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IN VITRO EVALUATION OF THE EFFICIENCY OF FUNGAL ENDOPHYTES AGAINST THE PHYTOPATHOGENS OF *SOLANUM TUBEROSUM* L.

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ABSTRACT

A study was conducted for two crop cycles to evaluate the efficiency of fungal endophytes against different phytopathogens of potato isolated from the leaf, stem, roots and tubers of *Solanum tuberosum* L. grown in South West Garo Hills district of Meghalaya. The endophytic fungal isolates were investigated *in vitro* against the selected phytopathogens of potato. *Humicola fuscoatra* recorded the highest percentage inhibition of 94.81% against *Sclerotium rolfsii*, followed by *Penicillium canescens* against *Alternaria solani* (93.33%), *Trichoderma viride* against *S. rolfsii* (93.33%), and *T. viride* against *Fusarium solani* (92.59%) and *P. lanosum* against *F. solani* (90%). Though all the isolates showed percentage of inhibition, *H. grisea* proved to be a good antagonist and showed maximum occurrence in suppressing the growth of *A. solani* (86.66%), *Rhizoctonia solani* (82.22%) and *Phytophthora infestans* (75.55%). The investigation revealed that the bioagents *T. viride* and *H. grisea* were able to inhibit the growth of the pathogen mycelia significantly.

Key words: Fungal endophytes, Antagonistic activity, *In vitro* screening, Phytopathogens, *Solanum tuberosum* L.

INTRODUCTION

Potatoes are widely cultivated and contribute to reduce worldwide food shortages (Han *et al*., 2005). However, potatoes are significantly lost both in quality and quantity due to many fungi causing diseases. They are susceptible to devastation by various diseases, such as black scurf caused by *Rhizoctonia solani* leading to wilting and plant death and dry rot caused by *Fusarium* dry rot that greatly affect tuber quality and severely reduce its market value (Wolf and Verret, 1999; Yao *et al*., 2002; Grosch *et al*., 2005 and Wharton and Kirk, 2007). Modern method of crop production has adverse effect on the environment and therefore a more approachable way of controlling the phytopathogens is the incorporations of effective microbial bioagents strains into successive disease management. Members of the genus *Penicillium* and *Trichoderma* have long been known for their potential to reduce the plant disease caused by fungal pathogens and they have gained considerable importance as potential antagonistic microorganisms (Pant and Mukhopadhyay, 2001). Biological control using antagonistic microorganisms as bioagents is a sustainable alternative to protect plants against soil borne pathogens (Eckwall and Schottel, 1997; Munimbazi and Bullerman, 1998; Emmert and Handelsman, 1999; Weller *et al*., 2002; Yao *et al*., 2002; Han *et al*., 2005; Grosch *et al*., 2005; El-Kot and Belal, 2006). The present study aimed to evaluate the efficiency of the isolated fungal endophytes against different phytopathogens of potato.

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MATERIALS AND METHODS

Isolation and identification of endophytic fungi

The isolation of fungal endophytes from plant parts were done following the method given by Suryanarayanan *et al.* (2003) and were identified based on the morphology of the fungal culture colony or hyphae, the characteristics of the spores structures using standard manuals by Subramaniam (1971), Barnett and Hunter (1972) and Domsch *et al*. (1980).The pure cultures of the isolates were maintained in Czapeck Dox Agar media. The colonization frequency (CF %) of a single endophyte species was calculated as given by Hata and Futai (1995) -

$$CF\ (\%) = (N_{COL}/N_t) \times 100$$

Where N_{COL} = number of segments colonized by each fungus; N_t = total number of segments

In vitro screening of biocontrol agents

Antagonism of the isolated fungal endophytes on phytopathogens was studied by dual culture technique (*in-vitro* culture) with the method proposed by Skidmore and Dickinson (1976). A mycelial disc (9mm diameter), obtained from the peripheral region of 5 days old cultures tested fungi and pathogens were placed simultaneously on the periphery, about 1 cm from the edges of the petridishes (9 cm diameter) at opposite sides. The petridishes containing the Czapek Dox Agar medium inoculated with the tested pathogen alone

