001). The endophytes assemblages of the leaf and the aerial root growing in the air and soil showed little overlap suggesting that the

nature of the host tissue as well as environment determine the endophyte of a host (Suryanarayanan and Vijaykrishna, 2001).

The present study was conducted to find out plant growth and biocontrol potential of endophytic fungi *Botrytis* sp against plant pathogens.

Materials and methods

Isolation of endophytic fungi

The plant sample was collected from Thiagarajar College, Madurai in sterile polythene bags and brought to the laboratory. After collected aerial root sample was washed thoroughly in running tab water and cut it into 1 cm segments. It was then surface sterilized with ethanol (70%) for 5 s and then in sodium hypochloride (0.5%) for 1 min and rinsed in sterile distilled water supplemented with tetracycline for 10 s (Krishnamoorthy and Hemalatha, 2003). The sterilized aerial root segment was placed aseptically on potato dextrose agar medium and incubated at room temperature for 5-7 days. The fungi appeared from the edges of the inoculated aerial root segment was isolated, identified and pure cultures were maintained on potato dextrose agar slant cultures.

Isolation of soil borne plant pathogen

Soil sample was collected from wilt symptom showing brinjal growing field at a depth of 5.08 cm (2”). Samples were collected by using sterile spatula in sterilized polythene bags.

Serial dilution plate technique

1 g of soil sample was aseptically transferred to 99 mL of sterile water taken in a conical flask. It was shaken well for about 15 min to release the spores and mycelium that adhere on the soil surface. From this soil wash, 1 mL was transferred aseptically with the help of sterile pipette to a test tube containing 9 mL of sterile distilled water to make the dilution 10^{-2} similarly; the serial dilutions were made upto 10^{-5} from the respective dilution, 1 mL of inoculums was aseptically transferred to sterile petriplates, sterilized and cooled potato dextrose agar medium was poured aseptically in to the plates. The plates were incubated at room temperature and were examined after 4 days for the development of fungal colonies.

Isolation of plant pathogen from infected rose leaves

Diseased leaf tissue of *Rosa indica* plant was surface sterilized in 70% ethanol and 0.1% sodium hypochloride solution for 1 min and rinsed in sterile distilled water for 10 s. The infected area was cut into small pieces and placed on the surface of a sterile potato dextrose agar media and incubated at room temperature for 4-5 days. The plates were examined for the growth of pathogenic organisms.

Pathogenecity of the isolated fungus

Healthy young leaves of *Rosa indica* plant were surface sterilized in 75% ethanol and 1% sodium hypochloride solution for 1 min and rinsed in sterile distilled water for 10 s. The sterilized leaves were placed in to a sterile petridishes containing moisturized filter paper. Then by using sterile inoculation needle fungal spores was removed from mother culture. It was placed on the leaf surface and incubated at room temperature for 4-5 days. After incubation leaves were observed for the symptom appearance.

Identification of the fungal pathogens

Based on their microscopic characters and their external morphology in the medium, they were identified as *Fusarium* sp, and *Diplocarpon rosae.*

Antagonistic activity - Dual culture method

The antagonistic activity between the endophytic fungi and isolated plant pathogens *Fusarium* sp and *Diplocarpon rosae* were tested following method suggested by Morton and Stroube (1955).

Seven day old grown cultures of pathogenic fungi *Fusarium* sp and *Diplocarpon rosae* were removed from the edges of the old colony aseptically by using 5 mm cork borer. These blocks were placed separately at one end of the petriplates containing potato dextrose agar medium.

5 mm diameter discs of the endophytic fungi isolated from *Ficus benghalensis* aerial root was removed from seven day old culture plates using a sterile cork borer (5 mm dia) and placed aseptically in the other end. Pathogen alone inoculated petriplates separately served as control. The plates were incubated at room temperature for 4-7 days. Three replicates were maintained for each treatment. After incubation the radial growths of pathogens were measured. The percentage of inhibition was calculated by the formula.

$$\text{Percentage of inhibition} = \frac{A1-A2}{A1} \times 100$$

Where:
A1 = Area covered by the pathogen in control.
A2 = Area covered by the pathogen in dual culture.

Volatile activity

The effect of volatile metabolites produced by isolated endophytic fungi was tested following method suggested by Dennis and Webstar (1971). Sterile potato dextrose agar plates were inoculated centrally with a disc of the isolated endophytic fungus (5mm dia) and incubated at 30 °C $\pm$ 2 °C for three days. After three days the lid of each plate was replaced by another same size sterilized bottom plate containing sterile medium inoculated with a disc of the pathogen. The two plates were wrapped by cellotape and incubated at room temperature for 4-5 days. Pathogen alone inoculated plates served as control. The volatile activities of endophytes were determined based on the growth of pathogen.

Herbicidal activity

This experiment was carried out by shoot-cut method of Vikrant et al. (2006). Different concentrations (1% - 5%) of endophytic filtrates were prepared and taken in different test tubes. Young *Parthenium hysterosporum* plant showing uniform growth was collected from field and brought to the laboratory in a polythene bag. The plants were cut at the bottom by placing them under water and the cut end was immersed in the fungal filtrates present in the test tube. Plants introduced in test tubes containing distilled water alone served as control. These tubes were incubated at 25 °C $\pm$ 1 °C. Plants were observed for 24 h for any visible change.

Seed germination test

Different concentration of culture filtrate (5%, 10%, 15%, and 20%) was prepared as described earlier. They were added into three sets of sterile petriplates containing filter paper. Uniform looking *Sorghum bicolor* seeds were washed with running water, and then they were surface sterilized with 1% mercuric chloride for 3-5 min. Then the seeds were washed with sterile distilled water. These seeds were placed in the petriplates (10 seeds/plate) containing different concentrations of culture filtrates. Plates with sterile distilled water alone served as control.

After 48 h, the number of seeds germinated in each plate was noted and the seed germination percentage was calculated by using formula:

$$\text{Seed germination \%} = (\text{No. of seeds germinated/Total no. of seeds}) \times 100.$$

After 4 days shoot and root length was measured.

