Int.J.Curr.Microbiol.App.Sci (2018) *7*(4): 3653-3663

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 04 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.704.411

Effect of Rice Fungal Endophytes on Seed Germination and Seedling Growth of Rice

Lalngaihawmi[1*], S. Banik[2], P. Chakruno[2] and Khatemenla[3]

[1]Department of Plant Pathology, Assam Agricultural University, Jorhat (785013), Assam, India
[2]Department of Plant Pathology, School of Agricultural Sciences and Rural Development (SASRD), Nagaland University, Medziphema Campus (797106), Nagaland, India
[3]Department of Horticulture, Assam Agricultural University, Jorhat (785013), Assam, India

**Corresponding author*

Keywords

Endophytes, Potato dextrose broth, Culture filtrate, Blotter method

Article Info

Accepted:
30 March 2018
Available Online:
10 April 2018

A B S T R A C T

An investigation was carried out to study the effect of rice fungal endophytes on the seed germination and seedling growth of rice at the department of Plant Pathology, SASRD, Medziphema campus, Nagaland. Culture filtrate of fourteen fungal endophytes *viz. Cladosporium cladosporioides, Penicillium citrinum, Fusarium moniliforme, Trichoderma asperellum, Penicillium pinophilum, Aspergillus niger, Aspergillus flavus, Drechslera specifera, Penicillium oxalicum, Geotrichum candidum, Curvularia lunata, Aspergillus amstelodami, Talaromyces* sp., and *Chaetomium ochraceum* were tested at different concentration (25, 50 and 100%) at different dipping periods (15, 30 and 60 minutes). The culture filtrates of the endophytes were prepared by growing the endophytes in Potato Dextrose Broth. Blotter method was used for testing the germination of seeds. The maximum per cent germination of rice seeds was recorded with the endophyte *P. citrinum* (96.65) when dipped for 15 and 60 minutes at 50 and 25% concentration respectively. The maximum shoot length was recorded with seeds treated with *C. cladosporioides* (13.74 mm) when dipped for 30 minutes at 100% concentration as compared to 6.74 mm shoot length recorded in control experiments. The maximum root length was recorded with seeds treated with *A. amstelodami* (42.59 mm) when dipped for 60 minutes at 100% concentration of culture filtrate as compared to control (41.19 mm).

Introduction

Endophytes are microorganisms living within plant tissues for all or part of their life cycle without causing any visible symptoms of their presence are defined as endophytes (Wilson, 1993; Saikkonen *et al.,* 2004a). Endophytes inhabit majority of healthy and symptomless plants, in various tissues, seeds, roots, stems and leaves (Johri, 2006). Plants benefit extensively by harbouring these endophytic microbes; they promote plant growth (Compant *et al.,* 2005) and confer enhanced resistance to various pathogens (Clay and Schardl, 2002; Höflich, 2000; Arnold *et al.,* 2003) by producing antibiotics (Ezra *et al.,* 2004). Endophytes also produce unusual secondary metabolites of plant importance

(Taechowisan *et al.,* 2005). The presence of fungal endophytes can cause higher rates of water loss in leaves (Anonymous, 2009). However, certain fungal endophytes help plant to survive drought and heat. Fungal endophyte-related host benefits are common phenomena, and have been focus of much research, particularly among the grass endophytes (Don, 2011). Endophytic fungi are very common and are well diversified. Every plant species is found to have at least host one fungal endophytes, but usually asymptomatic and sometimes systemically (Faeth and Fagan, 2002).

The possibility that plant associated microbial diversity is influenced by the diversity of plant species and environmental factors suggest a greater potential for harvesting unique potential secondary metabolites from endophytic microorganisms found in association with hitherto unexploited floristically diverse plant communities (Gunatilaka, 2006). Faeth and co-workers have recently isolated and partially identified over 400 endophytic fungal taxa from Arizona fescue (Schulthess and Faeth, 1998), more than 40 species of endophytic fungi from Emory oak (Faeth and Hammon, 1997) and 22 endophyte species from cacti (Suryanarayanan *et al.*,2005) growing in very dry regions of Arizona. It is noteworthy that endophytically established fungi, such as *Hypoxylon* spp. undergo active mycelial development in response to water stress in host organs (Chapela, 1989). Even endophytes in agronomic grasses grown under mesic conditions produce metabolites that increase resistance to drought stress (Clay and Holah, 1999; Bush *et al.,* 1997). Endophyti fungi *Phomopsis liquidambari* can establish a mutualistic symbiotic relationship with rice which in turn promotes the growth and yield of the host plant and reduces the amount of nitrogen (N) fertilizer required for plant growth (Bo *et al.,* 2014). Extracts of plant endophytes have also been found to have significant effect on the germination potential, vigour index of rice seeds seedling dry weight, fresh weight, root length, root dry weight, and the number of tillering (Na *et al.,* 2014). Understanding the need to exploit the potential and identification of endophytic fungi on the growth and development of rice this investigation was carried out.

Materials and Methods

The investigation was carried out in the Department of Plant Pathology, SASRD, Nagaland University, Medziphema Campus, Nagaland. Local cultivar of rice, Kemenya was used for this investigation.

