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# Isolation and Identification of Endophytic Fungi Associated with *Solanum tuberosum* L. of South-West Garo Hills, Meghalaya

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**Abstract:** A study was conducted for two years to isolate the fungal endophytes from different parts of *Solanum tuberosum* L.(potato) such as leaves, stem, roots and tubers grown in South West Garo Hills district of Meghalaya.44 endophytic fungal were isolated including the major phytopathogens of potato such as *Alternaria solani*, *Fusarium solani*, *Rhizoctonia solani* and *Sclerotium rolfsii.*Out of the total isolates the genus *Penicillium* recorded the maximum number of species with a total of 13 species followed by *Fusarium* with a total of 5 species.The fungal endophytes *Acremonium cereale*, *Cladosporium cladosporioides* and *Nectria ventricosa* have been isolated from all the parts of the potato plant throughout the planting season for two years.
**Keywords: "**Isolation", "identification", "fungal endophytes", "*Solanum tuberosum* L.", "Garo Hills"

## 1. Introduction

Fungal endophytes are microfungi and most belong to the ascomycetes and their anamorphs. They colonize the internal tissues of vascular plants without producing any apparent disease symptoms (Petrini 1991). Although several temperate plants have been studied for their endophyte assemblages,very few vegetable crops and tropical plants have been screened for endophytes(Rodrigues and Petrini 1997, Brown, Hyde and Guest 1998,Froshlich and Hyde 1999). Since the internal tissue of healthy plants is one of the little explored niches of fungi, the study of endophytes is expected to increase our knowledge of fungal biodiversity (Hawksworth 1991, Rodrigues and Samuels1992, 1994).

Agriculture is the mainstay of majority of the rural people in Meghalaya and potato (*Solanum tuberosum* L.) is one of the highly important crops in the state and is widely cultivated in South-West Garo Hills district of Meghalaya.The present study aimed at isolating and identifying fungal endophytes from different parts of the potato plant as such an investigation has not been carried out particularly in the potato crop of the said district in the state Meghalaya.

## 2. Materials and Methods

### *2.1 Soil temperature*

Soil temperature was noted at the time of soil sampling at monthly intervals with the help of soil thermometer.

### *2.2 Soil pH*

Ten grams of freshly collected soil was taken in a beaker containing 50ml of distilled water and stirred for twenty minutes on a magnetic stirrer. The solution was then kept overnight and the pH was read by using electronic digital pH meter.

 

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### *2.3 Soil Moisture Content*

The moisture content of the soil was determined by oven dry basis. Ten grams of freshly collected soil sample was kept in a hot air oven at 105ºC for 24 hours and reweighing the dried sample till a constant weight was obtained. The percentage moisture content was calculated as follows:

Moisture content (%) = $(W_1 x W_2)/ (W_1)$ x100

Where, $W_1$=initial weight

$W_2$=final weight

### *2.4 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

## 3. Results

### *3.1 Soil physico -chemical analysis*

Soil temperature showed variation in two crop cycles, gradually fluctuating in the first crop cycle (Table 1). It was recorded to be the highest in the month of october (27.66 ºC) and least in the month of march (24.16 ºC) for first crop cycle. In the second crop cycle highest was recorded in the month of october (38.33 ºC) and least in the months of december and january (20.33ºC).

pH of the soil ranged from 5.63-6.7(Table 1).pH of 6.7 was highest for the month of january and lowest for the month of march with a pH 6.06 for the first crop cycle. In the second year high pH was observed for the month of february (6.1) and least for the month of october (5.63).