Table 1. Colonisation frequencies isolated from different parts of potato plant

Sl.No.	Endophytic fungal species	Oct 2014- Mar 2015				Oct 2015-Mar 2016			
		L	S	T	R	L	S	T	R
		Zygomycota- 1Genera, 1 Species							
1	*Absidia cylindrospora*	-	-	-	-	5.55	1.85	-	-
		Ascomycota-13 Genera, 37 Species							
2	*Acremonium cereale*	13	11.11	5.55	-	24.07	7.4	9.25	16.66
3	*Apiospora montagnei*	3.7	-	-	-	3.7	-	-	1.85
4	*Aspergillus flavus*	-	14.81	3.7	-	-	5.55	3.7	-
5	*A. fumigatus*	-	-	-	-	-	-	-	12.96
6	*A. niger*	7.4	-	-	11.11	-	-	-	-
7	*Cladosporium macrocarpum*	-	-	-	1.85	1.85	-	5.55	3.7
8	*C. cladosporioides*	7.4	16.67	-	3.7	3.7	5.55	7.4	11.11
9	*C. sphaerospermum*	-	-	-	-	-	1.85	-	-
10	*Cochliobolus spicifer*	-	1.85	-	-	-	-	-	-
11	*Fusarium oxysporum*	-	3.7	-	12.96	3.7	14.81	-	5.55
12	*F. redolens*	-	-	-	9.25	-	-	-	-
13	*F. semitectum*	-	-	9.25	-	-	-	-	-
14	*F. solani*	-	-	12.96	-	-	-	-	-
15	*F. sporotrichioides*	-	14.81	-	12.96	-	-	-	-
16	*Alternaria alternata*	13	-	11.11	-	-	-	1.85	-
17	*A. brassicicola*	-	14.81	9.25	-	-	-	-	-
18	*A. solani*	5.55	5.55	-	-	-	-	-	-
19	*Gonytrichum macrocladum*	-	-	-	-	3.7	-	-	-
20	*Humicola fuscoatra*	16.7	-	-	14.81	11.11	9.25	7.4	5.55
21	*H. grisea*	-	-	-	-	-	1.85	-	-
22	*Nectria ventricosa*	11.11	7.4	11.11	20.36	-	3.7	1.85	5.55
23	*Penicillium brevicompactum*	11.11	-	1.85	-	7.4	-	-	3.7
24	*P. canescens*	-	-	5.55	-	3.7	5.55	9.25	-
25	*P.chrysogenum*	-	12.96	5.55	-	5.55	-	-	11.11
26	*P. expansum*	-	3.7	-	-	-	-	-	-
27	*P. funiculosum*	-	-	-	-	11.11	-	-	11.11
28	*P. janthinellum*	16.7	-	-	-	14.81	1.85	18.51	7.4
29	*P. jensenii*	-	-	1.85	-	3.7	5.55	1.85	3.7
30	*P. lanosum*	-	-	-	-	3.7	-	-	-
31	*P. purpureogenum*	3.7	-	-	-	3.7	16.66	3.7	-
32	*P. rubrum*	-	-	-	-	7.4	-	20.37	9.25
33	*P. sacculum*	-	-	-	-	-	3.7	-	-
34	*P. simplicissimum*	-	-	-	-	-	-	-	1.85
35	*P. stoloniferum*	-	-	-	-	-	-	-	1.85
36	*Phoma eupyrena*	16.7	12.96	-	-	-	-	-	1.85
37	*P. medicaginis*	-	-	-	-	-	5.55	1.85	-
38	*Trichoderma harzianum*	-	-	-	-	5.55	-	-	-
39	*T. viride*	16.7	-	-	11.11	-	-	-	-
		Oomycota- 2 Genera, 3 Species							
40	*Phytophthora cinnamomi*	11.11	5.55	-	5.55	-	-	-	-
41	*Pythium intermedium*	-	-	1.85	-	-	-	-	-
42	*P. irregulare*	11.11	-	-	-	-	-	5.55	-
		Basidiomycota-2 Genera, 2 Species							
43	*Rhizoctonia solani*	-	-	7.4	-	-	-	-	-
44	*Sclerotium rolfsii*	-	3.7	-	11.11	-	-	-	-

'+'= Fungal Species Present; '-'= Fungal Species Absent
L=Leaf, S=Stem, T=Tubers, R=Roots

served as control. All the plates were incubated at 28 °C and measurements were taken after 5 days. At the end of incubation period, radial growth was measured using the formula given below-

Percentage inhibition of growth calculated as-

% inhibition= $r-r^1/r \times 100$

r= growth of the fungus measured from the centre of the colony towards the centre of the plate in the absence of antagonistic fungus

r^1=growth of the fungus measured from the centre of the colony towards the antagonistic fungus

Diversity analysis

The species diversity and dominance were evaluated using Shannon and Weiner (1963) diversity index (H') and Simpson (1949) dominance index (D) respectively.