Isolation and identification of fungal endophyte

The rice plant was surfaced sterilized by sequential emersion in 70% ethanol for 5 minutes and Sodium hypochloride (0.9% available chlorine) for 20 minutes (Tian *et al.,* 2004). Different plant parts *viz.* leaves, shoots and roots were cut into fragment of 1 cm size. After which they were placed on PDA (Potato Dextrose Agar- distilled water 1000ml, potato 200g, dextrose 20g, agar agar 20 g) plates, each plates containing 10 numbers of fragment and were incubated at 25^{o}C for 2-3 days. The fungal colonies thus obtained were further purified and maintained in PDA slants. Identification of all the fungal endophytes isolated in this investigation was made by ITCC (Indian Type Cultural Collection), Division of Plant Pathology IARI (Indian Agricultural Research Institute), New Delhi – 110012, basing on their cultural and morphological characteristics.

Preparation of culture filtrate

The culture filtrates of the endophytes were prepared by growing the endophytes in Potato

Dextrose Broth (PDB: Peeled Potato 200g, Dextrose 20g and Distilled water 1000ml) in aseptic condition and were incubated at 25°C. The mycelium of the fungus was discarded once it covered up the entire broth. The broth was then filtered with the help of sterilized Whatman filter paper no.44 to extrude (100% concentration) the culture filtrate. The culture filtrates were later used for testing the effectiveness of the fungal endophytes on the rice plant.

Seed germination and seedling growth

Blotter method was used for testing the germination of seeds, where 30 numbers of seeds were treated with the culture filtrate of each fungal endophyte at different concentrations *viz.* 25, 50 and 100% and for different dipping periods i.e. 15, 30 and 60 minutes under each concentration.

Each of the treatment was maintained in three replications of 10 seeds each. Control plates were also maintained by dipping the seeds in sterile distilled water for comparison. The treated seeds along with the seeds dipped in sterile water (control) were then placed separately in moist blotter inside Petri plate and incubated at room temperature 28±2°C. The blotters were periodically re-wetted to prevent drying. Seed germination was determined on the fifth day from the treatment of the rice seeds with the endophytes and the number of germinated and un-germinated seeds were recorded and per cent germination of the seeds were calculated using an appropriate formula:

$$\text{Per cent germination} = \frac{\text{No. of seeds germinated}}{\text{Total number of seeds}} \times 100$$

On the fifth day after the treatment of the seeds with the culture filtrate of the endophytes. The length of the shoots as well as the length of the roots of each germinated seeds were measured separately and were recorded. Measurements of the seedlings from the control plates were also recorded.

Statistical analysis

Significance of different sources of variance was tested by Error mean square using Snedor's 'F' test of probability at 5 % level of significance. The percentage values were transformed into corresponding values by Arc sine and square root transformation.

Results and Discussion

Altogether 14 numbers of fungal endophytes of rice were isolated and identified in this investigation *viz. Cladosporium cladosporioides, Penicillium citrinum, Fusarium moniliforme, Trichoderma asperellum, Penicillium pinophilum, Aspergillus niger, Aspergillus flavus, Drechslera specifera, Penicillium oxalicum, Geotrichum candidum, Curvularia lunata, Aspergillus amstelodami, Talaromyces* sp., and *Chaetomium ochraceum.*

Effect of culture filtrate of fungal endophytes of rice on rice seed germination (%) in vitro

Significant influence on the germination percentage of rice seeds were observed for different concentration of culture filtrate of fungal endophytes *viz.* 25, 50 and 100% at three different dipping periods *viz.* 15, 30 and 60 minutes as depicted in Table 1. The maximum per cent germination of rice seeds was recorded with the endophyte *P. citrinum* (96.65) when dipped for 15 and 60 minutes at 50 and 25% concentration respectively and *P. pinophillum* when dipped for 30 minutes at 50% concentration as compared to 86.67% germination recorded in control experiments. The least percent germination of rice seeds was recorded with the endophyte *P. oxalicum*

(70) at 50 and 100% concentration of culture filtrate dipped for 60 minutes each and *T. asperellum* at 100% concentration dipped for 15 minutes. The highest mean percent germination was recorded in the rice seeds dipped in culture filtrate of *A. niger* (88.52) and the least mean percent germination was recorded in the rice seeds dipped in that of *P. oxalicum* (75.93) as compared to control that recorded 85.56% germination. Whereas, with respect to time and concentration, irrespective of the different culture filtrates of endophytes applied, the rice seeds dipped in 100% concentration of culture filtrate for 30 minutes recorded the highest mean percent germination (84.64%) and the least mean percent germination (82.00%) was recorded for rice seeds dipped in 100% concentration culture filtrates dipped for 15 minutes.

However, it was observed that different concentrations of the fungal endophytes had no significant difference of on the per cent germination of rice at different time intervals. But treatments with fungal endophyte species had significant influence on per cent germination while interactions between treatments applied and concentrations of the culture filtrate does not have significant effect on the per cent germination except for those rice seeds which were dipped in culture filtrates for 30 minutes.