Pot culture method

Preparation of pots. The plastic pots were washed with running water and sterilized with alcohol. Sand and red soil was mixed in the ratio 2:1 and it was sterilized by autoclaving at 120 lb pressures for 15 min at 121 °C and then the pots were filled with soil mixture.

Preparation of seeds. The surface sterilized *Sorghum bicolor* seeds were soaked in the culture filtrates (20%) for 24 h then, the treated seeds were sowed in the pots (20 seeds/pot) adequate watering was made for 15 days after germination to maintain the soil moisture. the plants were uprooted carefully, shoot and root lengths were measured. The fresh weights of the plants were noted immediately after harvest. Then, they were dried in hot air oven at 80°C for 12 h and weight was noted and this taken as dry weight.

Chlorophyll estimation

To find out the amount of photosynthetic pigments in treated plants, the following experiment was carried out as described by (Arnon, 1949).

50 mg of fresh leaf materials from the treated plants were ground with 80% acetone in a mortar and pestle. The extract was filtered through a muslin cloth and centrifuges at 5,000 rpm for 5 min to get a clean supernatant. The pellet was repeatedly extracted with 80% acetone, until it becomes colourless. The supernatant were cooled and made upto a final volume. The absorbance of the extract was measured at 645 nm and 665 nm against 80% acetone as blank. The chlorophyll content of the sample was calculated by using formula:

Chl a (mg/g) = (12.7 (A665)) – 2.69 (A645)/(1000 x W) x V

Chl b (mg/g) = (22.9 (A645)) – 4.68 (A665))/(1000 x W) x V

Total Chl (mg/g) = (20.2 (A645)) + 8.02 (A665))/(1000 x W) x V

Where:

V = Total volume of the Chl content
W = Weight of the leaves

Estimation of protein

The protein content of the treated plants was measured by Lowry et al. (1951) method. About 500 mg of fresh leaf material was taken in a mortar and pestle. To this, 15 mL of distilled water was added and ground well. The extract was centrifuged at 3,000 rpm for 10 min. The lower layer was discarded. To the supernatant equal volume of cold 5% TCA was added. It was left for 3 minutes in an ice bath. Test tubes were taken and centrifuged once again. The supernatant was discarded. The pellet was dissolved in 15 mL of 0.2 N NaOH. From this, 1 mL was taken and mixed with 4 mL of alkaline copper reagent. It was shaken and well, it was allowed to stand for 10 min at room temperature. Then 0.1 mL of distilled Folin's phenol reagent was added at 660 nm using by colorimeter. The amount of protein present in the plant materials were measured using a standard value.

Results

Isolation of Endophytic fungi

An endophytic fungus, which frequently appears on the medium, was isolated from the young healthy aerial root of *Ficus benghalensis* (L.). The isolated fungus was pure cultured and maintained on PDA medium in sland culture. Based on their colony morphology and microscopic spore characters, the fungus was identified as *Botrytis* sp belongs to the family sclerotiniaceae. It produced tree branching like conidiophores and conidia was dispersed after sporulation (Figure 1A, B).

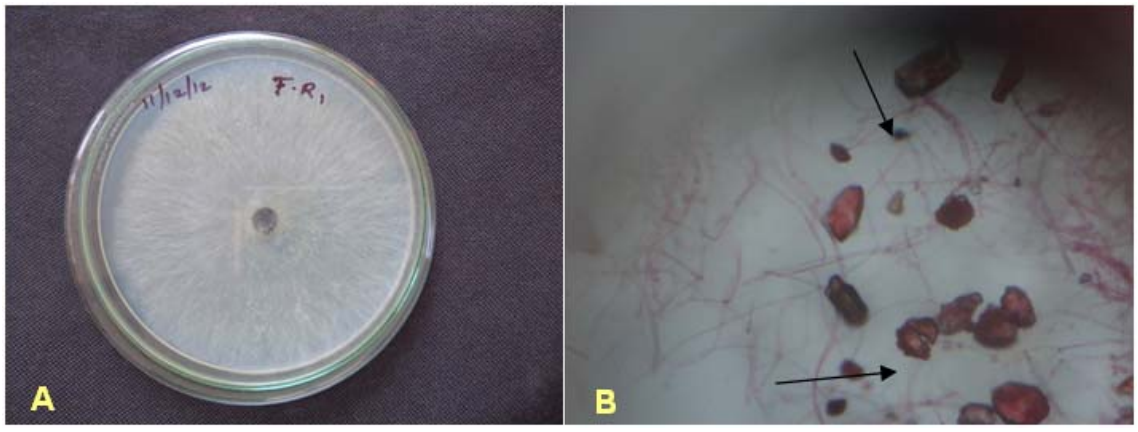

Figure 1. Endophytic fungi of *Botrytis* sp. (A); (B). Spores and hyphae.

Isolation of plant pathogens

i) A plant pathogen was isolated from pathogen infested brinjal field soil. Based on their microscopic spore characters and their external colony morphology, it was identified as *Fusarium* sp. It produced asexual microconidia spores like kidney shaped and aerial mycelia. It is a severe wilt disease causing soil pathogen to brinjal, tomato and legume plants (Figure 2).

Figure 2. *Fusarium* sp.

ii) A plant pathogen was isolated from infected rose leaves. Based on their microscopic spore characters and their external colony morphology, it was identified as *Diplocarpon rosae.* It produced ascospores. In Pathogenecity test, *Diplocarpon rosae* fungus was proved to be pathogenecity on the leaves of rose plant, because pathogen inoculated leaves were showed disease symptoms after 5 days. Disease symptoms were found as irregular brown spot initially appeared on the leaves (Figure 3).

