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Diversity of Rhizosphere fungal Mycoflora of Spinach

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ABSTRACT

The present investigation deals with the enumeration of fungal species and studies on their diversity in rhizosphere of Spinach. A total number of 14 fungal species were isolated. The most common genera were *Aspergillus, Trichoderma, Fusarium, Penicillium, Rhizopus, Chaetomium,* and *Cladosporium*. The study reveals the distribution pattern of fungal population in the soil during various treatments at 41, 76 days, and 111 days age of the plant.

Key words: Spinach, Rhizosphere, fungal mycoflora, diversity

INTRODUCTION

Spinach (*Spinacea Oleracea* L.) is one of the most important leafy vegetable crop plants.The term rhizosphere was first introduced by (Hiltner 1904; Hartmann *et al.,* 2008). He defined rhizosphere as a zone of influence next to the roots of plants where certain microorganisms become more abundant than in the soil.

According to Waksman (1952), abundance of microorganisms in soil is influenced by many factors like organic matter, soil reaction, moisture and temperature etc. High microbial density in rhizosphere is due to the presence of the organic compounds exuded by roots (Alaxender, 1977). Microorganisms growing on plant roots can influence plant growth (Tapwal *et al.,* 2003). The application of organic manure stimulates the microbial population and diversity (Zhong *et al.,* 2010). Soil fungal diversity was found to be a good and sensitive indicator of soil fertility (He *et al.,* 2008).Many evidences highlight the importance of the root micro biome in determining plant health and productivity (Berendsen *et al*., 2012).Present study reveals the diversity of fungi associated with spinach in rhizosphere and non rhizosphere soil samples. The objective of this study was to evaluate the effect of weed manures on rhizosphere mycoflora of spinach with respect to the soil fungal population.

MATERIALS AND METHODS

Collection of soil Samples

The Soil samples were collected from the rhizosphere and non rhizosphere regions from each experimental pot at 41, 76 and 111days intervals. The soil samples were stored in sterile polythene bags for laboratory study.

Isolation of microorganisms

The fungi were isolated by serial dilution method (Warcup 1960) using Potato Dextrose Agar (Dubey and Maheshwari, 2002).

Identification

The isolated fungi were identified on the basis of colony and morphological characters up to species level as per Gilman (1945) and Mukadam (1997).

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Enumeration

Enumeration of fungi in rhizosphere and non rhizosphere soil was done by following method:

1. Number of Colony Forming Units (CFUs) - (Dubey and Maheshwari, 2002)

$$CFU = \frac{\text{No. of colonies x Dilution factor}}{\text{Dry weight of soil (gm.)}}$$

2. Rhizosphere: Soil (R: S Ratio): (Dubey and Maheshwari, 2002)

$$R{:}Sratio = \frac{\text{CFU of rhizosphere soil}}{\text{CFU of non-rhizosphere soil}}$$

Diversity indices: (Poole, 1974)

a) Simpson's diversity index (D)

$$\sum_{i=1}^{s} \frac{n_i(n_i-1)}{N(N-1)}$$

b) Shannon's diversity index (H)

$$-\sum_{i=1}^{s} p_i \ln p_i$$

Where,

$$p_i = \frac{\text{No.of organisms in the species}}{\text{Total organisms}}$$

In pi = Natural log pi

c) Evenness Index (E_H): $E_H = H/H_{max} = H/lnS$

Where, H= Shannon's diversity index.

H_{max} = Maximum possible value of H = lnS

S = Species richness (Total no. of species in the community).

ABBREVIATIONS:

CON = Control; CFU = Chemical fertilizer urea; GMT = Green manure *Tephrosiaperpurea*; GMA = Green manure *Achyranthesaspera*; COT = Compost *Tephrosiaperpurea;* COA = Compost *Achyranthesaspera;* VMT = Vermicompost *Tephrosiaperpurea*; VMA = Vermicompost *Achyranthesaspera.*

RESULTS AND DISCUSSION

In this study of rhizosphere mycoflora of spinach 14 species of fungi viz. *Aspergillus niger* Link., *A. Flavus* Link., *A. terreus* Link., *A. roseus, Tricoderma viride* Pers, *Colletotrichum spinaceae, Rhizoctonia solani, Colletotrichum spinaceae, Rhizoctonia solani* Dc. Ex fries, *Alternaria spinaciae, Rhizopus oryzae, Mucor sp.*