The findings that describe the effect of fungal endophytes especially the endophytic species tested in the present investigation on germination of rice seeds has not been reported earlier. However, Lin *et al.,* (2007) reported that germination of rice seeds infected with endophytic *Phomopsis* sp. isolated from the inner bark of *Bischofia polycarpa* was significantly greater than that of endophyte free plants. Cheplick *et al.,* (1989) and Clay (1987) reported that seeds of tall fescue and perennial ryegrass germinate more rapidly and to higher levels when infected with endophytes. Though in the present investigation, the rapidity of germination was not recorded, the percent germination at higher levels has been achieved by some endophytes like *P. citrinum* and *P. pinophilum* both recording 96.65% germination of rice seeds when dipped in their culture filtrates compared to 86.67% germination in control. In the present experiment, rice seeds were dipped in culture filtrates of fungal endophytes that are contrary to the works of Cheplick *et al.,* (1989) and Clay (1987) who infected their planting materials with the endophytes directly. Na *et al.,* (2014) reported that germination potential and vigor index of rice seeds treated by mixture of 5 ng/ml of extracts of *Alternaria* (R) and 5 ng/ml of extracts of *Coprinus micaceus* (D) at germination stage were higher than those of control by 9.52% and 23.94%. In the present study, percent germination of rice seeds was higher by 10% (*P. citrinum* and *P. pinophilum* both recording 96.65% germination compared to control that is 86.67%) due to the influence of fungal endophytes.

Higher per cent germination of rice seeds in presence of culture filtrates of *P. citrinum* and *P. pinophilum* indicates the positive impact of these endophytes on seed health of rice. Endophytes are intricately associated with their host plants in tissue specific manner (Fisher and Petrini, 1992). Endophytes population of rice plant also differ in terms of diversity and quantity at different growth stages of rice plant (Imchen, 2015). It was observed earlier that *P. pinophilum* is specific to seedling stage of rice and not found in vegetative and maturity stage, whereas, *P. citrinum* is more abundant in seedling and vegetative stage of rice plant than maturity stage (Imchen, 2015). Thus, association of this *Penicillium* spp. with the seedling stage of rice plant perhaps explains their promotion effect on rice seed germination.

Table.1 Effect of culture filtrate of fungal endophytes of rice on rice seed germination (%) *in vitro*

Endophyte species	Per cent germination of rice seeds treated with culture filtrate of fungal endophytes									
	15 mins			30 mins			60mins			Mean
	25%	50%	100%	25%	50%	100%	25%	50%	100%	
1. *Cladosporium cladosporioides*	89.99 (9.48)*	80.00 (8.93)	83.33 (9.13)	86.67 (9.31)	86.67 (9.31)	86.67 (9.31)	90.00 (9.49)	83.33 (9.13)	83.33 (9.13)	**85.55**
2. *Penicillium citrinum*	83.33 (9.13)	96.65 (9.83)	76.67 (8.75)	73.33 (8.56)	90.00 (9.49)	73.33 (8.56)	96.65 (9.83)	80.00 (8.94)	80.00 (8.93)	**83.33**
3. *Fusarium moniliforme*	80.00 (8.94)	83.33 (9.13)	83.33 (9.13)	89.99 (9.48)	86.67 (9.31)	86.67 (9.31)	83.33 (9.13)	86.67 (9.31)	83.33 (9.13)	**84.81**
4. *Trichoderma asperellum*	76.67 (8.74)	76.67 (8.75)	70.00 (8.37)	83.33 (9.13)	73.33 (8.56)	86.67 (9.31)	80.00 (8.94)	83.33 (9.13)	83.33 (9.13)	**79.26**
5. *Penicillium pinophilum*	76.67 (8.74)	83.33 (9.13)	80.00 (8.94)	80.00 (8.94)	96.65 (9.83)	86.67 (9.31)	83.33 (9.13)	83.33 (9.13)	86.67 (9.31)	**84.07**
6. *Aspergillus niger*	86.67 (9.31)	89.99 (9.48)	89.99 (9.48)	93.33 (9.66)	86.67 (9.31)	86.67 (9.31)	86.67 (9.31)	86.67 (9.31)	90.00 (9.49)	**88.52**
7. *Aspergillus flavus*	80.00 (8.94)	83.33 (9.13)	76.67 (8.75)	86.67 (9.31)	76.67 (8.75)	86.67 (9.31)	83.33 (9.13)	80.00 (8.93)	83.33 (9.13)	**81.85**
8. *Drechslera specifera*	83.33 (9.13)	8.67 (9.31)	83.33 (9.13)	80.00 (8.93)	83.33 (9.13)	86.67 (9.31)	83.33 (9.13)	83.33 (9.13)	83.33 (9.13)	**83.70**
9. *Penicillium oxalicum*	83.33 (9.13)	76.67 (8.75)	76.67 (8.75)	73.33 (8.54)	76.67 (8.75)	83.33 (9.13)	73.33 (8.54)	70.00 (8.35)	70.00 (8.35)	**75.93**
10. *Geotrichum candidum*	86.67 (9.31)	76.67 (8.75)	83.33 (9.13)	80.00 (8.94)	83.33 (9.13)	76.67 (8.75)	83.33 (9.13)	80.00 (8.93)	80.00 (8.93)	**81.11**
11. *Culvularia lunata*	80.00 (8.94)	83.33 (9.13)	80.00 (8.94)	83.33 (9.13)	80.00 (8.93)	86.67 (9.31)	80.00 (8.94)	86.67 (9.31)	83.33 (9.13)	**82.59**
12. *Aspergillus amstelodami*	80.00 (8.94)	83.33 (9.13)	86.67 (9.31)	83.33 (9.13)	80.00 (8.93)	86.67 (9.31)	80.00 (8.94)	80.00 (8.94)	86.67 (9.31	**82.96**
13. *Talaromyces* sp.	83.33 (9.13)	80.00 (8.94)	86.67 (9.31)	83.33 (9.13)	86.67 (99.31)	86.67 (9.31)	83.33 (9.13)	80.00 (8.94)	83.33 (9.13)	**83.70**
14. *Chaetomium ochraceum*	93.33 (9.66)	86.67 (9.31)	86.67 (9.31)	80.00 (8.94)	86.67 (9.31)	86.67 (9.31)	83.33 (9.13)	83.33 (9.13)	86.67 (9.31)	**85.93**
15. Control	86.67 (9.31)	86.67 (9.31)	86.67 (9.31)	83.33 (9.13)	83.33 (9.13)	83.33 (9.13)	86.67 (9.31)	86.67 (9.31)	86.67 (9.31)	**85.56**
Mean	83.33	83.55	82.00	82.67	83.78	84.64	83.78	82.22	83.33	
	SEm±		CD(p=0.05)	SEm±		CD(p=0.05)	SEm±		CD(p=0.05)	
Concentration (C)	0.05		NS	0.05		NS	0.05		NS	
Treatment (T)	0.12		0.32	0.12		0.33	0.11		0.32	
C x T	0.20		NS	0.20		0.57	0.20		NS	