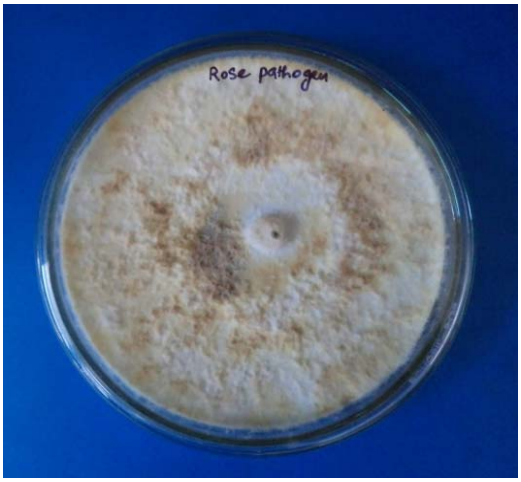

Figure 3. *Diplocarpon rosae.*

Antagonistic activity

a) Dual culture method. To find out the antagonistic ability of isolated endophytic fungi *Botrytis* sp, it was tested against plant pathogens of *Fusarium* sp and *Diplocarpon rosae* by dual culture method.

In antagonistic activity isolated endophytic fungi *Botrytis* sp showed significant inhibition against *Diplocarpon rosae* (1 cm) of rose plant when compare to control (10 cm) and endophytic fungi slight inhibition against wilt disease causing pathogen *Fusarium* sp of brinjal (7.4 cm). When compare to control (9 cm) (Figure 4, 5, 6, and 7).

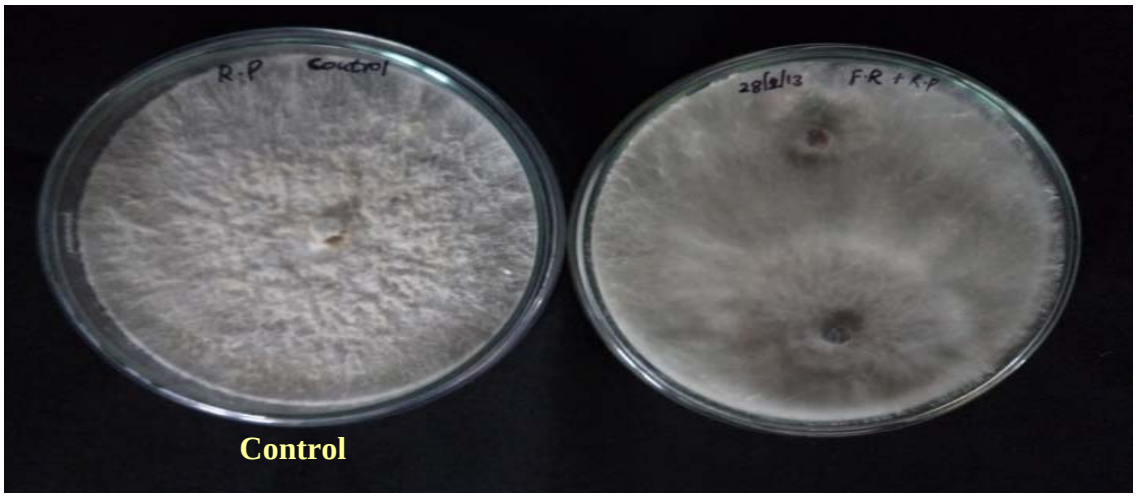

Figure 4. *Botrytis* sp antagonistic activity against *Diplocarpon rosae.*

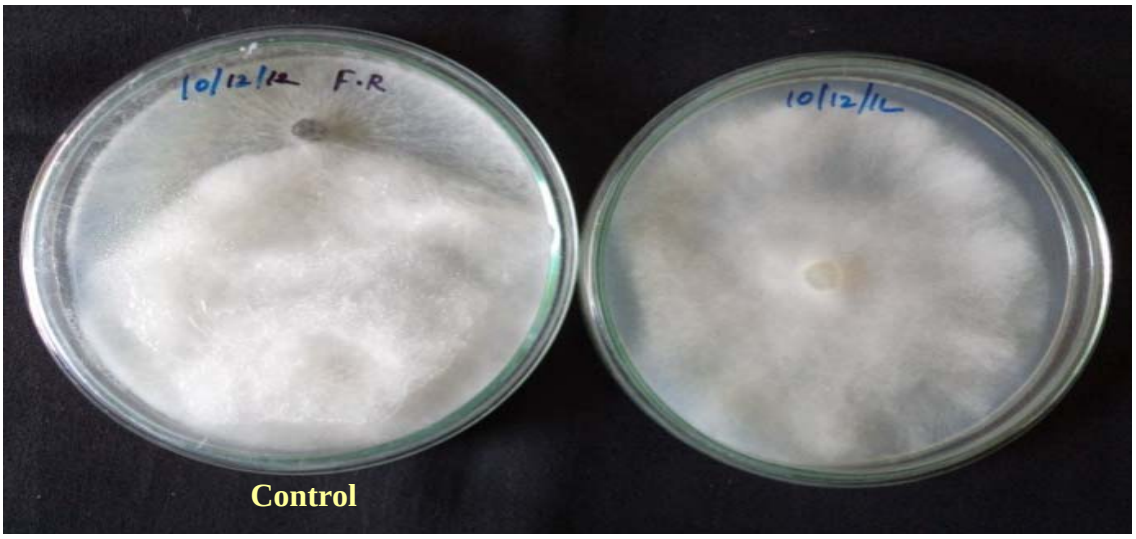

Figure 5. *Botrytis* sp antagonistic activity against *Fusarium* sp.

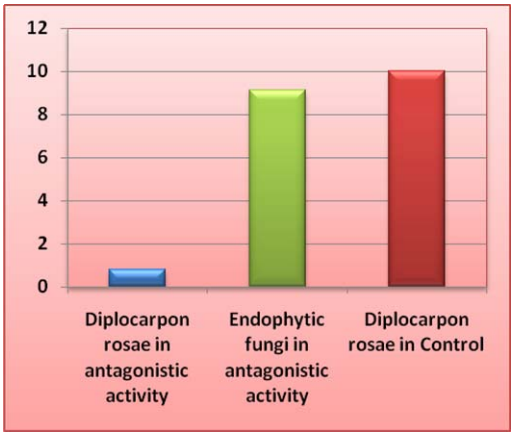

Figure 6. Endophytic fungi antagonistic activity against *Diplocarpon rosae.*

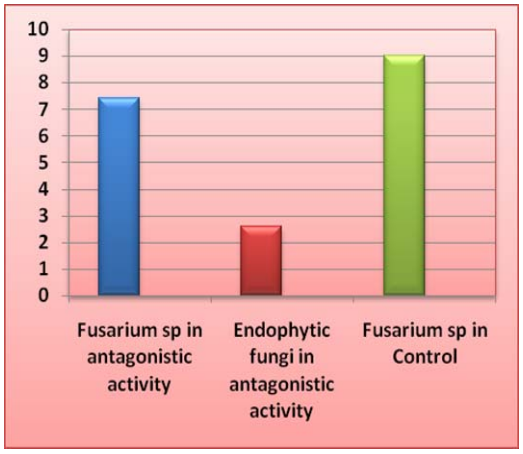

Figure 7. Endophytic fungi antagonistic activity against *Fusarium* sp.