Table 1: Impact of *Tephrosia* and *Achyranthus* weed on Rhizosphere mycoflora of spinach (41 days)

Fungi isolated (CFUX 10^{-4})	**Non rhizosphere**	**Rhizosphere**							
		Age of the plant (41days)							
		GMT	GMA	COT	COA	VMT	VMA	CFU	CON
Aspergillus niger	3.0	5.7	5.3	4.7	7.0	7.0	6.0	5.0	3.7
Aspergillus flavus	3.0	4.7	4.7	2.0	5.0	5.0	4.7	3.7	3.3
A. terrus	2.3	3.7		3.0	1.0	2.0		1.0	2.3
A. roseus	1.0		2.3	1.0		4.0	4.3	2.0	1.0
Trichodermaviride		1.0					2.7		
Colletotrichum spinaceae	1.0							2.0	1.3
Rhizoctonia solani	1.3								1.3
Fusarium oxysporum. f.sp.spinacia			1.0						
Alternaria sp.	0.3								1.3
Rhizopus oryzae	1.0		2.7	3.0	2.0	2.0			
Mucar		2.3	2.0	2.0			2.0	1.0	
Penicillium notatum				1.0	1.0	2.0	1.0		1.0
Chaetomium sp.	1.0	2.3			2.0			2.0	1.0
Cladosporium sp.	1.3			3.3				1.7	2.3
Sterile mycelium	1.0		1.0		2.0		1.0		

Table 2: Rhizosphere mycoflora of spinach (76 days)

Fungi isolated (CFUX 10^{-4})	**Non rhizosphere**	**Rhizosphere**							
		Age of the plant (76 days)							
		GMT	GMA	COT	COA	VMT	VMA	CFU	CON
Aspergillus niger	2.3	6.3	7.3	6.7	6.0	7.0	7.3	4.0	3.0
Aspergillus flavus	3.0	2.7	4.7	5.0	5.3	5.3	6.3	3.7	3.0
A. terrus		2.0		4.3	2.7				
A. roseus	2.0		4.0			3.7	3.0	2.7	2.3
Trichoderma viride		1.0				2.3	2.7		
Colletotrichum spinaceae				1.7					2.0
Rhizoctonia solani	0.3							2.0	
Fusarium oxysporum .f.sp.spinacia	2.7			0.3				1.7	2.3
Alternaria sp.	0.3								
Rhizopus oryzae	0.7	2.3	2.0		2.7	2.7	2.3		0.3
Mucar			1.3	0.3			1.3		
Penicillium notatum		1.3				1.3			0.3
Chaetomium sp.	1.0				3.7			3.0	1.3
Cladosporium sp.	1.7				2.3			2.0	2.3
Sterile mycelium			1.0						

Table 3: Rhizosphere mycoflora of spinach (111 days)

Fungi isolated (CFUX 10^{-4})	Non rhizosphere	Rhizosphere							
		Age of the plant (111 days)							
		GMT	GMA	COT	COA	VMT	VMA	CFU	CON
Aspergillus niger	3.0	6.0	6.0	6.7	6.3	7.3	7.0	4.0	3.7
Aspergillus flavus	2.0	5.0	5.3	5.7	6.3	6.3	7.0	3.0	2.7
A. Terrus	0.3					3.7	2.0		1.0
A. Roseus	2.0	4.3	4.7	4.0	3.7	2.0	3.0	2.7	2.0
Trichoderma viride			2.0						
Colletotrichum spinaceae	1.0							2.3	2.0
Rhizoctonia solani	1.3								1.0
Fusarium oxysporum f.sp.spinacia	2.0							2.0	1.7
Alternaria sp.	0.3								0.3
Rhizopus oryzae				3.3		3.3		1.3	0.3
Mucar		2.0					1.7		
Penicilliumnotatum			1.3			0.7			2.3
Chaetomium sp.	2.0		2.0		4.0		2.0		2.3
Cladosporium sp.	1.7			2.3			1.0	2.0	2.7
Sterile mycelium			1.0						

Penicillium notatum Link., *Chaetomium globosum*. Kunze and Schmidt, *Cladosporium fulvum* Link were recorded.