*Values in the parentheses indicate square root transformed values. NS = Non-significant

Table.2 Effect of culture filtrate of fungal endophytes of rice on growth of seedlings (shoots) *in vitro*

Endophyte species	Growth of roots (mm) of rice seeds treated with culture filtrate of fungal endophytes									
	15 mins			30 mins			60 mins			Mean
	25%	50%	100%	25%	50%	100%	25%	50%	100%	
1. *Cladosporium cladosporioides*	35.36 (36.48)*	39.29 (38.80)	36.41 (37.10)	36.41 (37.10)	36.32 (37.06)	34.81 (36.15)	41.48 (40.08)	36.76 (37.31)	33.05 (35.06)	36.66
2. *Penicillium citrinum*	26.23 (30.75)	27.97 (31.92)	34.15 (35.75)	38.85 (38.56)	30.70 (33.60)	23.96 (29.25)	24.67 (29.70)	29.69 (32.99)	28.75 (32.40)	29.44
3. *Fusarium moniliforme*	32.16 (34.51)	40.16 (39.32)	32.88 (34.98)	42.08 (40.44)	38.82 (38.54)	39.88 (39.15)	28.31 (32.13)	32.07 (34.49)	15.86 (23.44)	33.58
4. *Trichoderma asperellum*	32.85 (34.93)	22.35 (28.19)	30.85 (33.72)	29.87 (33.12)	27.59 (31.55)	34.51 (35.97)	30.72 (33.56)	34.07 (35.71)	34.69 (36.08)	30.83
5. *Penicillium pinophilum*	36.92 (37.42)	39.80 (39.11)	39.83 (39.13)	38.08 (38.11)	34.22 (35.80)	38.86 (36.78)	36.27 (37.02)	36.41 (37.11)	41.71 (40.23)	37.68
6. *Aspergillus niger*	38.60 (38.41)	35.08 (36.32)	27.15 (30.47)	38.61 (38.41)	37.40 (37.70)	40.87 (39.74)	40.01 (39.23)	38.09 (38.11)	39.34 (38.84)	37.24
7. *Aspergillus flavus*	37.54 (37.79)	38.56 (38.39)	39.26 (38.80)	37.45 (37.73)	40.08 (39.27)	39.45 (38.90)	34.77 (36.13)	35.45 (36.54)	37.23 (37.30)	37.75
8. *Drechslera specifera*	36.11 (36.92)	39.37 (38.86)	38.30 (38.23)	36.58 (37.21)	41.22 (39.93)	42.57 (40.73)	39.64 (39.01)	36.14 (36.95)	40.21 (39.35)	38.91
9. *Penicillium oxalicum*	27.39 (31.52)	27.39 (31.52)	29.20 (32.70)	28.07 (31.93)	24.63 (29.71)	24.72 (29.77)	28.85 (32.49)	30.13 (33.19)	27.77 (31.79)	27.57
10. *Geotrichum candidum*	36.35 (37.06)	39.39 (38.87)	42.21 (40.51)	35.62 (36.63)	36.62 (36.63)	28.28 (32.12)	38.62 (38.42)	38.98 (38.63)	39.01 (38.65)	37.12
11. *Culvularia lunata*	38.94 (38.61)	35.87 (36.79)	30.59 (33.55)	35.74 (36.71)	34.57 (36.00)	35.76 (36.72)	39.66 (39.03)	37.36 (37.67)	35.80 (36.74)	36.03
12. *Aspergillus amstelodami*	41.65 (40.19)	40.06 (39.26)	40.88 (39.74)	40.41 (39.46)	35.82 (36.74)	39.57 (38.96)	29.96 (33.17)	32.96 (35.03)	42.59 (40.73)	38.21
13. *Talaromyces* sp.	33.83 (35.54)	31.83 (34.32)	37.99 (38.03)	37.12 (37.53)	35.08 (36.31)	39.31 (38.82)	38.30 (38.23)	35.98 (36.85)	36.77 (37.33)	36.25
14. *Chaetomium ochraceum*	32.80 (34.93)	34.97 (36.24)	36.87 (37.38)	36.60 (37.21)	37.28 (37.63)	35.80 (36.75)	36.41 (37.11)	38.82 (38.54)	38.83 (38.54)	36.49
15. Control	42.22 (40.52)	42.22 (40.52)	42.22 (40.52)	38.95 (38.58)	38.95 (38.58)	38.95 (38.58)	41.19 (39.87)	41.19 (39.87)	41.19 (39.87)	40.79
Mean	35.26	35.62	35.92	36.70	35.22	35.13	35.62	35.61	35.52	
	SEm±	CD(p=0.05)		SEm±	CD(p=0.05)		SEm±	CD(p=0.05)		
Concentration (C)	0.03	NS		0.04	NS		0.03	NS		
Treatment (T)	0.08	0.21		0.09	0.24		0.07	0.21		
C x T	0.13	0.37		0.15	0.42		0.13	0.36		