1. Shannon-Weiner Diversity Index (H') = $-\Sigma pi \ln pi$,

Where, pi = the proportion of important value of the i^{th} species ($pi=ni/N$, ni is the important value index of i^{th} species and N is the important value index of all the species)

2. Simpson Dominance Index (D) = $\Sigma (n_i/N)^2$

Where, n_i is the number of individuals of species and N is the total number of species in community

Table 2. *In vitro* antagonistic efficiencies of the fungal endophytes against the selected phytopathogens

Sl. No	Test Antagonists	Growth inhibition (%) of pathogens				
		RS	SR	PI	FS	AS
1	*Absidia cylindrospora*	71.11	78.88	62.22	57.77	70
2	*Acremonium cereale*	80.74	71.85	44.44	71.85	85.18
3	*Alternaria alternata*	48.88	45.55	55.55	60	62.22
4	*A. brassicicola*	57.77	34.81	40	56.29	39.50
5	*Apiospora montagnei*	62.22	54.07	51.11	70.37	62.96
6	*Aspergillus flavus*	47.77	50	44.44	47.77	51.11
7	*A. fumigatus*	57.77	56.66	46.66	56.66	50
8	*A. niger*	80	57.03	35.55	44.44	67.90
9	*Cladosporium cladosporioides*	60	48.14	11.85	22.96	24.69
10	*C. macrocarpum*	45.92	34.07	24.44	53.33	43.20
11	*Cochliobolus spicifer*	5.92	28.88	38.51	38.51	23.45
12	*Fusarium oxysporum*	48.14	59.25	21.48	50.37	26
13	*F. redolens*	45.18	49.62	33.33	58.51	62.96
14	*F.semitectum*	65.18	30.37	41.48	54.81	27.16
15	*F.sporotrichioides*	49.62	53.33	43.70	60.74	20.98
16	*Gonytrichum macrocladum*	43.33	47.77	56.66	43.33	47.77
17	*Humicola fuscoatra*	74.07	94.81	73.33	64.44	75.21
18	*H. grisea*	82.22	46.66	75.55	71.11	86.66
19	*Nectria ventricosa*	62.96	62.22	35.55	61.48	66.66
20	*Penicillium brevicompactum*	73.33	59.25	34.81	57.77	41.97
21	*P. janthinellum*	77.03	54.81	24.44	31.11	23.45
22	*P.canescens*	43.33	56.66	65.55	85.55	93.33
23	*P. chrysogenum*	69.62	60	37.03	26.66	27.16
24	*P. expansum*	45.18	50.37	18.51	34.81	48.14
25	*P. jensenii*	83.33	58.88	62.22	80	67.77
26	*P. lanosum*	60	42.22	46.66	90	64.44
27	*P. medicaginis*	42.22	52.22	55.55	43.33	52.22
28	*P. purpureogenum*	81.11	70	58.88	63.33	83.33
29	*P. rubrum*	78.88	65.55	56.66	64.44	45.55
30	*P. simplicissimum*	44.44	57.77	68.88	72.22	55.55
31	*P. stoloniferum*	50	75.55	61.11	74.44	46.66
32	*Phoma eupyrena*	62.96	53.33	55.55	62.96	65.43
33	*Phytophthora cinnamomi*	65.18	57.77	40	54.07	59.25
34	*Pythium irregulare*	32.59	30.37	39.25	29.62	29.62
35	*Trichoderma viride*	88.88	93.33	72.59	92.59	73.32

*Each value is mean of three replicates

RS = *Rhizoctonia solani*, SR = *Sclerotium rolfsii,* PI =*Phytophthora infestans,* FS = *Fusarium solani,* AS =*Alternaria solani*