The moisture content of the soil (Table 1) was highest in the month of october (16.13%) and lowest for the month of december (10.63%) in the first crop cycle. However in the second year, it recorded the maximum in the month of october (23.48%) and minimum in the month of december (3.89%). It can be observed the active months for soil physico-chemical analysis for the soil temperature, pH and moisture content fall under the months of october,december,and January (Figure 1).

| Soil properties | 2014 | | | | | | 2016 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Oct | Nov | Dec | Jan | Feb | Mar | Oct | Nov | Dec | Jan | Feb | Mar |
| Soil temp (ºC) | 27.66 | 27 | 26.33 | 26.33 | 25.86 | 24.16 | 38.33 | 24.33 | 20.33 | 20.33 | 22.66 | 34.66 |
| pH | 6.23 | 6.23 | 6.31 | 6.7 | 6.1 | 6.06 | 5.63 | 5.81 | 6 | 5.9 | 6.1 | 5.86 |
| Moisture content (%) | 16.13 | 11.93 | 10.63 | 12 | 13.8 | 13 | 23.48 | 4.68 | 3.89 | 4.39 | 17.23 | 6.01 |

**Table 1: Physico- chemical properties of soil during the study period 2014-2016**

 

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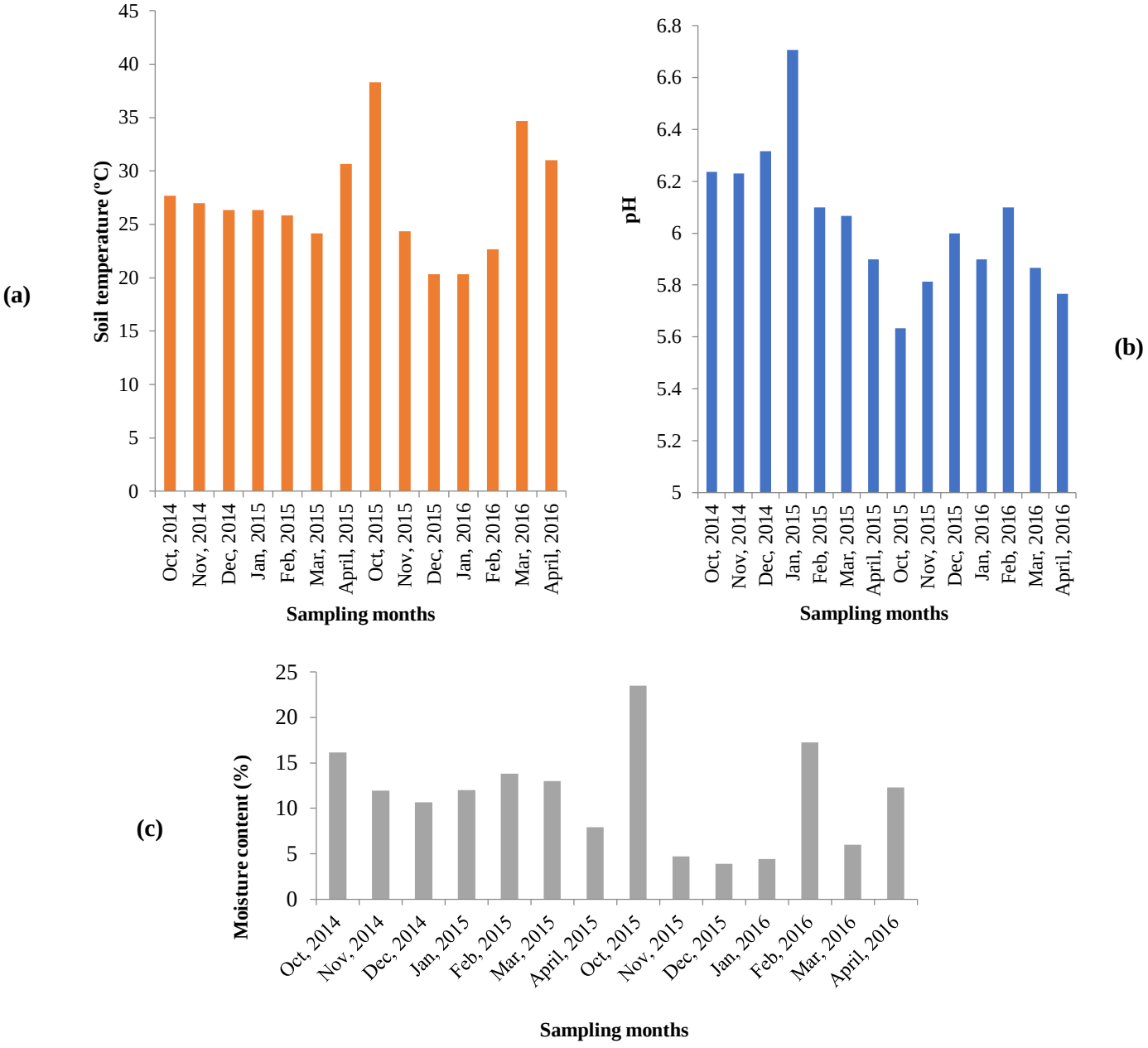