*Values in the parentheses indicate square root transformed values. NS = Non-significant

Table.3 Effect of culture filtrate of fungal endophytes of rice on growth of seedlings (roots) *in vitro*

Endophyte species	Growth of roots (mm) of rice seeds treated with culture filtrate of fungal endophytes									
	15 mins			30 mins			60mins			Mean
	25%	50%	100%	25%	50%	100%	25%	50%	100%	
1. *Cladosporium cladosporioides*	35.36 (36.48)*	39.29 (38.80)	36.41 (37.10)	36.41 (37.10)	36.32 (37.06)	34.81 (36.15)	41.48 (40.08)	36.76 (37.31)	33.05 (35.06)	36.66
2. *Penicillium citrinum*	26.23 (30.75)	27.97 (31.92)	34.15 (35.75)	38.85 (38.56)	30.70 (33.60)	23.96 (29.25)	24.67 (29.70)	29.69 (32.99)	28.75 (32.40)	29.44
3. *Fusarium moniliforme*	32.16 (34.51)	40.16 (39.32)	32.88 (34.98)	42.08 (40.44)	38.82 (38.54)	39.88 (39.15)	28.31 (32.13)	32.07 (34.49)	15.86 (23.44)	33.58
4. *Trichoderma asperellum*	32.85 (34.93)	22.35 (28.19)	30.85 (33.72)	29.87 (33.12)	27.59 (31.55)	34.51 (35.97)	30.72 (33.56)	34.07 (35.71)	34.69 (36.08)	30.83
5. *Penicillium pinophilum*	36.92 (37.42)	39.80 (39.11)	39.83 (39.13)	38.08 (38.11)	34.22 (35.80)	38.86 (36.78)	36.27 (37.02)	36.41 (37.11)	41.71 (40.23)	37.68
6. *Aspergillus niger*	38.60 (38.41)	35.08 (36.32)	27.15 (30.47)	38.61 (38.41)	37.40 (37.70)	40.87 (39.74)	40.01 (39.23)	38.09 (38.11)	39.34 (38.84)	37.24
7. *Aspergillus flavus*	37.54 (37.79)	38.56 (38.39)	39.26 (38.80)	37.45 (37.73)	40.08 (39.27)	39.45 (38.90)	34.77 (36.13)	35.45 (36.54)	37.23 (37.30)	37.75
8. *Drechslera specifera*	36.11 (36.92)	39.37 (38.86)	38.30 (38.23)	36.58 (37.21)	41.22 (39.93)	42.57 (40.73)	39.64 (39.01)	36.14 (36.95)	40.21 (39.35)	38.91
9. *Penicillium oxalicum*	27.39 (31.52)	27.39 (31.52)	29.20 (32.70)	28.07 (31.93)	24.63 (29.71)	24.72 (29.77)	28.85 (32.49)	30.13 (33.19)	27.77 (31.79)	27.57
10. *Geotrichum candidum*	36.35 (37.06)	39.39 (38.87)	42.21 (40.51)	35.62 (36.63)	36.62 (36.63)	28.28 (32.12)	38.62 (38.42)	38.98 (38.63)	39.01 (38.65)	37.12
11. *Culvularia lunata*	38.94 (38.61)	35.87 (36.79)	30.59 (33.55)	35.74 (36.71)	34.57 (36.00)	35.76 (36.72)	39.66 (39.03)	37.36 (37.67)	35.80 (36.74)	36.03
12. *Aspergillus amstelodami*	41.65 (40.19)	40.06 (39.26)	40.88 (39.74)	40.41 (39.46)	35.82 (36.74)	39.57 (38.96)	29.96 (33.17)	32.96 (35.03)	42.59 (40.73)	38.21
13. *Talaromyces* sp.	33.83 (35.54)	31.83 (34.32)	37.99 (38.03)	37.12 (37.53)	35.08 (36.31)	39.31 (38.82)	38.30 (38.23)	35.98 (36.85)	36.77 (37.33)	36.25
14. *Chaetomium ochraceum*	32.80 (34.93)	34.97 (36.24)	36.87 (37.38)	36.60 (37.21)	37.28 (37.63)	35.80 (36.75)	36.41 (37.11)	38.82 (38.54)	38.83 (38.54)	36.49
15. Control	42.22 (40.52)	42.22 (40.52)	42.22 (40.52)	38.95 (38.58)	38.95 (38.58)	38.95 (38.58)	41.19 (39.87)	41.19 (39.87)	41.19 (39.87)	40.79
Mean	35.26	35.62	35.92	36.70	35.22	35.13	35.62	35.61	35.52	
	SEm±	*CD(p=0.05)*		*SEm±*	*CD(p=0.05)*		*SEm±*	*CD(p=0.05)*		
Concentration (C)	0.36	NS		0.31	NS		0.32	NS		
Treatment (T)	0.81	2.27		0.69	1.94		0.71	1.99		
C x T	1.40	3.94		1.19	3.35		1.23	3.45		