Volatile activity

In volatile activity, endophytic fungi *Botrytis* sp showed positive inhibition against *Diplocarpon rosae* because on fourth day of incubation, endophytic fungi inhibited mycelia growth and sporulation of *Diplocarpon rosae* (2.1 cm). When compare to control (3.3 cm). It was determined endophytic fungus *Botrytis* sp was produced volatile compounds against *Diplocarpon rosae*. But endophytic fungi slight inhibition against *Fusarium* sp (8.2 cm), when compare to control (10 cm).

Herbicidal activity

The endophytic fungal *Botrytis* sp was tested for their herbicidal activity following shoot cut bioassay method. Secondary metabolites of *Botrytis* sp showed no herbicidal activity against *Parthenium hysterophorous* within 12 h, after treatment (Figure 8).

Growth promoting activity

To find out the effect of endophytic fungal metabolites on the germination of *Sorghum bicolor* and *Vigna unguiculata* seeds, seed germination test was carried out.

The endophytic fungal culture filtrate was found to be efficient in enhancing germination of *Sorghum bicolor* and *Vigna unguiculata* seeds. In sorghum seeds 80-90% germination was noticed at 5%, 10% and 15% treatments and 70% germination at 20% treatment seeds treated with water alone showed 70% of germination (Figure 9; Table 1).

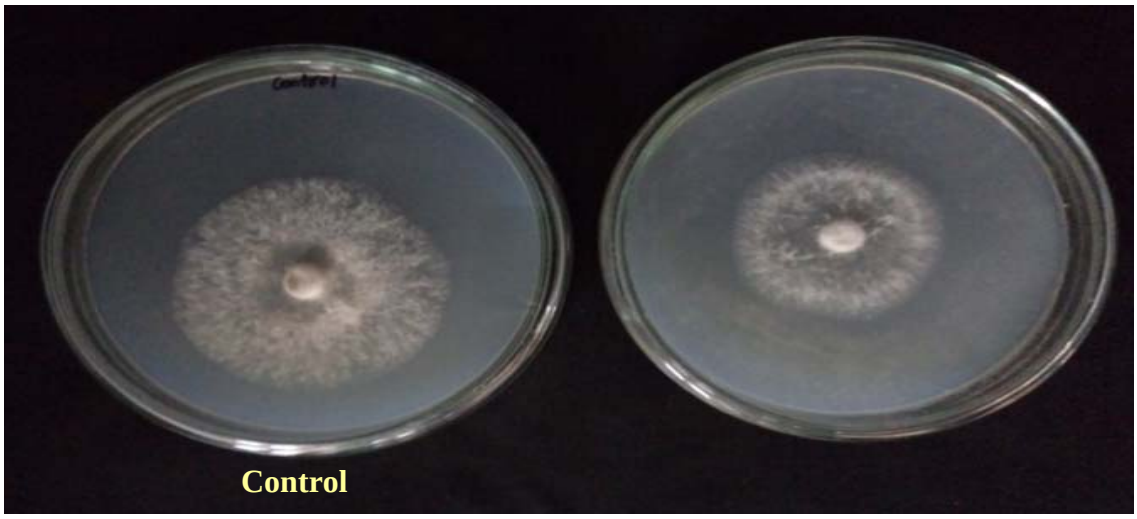

Figure 8. *Botrytis* sp volatile activity against *Diplocarpon rosae*.

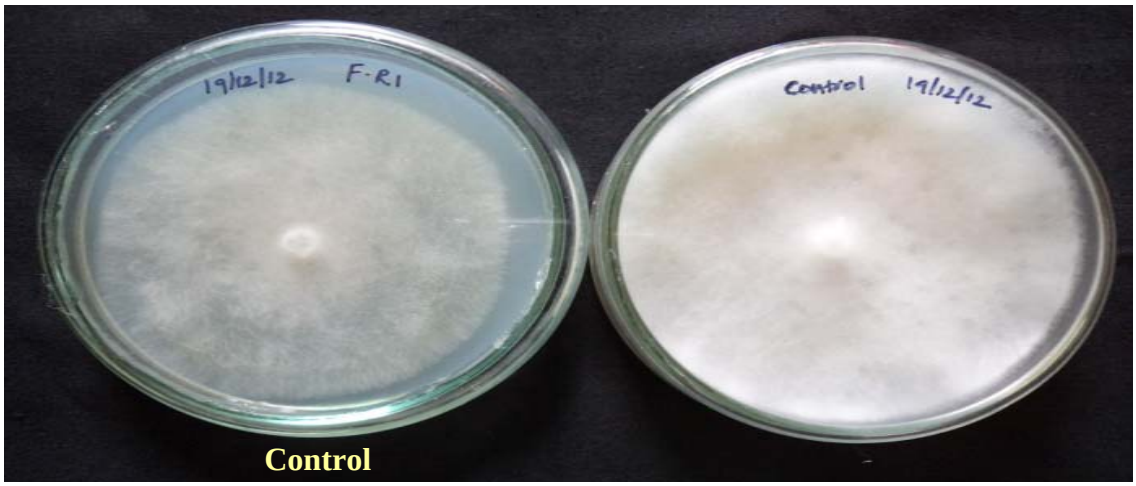

Figure 9. *Botrytis* sp volatile *activity against Fusarium sp.*

Table 1. Percentage of seed germination of S*orghum bicolor* at different concentrations of culture filtrate.