**Figure 1: Graphical representation showing the physico-chemical properties of (a) soil temperature, (b) pH and (c) moisture content during the study period 2014-2016**

 

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### *3.2 Isolation and identification of fungal endophytes*

A total of 44 endophytic fungal were isolated from the different parts of the potato plant with atotalisolates of 18 genera that included the isolation of major phytopathogens of potato plant viz. *Alternaria solani*, *Fusarium solani*, *Rhizoctonia solaniand Sclerotium rolfsii* (Figure 2). *Penicillium* recorded the highest occurrence with a total of 13 species followed by *Fusarium* with an occurrence of 5 species (Table 2). The genera *Alternaria, Aspergillus, Cladosporium* recorded with 3 species each whereas *Humicola, Phoma, Pythium* and *Trichoderma* occurred with 2 species (Table 2). However the genera *Absidia, Acremonium, Apiospora, Cochliobolus, Gonytrichum, Nectria,Phytophthora, Rhizoctonia* and *Sclerotium* had minimum number of occurrence with 1 species (Table 2). The maximum occurrence in the fungal endophytes *Acremoniumcereale, Cladosporium cladosporioides* and *Nectria ventricosa* has been isolated from all the parts of the potato plant (Table 2). Howeverthe species such as *Aspergillus fumigatus,Cochliobolus spicifer,Cladosporium sphaerospermum,Humicola grisea, Gonytrichum macrocladum, Fusarium solani, F. semitectum, F.redolens, Pythium irregulare, P.intremedium, Penicillium stoloniferum,P. simplicissimium, P.sacculum, P.lanosum, P.expansum,Rhizoctonia solani, Trichoderma harzianum* have been isolated only from a single part of the potato plant throughout the collecting season (Table 2).

**Table 2: Colonisation frequencies of fungal endophytes isolated from the different parts of the potato plant during the study periods 2014-2016**

| | | 2014 | | | | 2016 | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sl.No. | *Endophytic fungal species* | Leaf | Stem | Tuber | Roots | Leaf | Stem | Tuber | Roots |
| | Zygomycota- 1Genera, 1 Species | | | | | | | | |
| 1 | *Absidia cylindrospora* | - | - | - | - | + | + | - | - |
| | Ascomycota-13 Genera, 37 Species | | | | | | | | |
| 2 | *Acremonium cereale* | + | + | + | - | + | + | + | + |
| 3 | *Apiospora montagnei* | + | - | - | - | + | - | - | + |
| 4 | *Aspergillus flavus* | - | + | + | - | - | + | + | - |
| 5 | *A. fumigatus* | - | + | - | - | - | - | - | - |
| 6 | *A. niger* | + | - | - | + | - | - | - | - |
| 7 | *Cladosporium macrocarpum* | - | - | - | + | + | - | + | + |
| 8 | *C. cladosporioides* | + | + | - | + | + | + | + | + |
| 9 | *C. sphaerospermum* | - | - | - | - | - | + | - | - |
| 10 | *Cochliobolus spicifer* | - | + | - | - | - | - | - | - |
| 11 | *Fusarium oxysporum* | - | + | - | + | + | + | - | + |
| 12 | *F. redolens* | - | - | - | + | - | - | - | - |
| 13 | *F. semitectum* | - | - | + | - | - | - | - | - |
| 14 | *F. solani* | - | - | + | - | - | - | - | - |
| 15 | *F. sporotrichioides* | - | + | - | + | - | - | - | - |
| 16 | *Alternaria alternata* | + | - | + | - | - | - | + | - |
| 17 | *A. brassicicola* | - | + | + | - | - | - | - | - |
| 18 | *A. solani* | + | + | - | - | - | - | - | - |
| 19 | *Gonytrichum macrocladum* | - | - | - | - | + | - | - | - |
| 20 | *Humicola fuscoatra* | + | - | - | + | + | + | + | + |