*Values in the parentheses indicate square root transformed values. NS = Non-significant

The *Penicillium* spp. as endophytes may also produce metabolites in their culture filtrates that would give protection to the rice seeds from harmful seed microflora at the time of germination (Saikkonen *et al.*, 2004b).

Seedling growth

Growth of seedlings (shoots) of rice seeds treated with culture filtrate of fungal endophytes

It is evident from the datas from Table 2 that various treatments with culture filtrates at different period of dipping showed significant influence on the growth of shoots. The maximum shoot length was recorded with seeds treated with *C. cladosporioides* (13.74 mm) when dipped for 30 minutes at 100% concentration as compared to 6.74 mm shoot length recorded in control experiments. The least shoot length of rice seed was recorded with endophyte *G. candidum* (3.02 mm) at 25% concentration dipped for 15 minutes. The highest mean shoot length was recorded with rice seeds dipped in culture filtrate of *C. cladosporioides* (11.29) and the least mean shoot length was recorded in the rice seeds dipped in that of *G. candidum* (3.68) as compared to control that recorded 6.76 mm shoot length. Whereas, with respect to time and concentration, irrespective of the different culture filtrates of endophytes applied, the rice seeds dipped in 50% concentration of culture filtrate for 30 minutes recorded the highest mean shoot length (8.04 mm) and the least mean shoot length (7.33) was recorded for rice seeds dipped in 100% concentration culture filtrates dipped for 30 minutes. Statistical analysis revealed that different concentrations of the fungal endophytes *viz.* 25, 50 and 100% had no significant influence of on the length of shoot of rice seeds at different time intervals of 15, 30 and 60 minutes. On the contrary, treatments with different fungal endophyte species and interactions between treatments applied and concentrations of the culture filtrate were found to have significant effect on the growth of shoots of rice seeds.

Growth of seedlings (roots) of rice seeds treated with culture filtrate of fungal endophytes

The datas pertaining to the effect of culture filtrate of fungal endophytes on the growth of roots of rice seeds are presented in Table 3. The maximum root length was recorded with seeds treated with *A. amstelodami* (42.59 mm) when dipped for 60 minutes at 100% concentration of culture filtrate as compared to 41.19 mm of root length recorded in control. The minimum root length was recorded with seeds dipped in *F. moniliforme* (15.86) at 100% concentration dipped for 60 minutes. The highest mean root length was recorded with rice seeds dipped in culture filtrate of *D. specifera* (38.91) and the least mean root length was recorded in the rice seeds dipped in that of *T. asperellum* (30.83) as compared to control that recorded 40.79 mm root length. Whereas, with respect to time and concentration, irrespective of the different culture filtrates of endophytes applied, the rice seeds dipped in 100% concentration of culture filtrate for 15 minutes recorded the highest mean root length (35.92) and the least mean root length (35.13) was recorded for rice seeds dipped in 100% concentration culture filtrates dipped for 30 minutes. No significant influence was observed for different concentrations of the fungal endophytes at different time intervals. On the contrary, treatments with different fungal endophyte species and interactions between treatments applied and concentrations of the culture filtrate were found to have significant effect on the growth of shoots of rice seeds.