Endophytic fungi	Treatment				
	Water alone	Concentration of culture filtrates			
Botrytis sp		5%	10%	15%	20%
	7 (70%)	8 (80%)	9 (90%)	8 (80%)	7 (70%)

Vigna unguiculata seeds showed 90-100% germination was noticed at 5%, 10% and 15% treatments and 70% germination at 20% treatment. Seeds treated with water alone showed 90% of germination (Figure 9; Table 2).

After 4 days of treatment, the root length and shoot length was measured for the germinated seeds. Culture filtrate treated seeds produced significant increase in root and shoot length. In the case of *Sorghum bicolor*, maximum shoot length (1.5, 1.4 cm) was observed for the metabolite treatments at 5%, 10%, 15%, and 20% when compared to control (water alone 1.3 cm) (Table 3).

Maximum root length (1.9, 1.8, and 1.7 cm) was observed for the metabolite treatments at 5%, 10% and 20% and 1.6 cm root length at 15% treatment when compared to control (water alone 1.6 cm).

Table 2. Percentage of seed germination of *Vigna unguiculata* at different concentration of culture filtrate.

Endophytic fungi	Treatments				
	Water alone	Concentration of culture filtrates			
		5%	10%	15%	20%
Botrytis sp	9 (90%)	10 (100%)	9 (90%)	9 (90%)	7 (70%)

Table. 3. Shoot and root length of germinated *Sorghum bicolor* seeds (after 4 days) at different concentration of culture filtrates

Endophytic fungi	Parameter	Treatments (cm)				
		Water alone	Concentration of culture filtrates			
			5%	10%	15%	20%
Botrytis sp	S. L.	1.3	1.4	1.5	1.4	1.4
	R. L.	1.6	1.8	1.9	1.6	1.7

S. L. = Shoot length; R. L. = Root length.

Table. 4. Shoot and root length of germinated *Vigna unguiculata* seeds (after 4 days) at different concentration of culture filtrates.

Endophytic fungi	Parameter	Treatments (cm)				
		Water alone	Concentration of culture filatrates			
			5%	10%	15%	20%
Botrytis sp	S. L.	2.1	2.8	2.7	2.5	2.3
	R. L.	2.9	3.9	3.6	2.7	2.6

S. L. = Shoot length; R. L. = Root length.

For cow pea, treated seeds showed maximum shoot lengths (28, 2.7, and 2.5 cm) for the metabolite treatments at 5%, 10% and 15%, when compared to control (water 2.1) (Table 4).

Maximum root length (3.9, 3.6 cm) was observed for the metabolite treatments at 5% and 10%, when compared to control (water 2.9 cm). Minimum root length (2.7, 2.6 cm) was noticed at 15% and 20% treatments.

Pot culture method

Sorghum bicolor plants treated with endophytic culture filtrate showed significant growth when compared to control. Maximum increase in fresh weight and dry weight was observed in the plants treated with the culture filtrate of *Botrytis* sp. The root and shoot length was also found to be more in the culture filtrate treatment when compared to control (Figure 10; Table 5, 6).

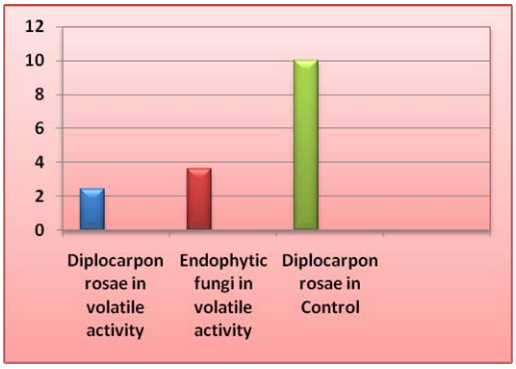

Figure 10. Endophytic fungi volatile activity against *Diplocarpon rosae*.

Endophytic fungi culture filtrate treated *Sorghum bicolor* plants showed higher chlorophyll content at 20% treatment. At 10% and 30% treatments chlorophyll amount is low. The amount of protein was found to be higher in all concentrations (10%, 20%, and 30%). When compared to control (0.30) (Table 7).

Table 5. Biomass in endophytic fungal treated *Sorghum bicolor* plants.

Nature of seed	Treatments (seed treated with culture filtrate)	Fresh weight (g)			Dry weight (g)		
Sorghum bicolor	Control	0.56			0.18		
	Botrytis sp	10%	20%	30%	10%	20%	30%
		1.38	1.88	1.59	0.28	0.36	0.30

Table 6. Growth parameters in endophytic fungal treated *Sorghum bicolor* plants.

Nature of seed	Treatment (seed treated with culture filtrate)	Shoot length (cm)			Root length (cm)		
Sorghum bicolor	Control	26.9			9.4		
	Botrytis sp	10%	20%	30%	10%	20%	30%
		57.5	58.1	57	12.6	16.2	15.2

Table 7. Estimation of photosynthetic pigments and protein in endophytic fungal treated *Sorghum bicolor* plants.

Nature of seed	Treatment (seed treated with culture filtrate)	Chl. a (mg/g)			Chl. b (mg/g)			Total Chl. (mg/g)			Protein µg/g		
Sorghum bicolor	Control	0.00018			0.00015			0.00033			0.38		
	Botrytis sp	10%	20%	30%	10%	20%	30%	10%	20%	30%	10%	20%	30%
		0.00032	0.00064	0.00027	0.00057	0.0011	0.00043	0.00084	0.00076	0.00072	0.44	0.70	0.63