 

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 21 | *H. grisea* | - | - | - | - | - | + | - | - |
| 22 | *Nectria ventricosa* | + | + | + | + | - | + | + | + |
| 23 | *Penicillium brevicompactum* | + | - | + | - | + | - | - | + |
| 24 | *P. canescens* | - | - | + | - | + | + | + | - |
| 25 | *P.chrysogenum* | - | + | + | - | + | - | - | + |
| 26 | *P. expansum* | - | + | - | - | - | - | - | - |
| 27 | *P. funiculosum* | - | - | - | - | + | - | - | + |
| 28 | *P. janthinellum* | + | - | - | - | + | + | + | + |
| 29 | *P. jensenii* | - | - | + | - | + | + | + | + |
| 30 | *P. lanosum* | - | - | - | - | + | - | - | - |
| 31 | *P. purpureogenum* | + | - | - | - | - | - | - | + |
| 32 | *P. rubrum* | - | - | - | - | + | - | + | + |
| 33 | *P. sacculum* | - | - | - | - | - | + | - | - |
| 34 | *P.simplicissimum* | - | - | - | - | - | - | - | + |
| 35 | *P. stoloniferum* | - | - | - | - | - | - | - | + |
| 36 | *Phoma eupyrena* | + | + | - | - | - | - | - | + |
| 37 | *P. medicaginis* | - | - | - | - | - | + | + | - |
| 38 | *Trichoderma harzianum* | - | - | - | - | + | - | - | - |
| 39 | *T. viride* | + | - | - | + | - | - | - | - |
| | Oomycota- 2 Genera, 3 Species | | | | | | | | |
| 40 | *Phytophthora cinnamomi* | + | + | - | + | - | - | - | - |
| 41 | *Pythium intermedium* | - | - | + | - | - | - | - | - |
| 42 | *P. irregulare* | - | - | - | - | - | - | + | - |
| | Basidiomycota-2 Genera, 2 Species | | | | | | | | |
| 43 | *Rhizoctonia solani* | - | - | + | - | - | - | - | - |
| 44 | *Sclerotium rolfsii* | - | + | - | + | - | - | - | - |

'+'= Fungal Species Present; '-'= Fungal Species Absent

 