Earlier works suggests that endophyte species have positive effect on the growth of

seedlings (Bandar *et al.,* 2006, Lin *et al.,* 2007, Bo *et al.,* 2014 and Na *et al.,* 2014). The present findings are also in conformity with similar explanation. Endophytic species like *Phomopsis* sp. (Lin *et al.,* 2007, Chao *et al.,* 2008 and Bo *et al.,* 2014) have already been shown to promote the growth of rice plant. Kumar *et al.,* (2012) reported that culture filtrate of *Piriformospora indica* (a root endophytic fungus) had significant enhancement in lignan production and growth of *Linum album* hairy root cultures. Bagheri *et al.,* (2013) also reported that endophytic fungus, *Piriformospora indica* has a pronounced growth-promoting activity on rice plant. The growth of seedlings upon treatment with different culture filtrate of fungal endophytes may be due to the increment in the production lignans like podophyllotoxin and 6 –methoxypodophyllotoxin concentratation which also coincides with the increase in phenylalanine ammonia lyase activity in the rice plant (Kumar *et al.,* 2012). Bagheri *et al.,* (2013) reported that endophytes increase the biomass of aerial parts and root, total soluble proteins, Relative Water Content (RWC), free proline content and enzyme antioxidants activity of inoculated rice as compared to un-inoculated ones. Since the data obtained in the present investigation also indicates significant increase in the growth of seedlings (shoot), thus it corroborates with the findings of the earlier workers.

With respect to root length of rice seedlings, the mean root length was shorter in all the treatments with culture filtrate of fungal endophytes than that of control. This indicates that seed treatment with culture filtrate of endophytes may not be a viable idea to obtain increased root length that would actually reflect increased plant establishment in an area. As our experiment was limited to only five days after germination and endophytic fungi were not directly involved, and there was a mixtures of fungal metabolites in their culture filtrate, effect of endophytes on rice root length was perhaps not translated well. Other workers have reported growth promoting effect of various fungal endophytes *viz. P. indica* on rice plant (Bagheri *et al.,* 2013) and *Linum album* (Kumar *et al.,* 2012). *P. indica* is a root endophytic fungus. Endophytic fungi *Phoma glomerata* and *Penicillium* sp. significantly promoted shoot growth in gibberellic acid deficient dwarf mutant variety of rice indicating role of endophytes in producing plant growth hormone like GA that is known for inducing shoot growth (Waqas *et al.,*2012).

The present study manifests the effects of fungal endophytes on the germination of rice seedlings as well as shoot and root length of rice seedlings upto five days after germination. In order to observe the full beneficial effects of fungal endophytes, a further elaborative study is required to be taken up till maturity stage of rice plant including the biochemical analysis of culture filtrate of the endophytes. Individual endophytes may be screened for their beneficial effects on rice plant and further explored for commercialization.

References

Anonymous. 2009. Brookhaven National Laboratory and their Belgian colleagues at Hasslet University. *Applied and Environmental Microbiology*. Retrieved 24 July 2012.

Arnold, A, E., Meffa, L. C., Kyollo, D., Rojas, E. I., Maynard, Z., Robbins, N and Herre, E. A. 2003. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National. Academy of Sciences. USA* 100: 15649–15654.

Bagheri, A. A., Saadatmand, S., Niknam, V., Nejadsatari, T and Babaeizad, V. 2013.

Effect of endophytic fungus, *Piriformospora indica,* on growth and activity of antioxidant enzymes of rice (*Oryza sativa* L.) under salinity stress. *International journal of Advanced Biological and Biomedical Research* 1 (11): 1337-1350

Bandara, W. M. M. S., Seneviratne, Gamini and Kulasooriya, S. A. 2006. Interactions among endophytic bacteria and fungi: Effects and potentials. *Journal of Biosciences* 31(5):645-650.

Bo, Y., Mi, W. X., Yan, M. H., Yong, J., Xia, L and Chao, D, C. 2014. Effects of the fungal endophyte *Phomopsis liquidambari* on nitrogen uptake and metabolism in rice. *Plant Growth Regulation* 73(2):165-179.

Bush, L. P., Wilkinson, H, H and Schardl, C. L. 1997. Bioprotective alkaloids of grass-fungal endophyte symbioses. *Plant Physiology* 114:1- 7.

Chao, D. C., Lin, Z, Y., Yin, Y. Q., Shan, S. Q and Xia, L. (2008). The effects of increasing production of endophytes Phomopsis B3 on rice. *Agricultural Science & Technology - Hunan* 9(1):39-42.

Chapela, I. H. 1989. Fungi in health stems and American beech and aspen: a comparative study. *New Phytologist* 113:65- 75.

Cheplick, G. P., Clay, K and Wray, S. 1989. Interactions between fungal endophyte infection and nutrient limitation in the grasses *Lolium perenne* and *Festuca arundinacea*. *New Phytologist* 111:89-97 27.

Clay, K and Holah, J. 1999. Fungal endophyte symbiosis and plant diversity in successional fields. *Science* 285: 1742-1744.

Clay, K and Schardl, C. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist* 160: 99–127.