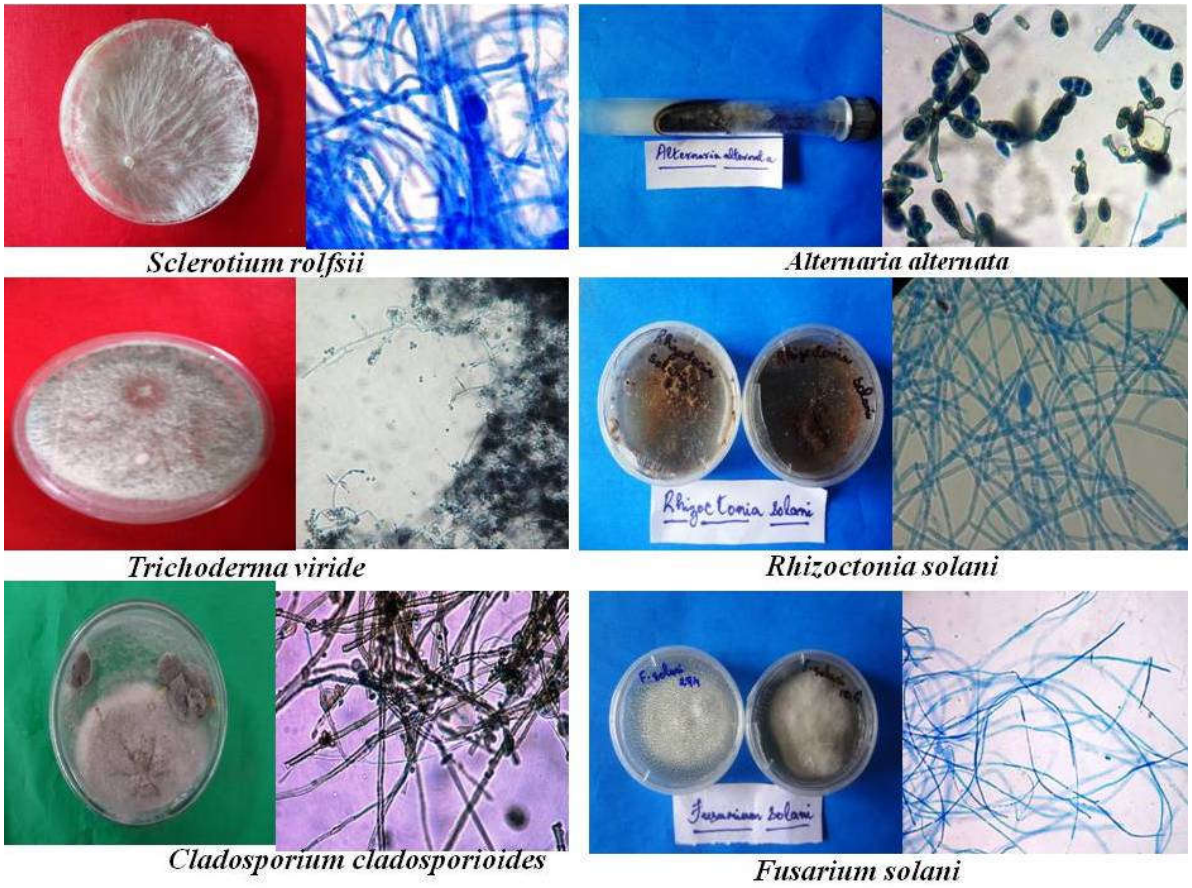
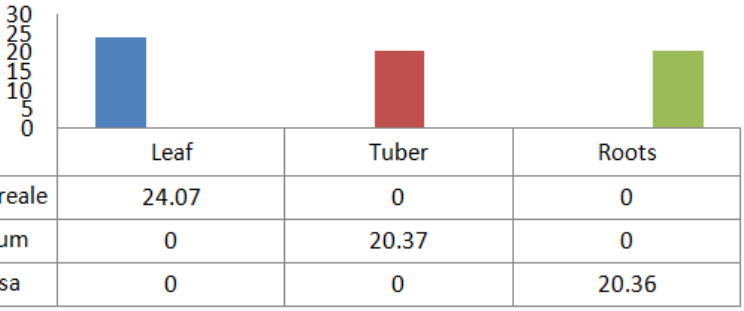