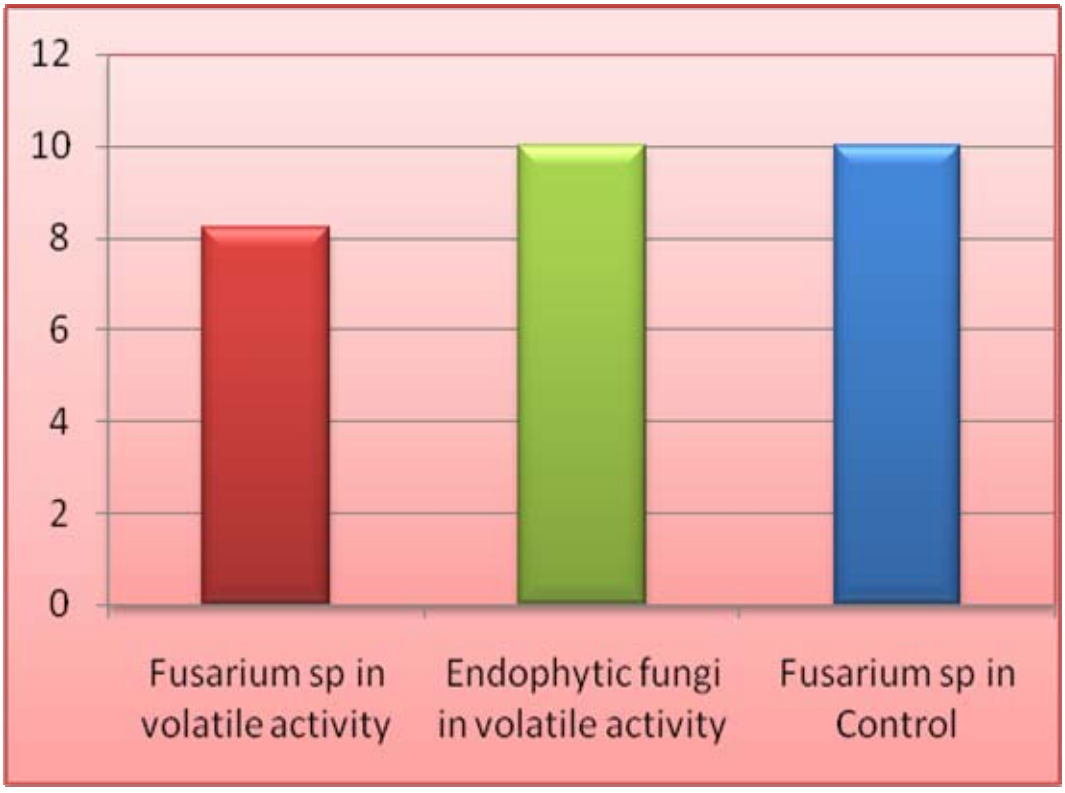

Figure 11. Endophytic fungi volatile activity against *Fusarium* sp.

Figure 12. Herbicidal activity of endophytic fungi.

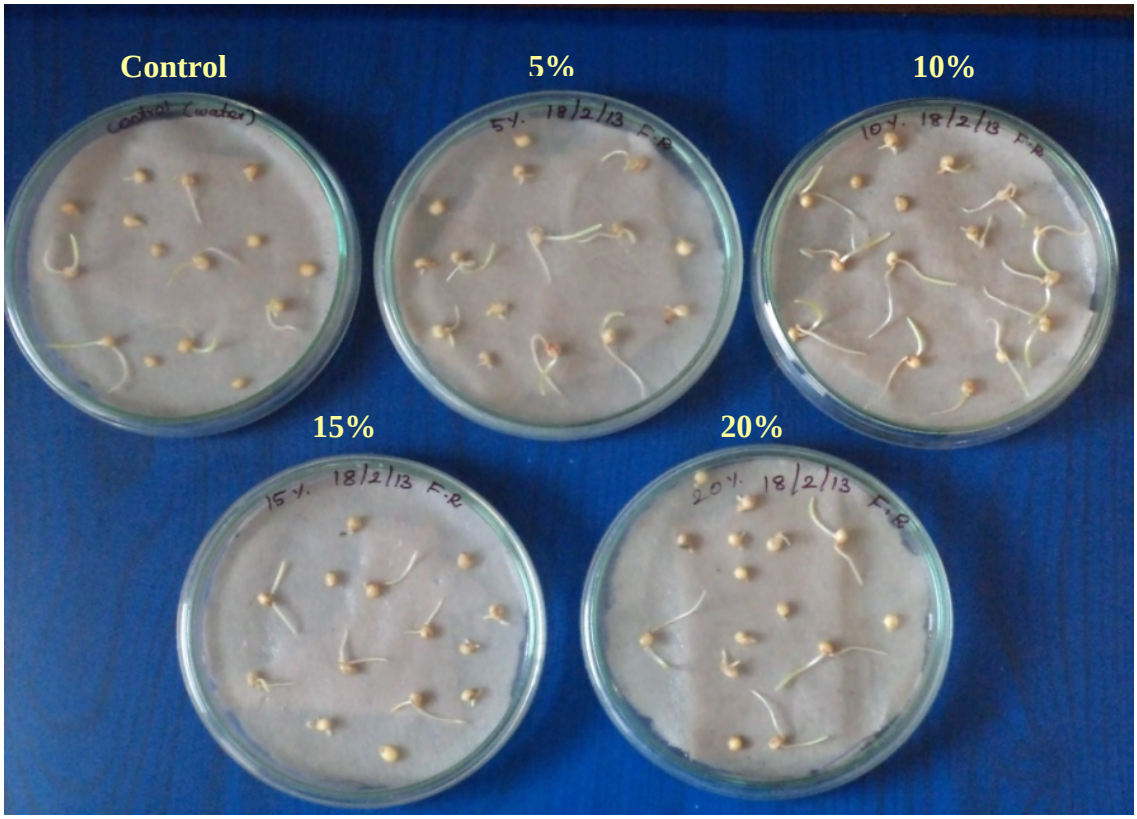

Figure 13. Effect of endophytic fungal metabolites on seed germination of *Sorghum bicolor* seeds.