Clay, K. 1987. Effects of fungal en-dophytes on the seed and seedling biolo-gy of *Lolium perenne* and *Festuca arundinacea*. *Oecologia* 73:358-62.

Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clément, C and Barka, E. A. 2005. Endophytic colonization of *Vitis vinifera* L. by plant growth- promoting bacterium *Burkholderia* sp. strain PsJN. *Applied and Environmental Microbiology* 71:1685–1693.

Don, C. 2011. Dairy Farmer Finds Unusual Forage Grass. *Casler*. USDA. Retrieved August 1, 2012.

Ezra, D., Hess, W. M and Strobel, G. A. 2004. New endophytic isolates of *Muscodor albus*, a volatile-antibiotic-producing fungus. *Microbiology* 150: 4023–4031.

Faeth, S. H and Fagan, W. F. 2002. Fungal endophytes: Common host plant symbionts but uncommon mutualists. *Integrative and Comparative Biology* 42(2): 360-368.

Faeth, S. H and Hammon, K. E. 1997. Fungal endophytes in oak trees. Long-term patterns of abundance and associations with leafminers. *Ecology* 78*:*810- 819.

Fisher, P. J and Petrini, O. 1992. Fungal saprobes and pathogens as endophytes of rice (*Oryza sativa* L.). *New Phytologist* 120(1):137-143.

Gunatilaka, A. A. L. 2006. Natural products from plant-associated microorganisms: Distribution, structural diversity, bioactivity and implication of their occurance. *Journal of Natural Products.* 69: 509-526.

Höflich, G. 2000. Colonization and growth promotion of nonlegumes by *Rhizobium* bacteria. Microbial Biosystems: New Frontiers; in *Proceedings of the 8th International Symposium on Microbial Ecology* (eds) C R Bell, M., Brylinsky and Johnson-Green, P. *(Halifax:*

Atlantic Canada Society for Microbial Ecology). pp 827–830.

Imchen, R. 2015. Fungal endophytes of rice. M.Sc Thesis. Department of Plant Pathology, SASRD, Nagaland University.

Johri, B. N. 2006. Endophytes to the rescue of plants. *Current Science* 90: 1315–1316.

Kumar, V., Rajauria, G., Sahai, V and Bisaria, V. S. 2012. Culture filtrate of root endophytic fungus *Piriformospora indica* promotes the growth and lignan production of *Linum album* hairy root cultures. *Process Biochemistry* 47: 901‑907

Lin, Y. Z., Chao, D. C., Xia, L., Shuang, T. L and Xiang, W. X. 2007. Extensive host range of an endophytic fungus affects the growth and physiological functions in rice (*Oryza sativa* L.). *Symbiosis (Rehovot)* 43(1):21-28.

Na, W., Zhen, Y., Jun, C., Xun, C., Na, G and Tao, Y. 2014. Effects of extracts of plant endophyte on growth of rice. *Journal of Jilin Agricultural University* 36(1): 10-16.

Saikkonen, K., Helander, M and Faeth, S. H. 2004a. Fungal endophytes: hitchhikers of the green world. *Plant microbiology* (eds) Gillings, M. and Holmes, A. *(Oxford: BIOS Scientific Publishers).* pp 77–95.

Saikkonen. K., Wa, L. P., Helander, M and Faeth, S. H. 2004b. Evolution of endophyte–plant symbioses. *Trends in Plant Science* 9:275-280.

Schulthess, F and Faeth, S. H. 1998. Distribution, abundances and associations of the endophytic fungal community of Arizona fescue *(Festuca arizonica). Mycologia* 90*:* 569- 578.

Suryanarayanan, T. S., Wittlinger, S., Faeth, S. H. 2005. Endophytic fungi associated with cacti of Arizona. *Mycological Research* 109*:* 635- 639.

Taechowisan, T., Chunhua, L. U., Shen, Y and Lumyong, S. 2005. Secondary metabolites from endophytic *Streptomyces aureofaciens* CMUAc130 and their antifungal activity. *Microbiology* 151: 1691–1695.

Tian, X. L., Cao, L. X., Tan, H. M., Zeng, Q. G., Jia, Y. Y., Han, W. Q and Zhou, S. N. 2004. Study on the communities of endophytic fungi and endophytic actinomycetes from rice and their antipathogenic activities *in vitro. World Journal of Microbiology & Biotechnology 20*(3):303-309.

Waqas, M., Khan, A. L., Kamran, M., Hamayun, M., Kang, S. M., Kim, Y. H and Lee, I. J. 2012. Endophytic fungi produce Gibberellins and Indoleacetic acid and promotes host-plant growth during stress. *Molecules* 17: 10754-10773.

Wilson, D. 1993. Fungal endophytes: out of sight but should not be out of mind? *Oikos* 68: 379–384.

How to cite this article:

Lalngaihawmi, S. Banik, P. Chakruno and Khatemenla. 2018. Effect of Rice Fungal Endophytes on Seed Germination and Seedling Growth of Rice. *Int.J.Curr.Microbiol.App.Sci.* 7(04): 3653-3663. doi: https://doi.org/10.20546/ijcmas.2018.704.411