Figure 1. Pure cultures of endophytic fungi isolated from different parts of the potato plant

Table 3. Data analysis in different parts of the potato plant

Diversity Indices	Leaf	Stem	Tubers	Roots
Shannon -Weiner Diversity Index (H')	2.726598	2.478459	4.646832	2.679616
Simpson's Dominance Index (D)	0.080351	0.101208	0.11454	0.07959

Figure 2: Graphical reprentation showing the highest occurence of colonisation frequencies of an endophytic fungi in different parts of a potato plant



	Leaf	Tuber	Roots
Acremonium cereale	24.07	0	0
Penicillium rubrum	0	20.37	0
Nectria ventricosa	0	0	20.36

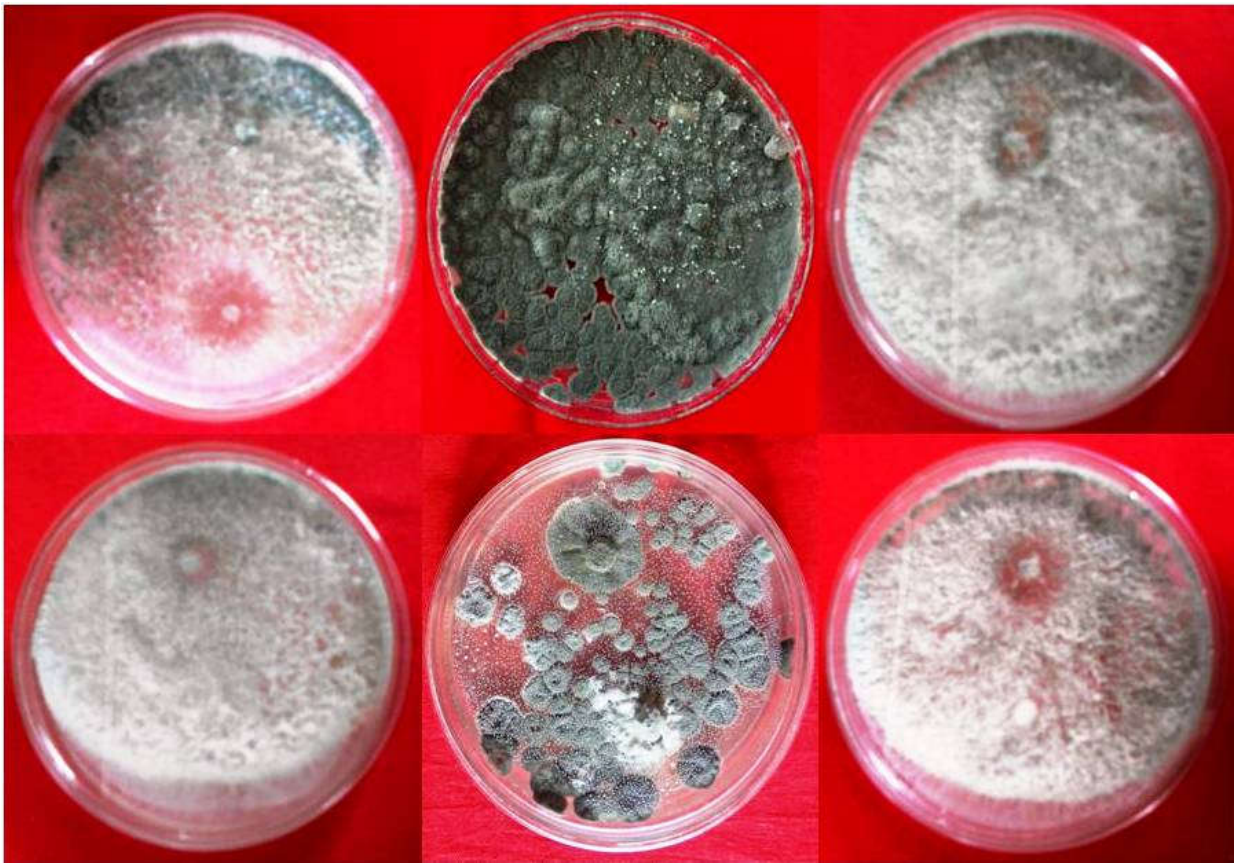


Figure 3. Dual culture method showing antagonistic activity of
(a) *H. fuscoatra* v/s *S. rolfsii* (b) *P. canescens* v/s *A. solani*
(c) *T. viride* v/s *S.rolfsii* (d) *T.viride* v/s *F. solani*
(e) *P.lanosum* v/s *F.solani* (f) *T.viride* v/s *R. solani*