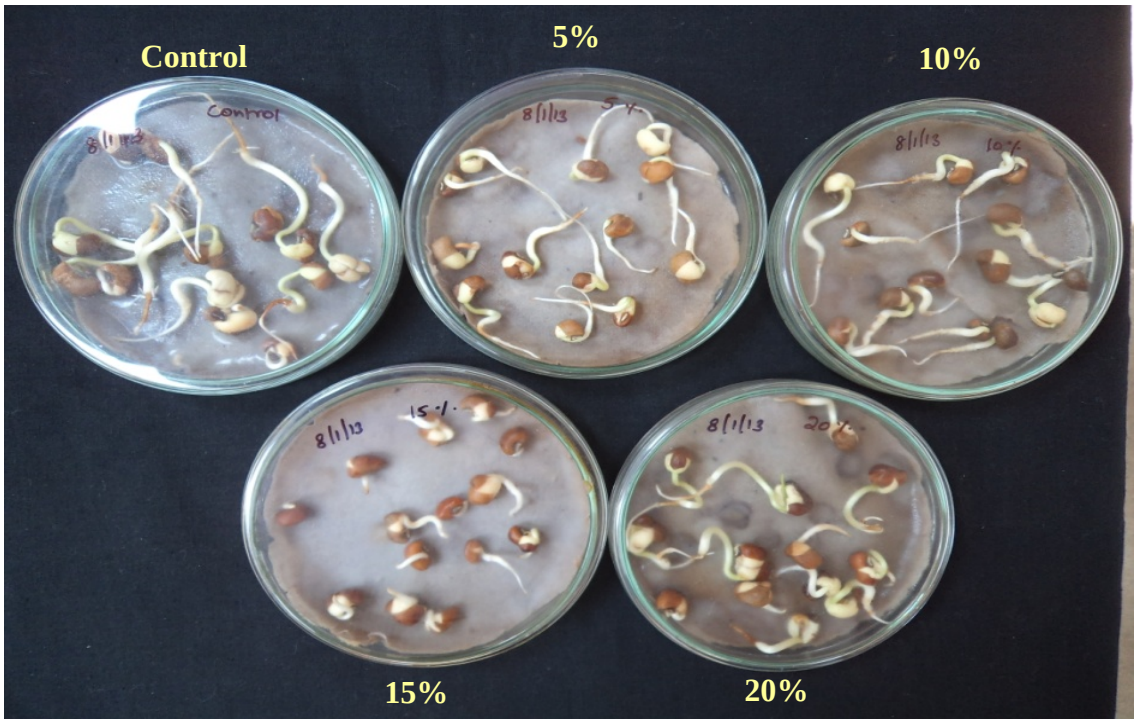

Figure 14. Effect of endophytic fungal metabolites on seed germination of *Vigna unguiculata* seeds.

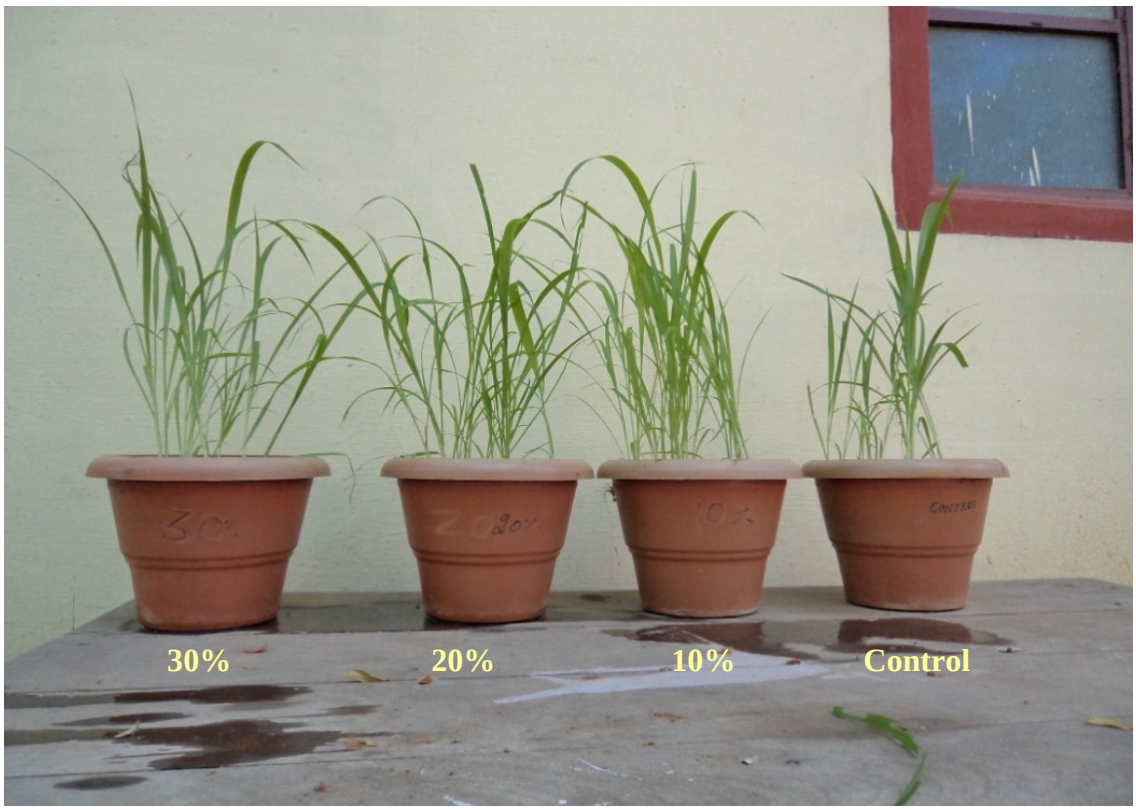

Figure 15. Pot culture experiment growth promoting activity of endophytic fungi on *Sorghum bicolor* plants.

Discussion

In this study, an endophytic fungus was isolated from aerial root of *Ficus benghalensis* (Indian banyan). Our results correlate to this, Mitosporic fungi and several sterile forms were isolated as endophytes from the leaf tissues and aerial

roots of *Ficus benghalensis* (Moraceae). The endophytic fungi more densely colonized the petiole, the species composition and colonization frequency of the endophytes were more for aerial roots entering the soil, when compared those growing in the air since the roots recruited some endophytes from the soil. The endophytes assemblages of the leaf and the aerial root growing in the air and soil showed little overlap suggesting that the nature of the host tissue as well as environment determine the endophyte of a host.