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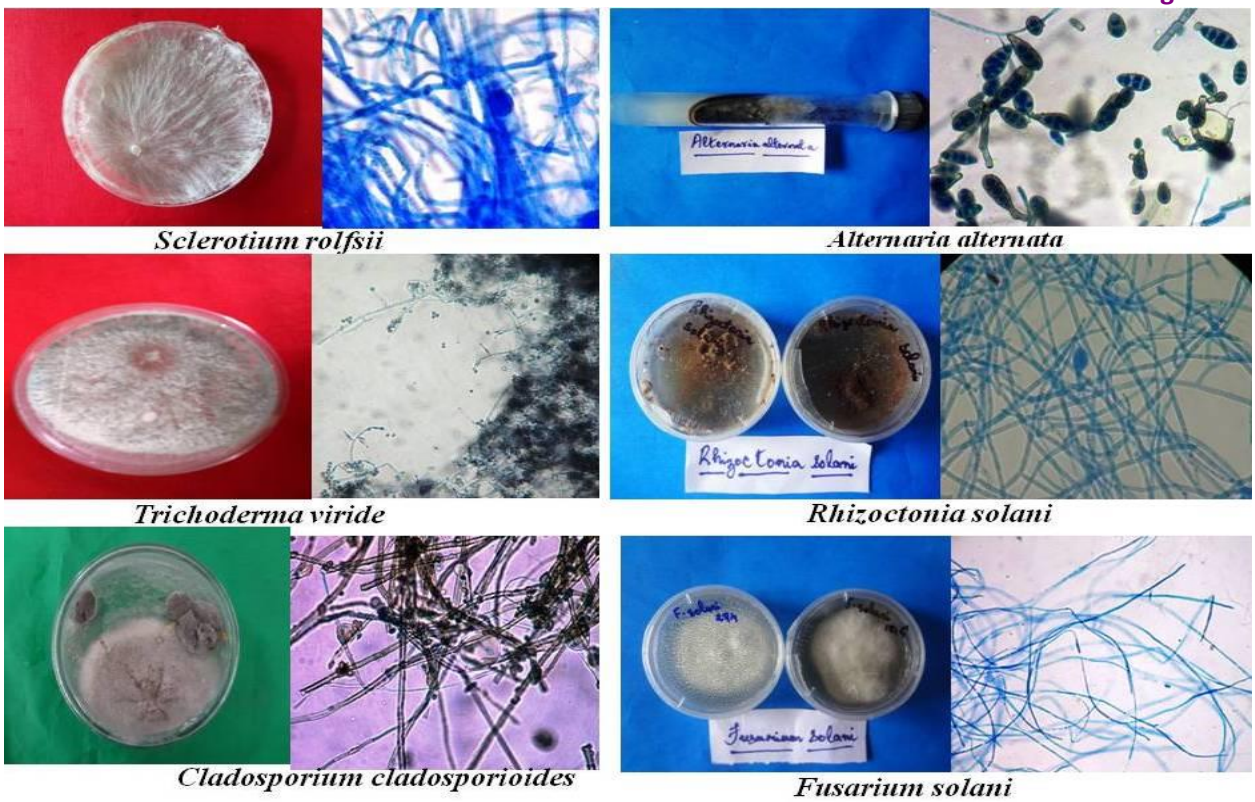


**Figure 2: Pure cultures isolates and identification of few endophytic fungi isolated from the different parts of the potato plant during the study periods 2014-2016**

## 4. Conclusion

Soil temperature was observed to be the highest in the month of October that is the pre-planting season for the two crop cycles thus the soil has less vegetation cover exposing the bare soil which allows direct penetration of the solar rays and increases its soil temperature.

The pH of the soil was found to be moderately to slightly acidic and the soil being cultivable showed increase soil acidity due to increased oxidation of organic matter (Bullock, 1992). The optimum pH range commonly reported for potatoes is 5.5 to 7.5 (Smith 1940), with higher pH levels causing significant yield reductions in some cases (Odland and Albritton 1950) and little effect in others (Blodgett and Cowan 1935).The change in pH is governed by plant roots (Smiley, 1974; Romheld, 1986; Hinsinger, 1998; Jaillard *et al*., 2001) which arise mostly from the release of $H^+$ or $OH^-$/$HCO_3$- to counterbalance a net excess of cations or anions entering the roots (Nye, 1981; Haynes, 1990; Hinsinger, 1998).

The result showed that the soil moisture content was highest in the month of pre-planting month which is October because some terrestrial ecosystems can exhibit higher water content than that of the surrounding soil as result of ´´hydraulic redistribution´´ (Caldwell and Richards, 1989).Water from deeper soil profile is accessed by deep roots, transported to roots in surface soil, and can ultimately move out into root surface soil. Papendick and Campbell (1975) also reported than an actively transpiring plant removes huge quantities of water from the soil.

The maximum colonization for the two crop 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*).*

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# A Brief Author Biography

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**2nd Author –** Prof. Highland Kayang joined the Department of Botany as a Lecturer in the year 1997, Reader in the year 2006 and as a Professor in the year 2012. Since the inception of his lectureship, Prof. Kayang has been actively involved in research in soil microbiology, microbial diversity, biodiversity conservation.

 