RESULTS

Isolation and identification of fungal endophytes

A total of 44 endophytic fungal were isolated from the different parts of the potato plant with a total isolates of 18 genera (Figure1). *Penicillium* recorded the highest occurrence with a total of 13 species followed by *Fusarium* with an occurrence of 5 species (Table 1). The genera *Alternaria, Aspergillus, Cladosporium* recorded with 3 species each whereas *Humicola, Phoma, Pythium* and *Trichoderma* occurred with 2 species. However the genera *Absidia, Acremonium, Apiospora, Cochliobolus, Gonytrichum, Nectria, Phytophthora, Rhizoctonia* and *Sclerotium* had minimum number of occurrence with 1 species.

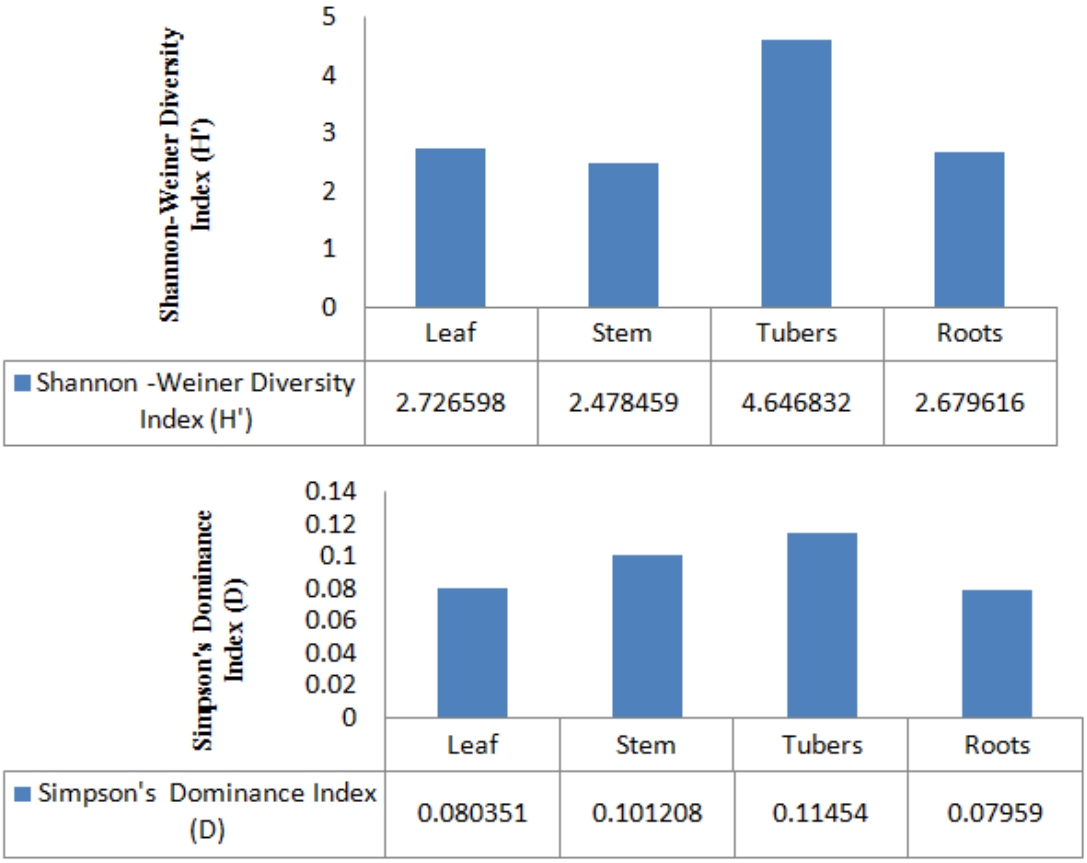


Figure 4. Graphical representation showing diversity indices in different parts of potato plant

Acremonium cereale recorded the highest colonization frequency of 24.07% in leaf followed by *Penicillium rubrum* (20.37%) in tubers and *Nectria ventricosa* (20.36%) in roots (Figure 2). The maximum occurrence in the fungal endophytes of *A. cereale, C. cladosporioides* and *N. ventricosa* have been isolated from all the parts of the potato plant. However the genera *Absidia, Alternaria, Apiospora, Cladosporium, Cochliobolus, Humicola, Nectria, Penicillium, Phoma* and *Pythium* showed the least colonization frequencies of 1.85%.