In antagonistic activity, endophytic fungi *Botrytis* sp inhibited mycelia growth and sporulation of *Diplocarpon rosae*. Our results similar to this, antagonistic isolates showed different inhibition rates when confronted with *R. solani*. The highest inhibition rate was observed with *Trichoderma atroviride*, followed by *Phomopsis* sp, *A. longipes*, and *Epicoccum nigrum* (Lahlali and Hijri, 2010).

In volatile activity, endophytic fungi *Botrytis* sp produced volatile compounds against *Diplocarpon rosae* but endophytic fungi slight volatile activity against *Fusarium* sp. As same to this, all antagonistic fungal isolates is capable of producing volatile compounds when grown on PDA media shows a significant difference between various antagonist isolates. The highest inhibition was recorded by *Trichoderma atrovirde* (81.81%), followed by *Phomopsis* sp. (38.63%), *A. longipes* (21.02%), and *E. nigrum* E18 (20.73%), E1 (11.36%), and E8 (10.22%), respectively (Rachid and Mohamed, 2010).

The seed germination test analysis of endophytic fungi *Botrytis* sp culture filtrate was found to be efficient enhancing germination of *Sorghum bicolor* and *Vigna unguiculata* seeds. In pot culture experiment, *Sorghum bicolor* plants were treated with endophytic fungi culture filtrate promoted plant height and shoot length, when compared to control. Our results similar to this, 101endophytic fungal isolates (82.7%) promoted plant height and shoot length of Waito-C rice, major proportion of endophytic fungi inhabiting sand dune plants produce metabolites, which are helpful in plant growth and development (Khan et al., 2012).

Conclusion

Endophytic fungi *Botrytis* sp has biocontrol potential and may be produced some growth hormones in the culture filtrate. So it provides a base for further investigation. In future we will analyze endophytic fungi culture filtrate by analytical techniques (thin layer chromatography, column chromatography, high performance liquid chromatography, fourier transmission infra red and gas chromatography-mass spectroscopy). After isolation and purification process of fractions will be tested for biofertilizer and biocontrol agent against plant pathogens in field.

Conflict of interest statement

Authors declare that they have no conflict of interests.

References

Amirita, A.; Sindhu, P.; Swetha, J.; Vasanthi, N. S.; Kannan, K. P. Enumeration of endophytic fungi from medicinal plants and screening of extracellular enzymes. **World Journal of Science and Technology**, v. 2, n. 2, p. 13-19, 2012.

Arnold, A. E. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. **Fungal Biology Reviews**, v. 21, p. 51-66, 2007.

Arnon, D. I. Copper enzymes in isolated choloroplasts, polyphenol oxidase in *Beta vulgaris*. **Plant. Physiol.**, v. 24, p. 4-15, 1949.

Dennis, C.; Webstar, J. Antagonistic properties of species group of *Trichoderma*. II. Production of volatile antibiotics. **Trans. Brit. Mycol. Soc.**, v. 57, p. 41-48, 1971.

Huang, Y.; Wang, J.; Li, G.; Zheng, Z.; Su, W. Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants *Taxus mairei*, *Cephalotaxus fortune* and *Torreya grandis*. **FEMS Immunol. Med. Microbiol.**, v. 31, p. 163-167, 2001.

Huang, W. Y.; Cai, Y. Z.; Hyde, K. D.; Corke, H.; Sun, M. Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. **Fungal Diversity**, v. 33, p. 61-75, 2008.

Jalgaonwalal, R. E.; Mohite, B. V., Mahajan, R. T. Evaluation of endophytes for their antimicrobial activity. **Afr. J. Biotechnol.**, v. 10, n. 1, p. 103-107, 2011.

Khan, S. A.; Hamayun, M.; Khan, A. L.; Lee, I.-J.; Shinwari, Z. K.; Kim, J.-G. Isolation of plant growth promoting endophytic fungi from dicots inhabiting costal sand dunes of Korea. **Pak. J. Bot.**, v. 44, n 4, p. 1453-1460, 2012.

Krishnamurthy, Y. L.; Hemalatha, T. V. Isolation of endophytic fungi from some grasses. **J. Mycol. Plt. Pathol.**, v. 33, n. 2, p. 305-306, 2003.

Krishnamurthy, Y. L.; Naik, S. B.; Jayaram, S. Fungal communities in herbaceous medicinal plants from the Malnad Region', Southern India. **Microbes Environ.**, v. 23, p. 24-28, 2008.

Lahlali, R.; Hijri., M. Screening, identification and evaluation of potential biocontrol fungal endophytes against *Rhizoctonia solani* AG3 on potato plants. **FEMS Microbiol. Lett.**, v. 311, n. 2, p. 152-159, 2010.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with folin phenol reagent. **J. Bio. Chem.**, v. 193, p. 265-275, 1951.

Morton, D. T.; Strobe, W. H. Antagonistic and stimulatory effects of soil microorganism upon *Sclerotium*. **Phytopatho.**, v. 45, p. 417-420, 1995.

Saikkonen, K.; Wali, P.; Helander, M.; Faeth, S. H. Evolution of endophyte-plant symbioses. **Trends in Plant Science**, v. 9, p. 275-280, 2004.

Strobel, G.; Daisy, B. Bioprospecting for microbial endophytes and their natural products. **Microbiol. Mol. Biol. Rev.**, v. 67, p. 491-502, 2003.

Suryanarayanan, T. S.; Vijayakrishna, D. Fungal endophytes of aerial roots of *Ficus benghalensis*. **Fungal Diversity**, v. 8, p. 155-161, 2001.

Vikrant, P.; Verma, K. K.; Rajak, R. C.; Pandey, A. K. Characterization of a phytotoxin from *Phoma herbarum* for management of *Parthenium hysterophorus* L. **J. Phytol.**, v. 154, p. 461-468, 2006.

Worpong, J.; Strobel, G. A.; Ford, E. J.; Li, J. Y., Baird, G.; Hess, W. M. *Muscodor albus* gen. et sp. nov., an endophyte from *Cinnamomum zeylanicum*. **Mycotaxon**, v. 79, p. 67-79, 2001.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.