In-vitro experiments

In the dual culture experiment the antagonist fungal bioagents have considerable effect in the reduction of the growth of different test phytopathogens compared to their respective control. The endophytic fungal isolates were investigated *in vitro* against the selected phytopathogens of potato viz. *Alternaria solani*, *Phytophthora infestans* , *Rhizoctonia solani*, *Fusarium solani* and *Sclerotium rolfsii* (Figure 3). The mycelial growth of pathogenic isolates was noticeably constrained after a period of 10 days at a temperature of 25ºC. The results in Table 2 indicate out of the 35 fungal isolates *Humicola fuscoatra* recorded highest percentage inhibition of 94.81% against *S. rolfsii*, followed by *Penicillium canescens* against *A.solani* (93.33%), *T. viride* against *S.rolfsii* ((93.33%), and *T. viride* against *F. solani* (92.59%) and *P. lanosum* against *F. solani* (90%). Though all the isolates showed percentage of inhibition, *H. grisea* proved to be a good antagonist and showed maximum occurrence in suppressing the growth of *A. solani* (86.66%), *R. solani* (82.22%) and *P. infestans* (75.55%). The species of *Absidia, Acremonium, Aspergillus, Humicola, Penicillium* and *Trichoderma* have been found to have a comparable maximum percentage inhibition of mycelial growth against the tested phytopathogens. Thus, the target organisms *T. viride* and *H.grisea* are promising biological agents restricting the growth of pathogens in potato.

Data Analysis

The data analysis for species diversity and dominance were done for leaf, stem, tubers and roots parts of the potato plant (Table 3). The results reported species diversity to be highest in the tubers and lowest in the stem. It ranged from 2.47 to 4.64. The dominance index was highest in the tubers and least in the roots. It ranged from 0.07-0.11.Throughout the sampling months both Shannon-Weiner diversity index and Simpson's dominance index showed maximum values in the tubers and variation in the other parts of the potato plant (Figure 4).

DISCUSSION

The maximum colonization for the two cop cycles has been isolated from the leaves and has been reported in genera *Acremonium, Nectria* and *Penicillium* where they occur endophytically in all the parts of the plant especially in leaves (Petrini 1984). The reason for the moderate occurrence of fungal endophytes *Absidia, Alternaria, Apiospora, Cladosporium, Cochliobolus, Humicola, Nectria, Penicillium, Phoma* and *Pythium* reported least colonization frequencies might be due to the environmental and climatic factors prevailing in the region during collection such as site moisture, rainfall, wind exposure that influence endophytic

infestation and thereby affect the distribution and diversity of fungal endophytes (Fisher *et al.* 1995*)*. The diversity indices of endophytic colonization is reported to be the highest in the tubers than any parts of the plant because of its sensitivity to leaf size, age, methodology (Lodge *et al*. 1996; Gamboa *et al*. 2002) and different environmental conditions also resulted in different fungal species richness and different fungal species composition in the stands (Saikkonen 2007). The endophytic fungi *Trichoderma* and *Humicola* has been reported as good biocontrol agents in controlling plant diseases and competing efficiently for space and nutrients where they overgrew and sporulated on the pathogen colonies and inhibited the growth of the target organisms enabling it to grow much faster than the pathogenic fungi such as *S.rolfsii* and *F.solani* (El-Gali, 2003; Bendahmane and Mahiout, 2012; El-Gali *et.al*, 2015 and Benítez et al., 2004; Simon and Sivasithaparam, 1988). The present study clearly indicates the high potential of the isolated fungal isolates as good biocontrol agents so as to enhance the productivity and in controlling different plant pathogens of *S.tuberosum* L.

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