The results indicated that the population of *A. niger*, *A. flavus*, *A. roseus*, increased significantly in all the treatments as compared to control and non rhizosphere. This may be due to the root exudates of spinach which might have enhanced the population of fungi. Maximum number of colonies *of A. niger*, *A. flavus*, and *A. roseus* was observed in VMT followed by VMA, GMT, GMA, COA, COT as compared to CFU, Control and non rhizosphere. *Trichoderma* was maximum in VMT and VMA after 111 days but it was not observed during first two harvest.

Pathogenic fungi *like Colletotrichum spinaceae, Rhizotonia solani, Fusarium oxysporum, Alternaria sp*. was more in control, non rhizosphere and CFU in all 3 harvests. These were Found minimum after 76 days and are totally absent during harvest 3 due to increased microbial activity. *Rhizopus* is saprophytic as well as pathogenic fungus was observed in compost and vermicompost treatment but it was absent in green manure. It was observed irregularly in remaining treatments. *Chaetomium* and *Cladosporium* observed in control, non rhizosphere and most of the treatments but *chaetomium* was maximum in GMA and *Cladosporium* was maximum in the treatment of GMC.

To study the diversity of fungi in the Rhizosphere and Non Rhizosphere region, the soil samples was analyzed for number of genera, number of species and total number of colonies. The results are tabulated in Table 1, 2 and 3. The increased microbial population in the rhizosphere as compared to non Rhizosphere is called Rhizosphere Effect (Rangaswami and bagyaraj, 1998).

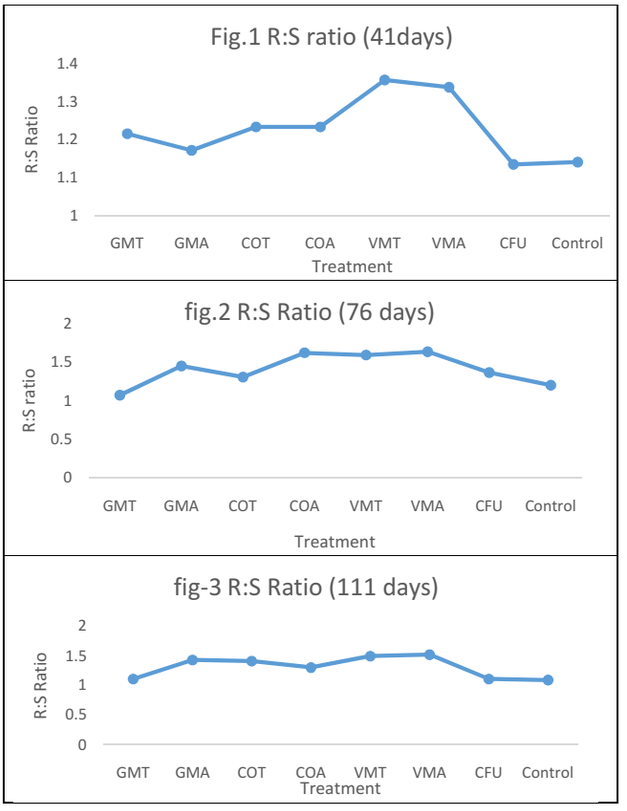

Fig. : Rhizosphere: Soil (R: S Ratio)

Table 4: Statistical analysis of diversity of fungi

Diversity	Simpson's Index (D)			Shannon's Diversity Index (H)			Evenness (E_H)		
	Age of the plant(days)			Age of the plant (days)			Age of the plant (days)		
	41	76	111	41	76	111	41	76	111
Non Rhizosphere	0.063	0.064	0.066	2.19	1.9	1.98	0.82	0.71	0.75
GMT	0.163	0.19	0.234	1.75	1.55	1.29	0.66	0.58	0.48
GMA	0.145	0.167	0.156	1.72	1.53	1.67	0.65	0.57	0.63
COT	0.107	0.231	0.189	1.92	1.37	1.51	0.72	0.51	0.57
COA	0.178	0.152	0.226	1.67	1.67	1.33	0.63	0.63	0.5
VMT	0.173	0.174	0.19	1.62	1.62	1.61	0.61	0.61	0.61
VMA	0.151	0.226	0.176	1.72	1.6	1.66	0.65	0.6	0.62
CFU	0.116	0.109	0.106	1.85	1.84	1.84	0.7	0.69	0.69
Control	0.076	0.086	0.072	2.08	1.93	2.11	0.78	0.73	0.80

The Similar effects were also observed in the mycoflora associated with Spinach. There was increase in the Rhizosphere: Soil (R: S Ratio) in all the treatments as compared to CFU and control. (Fig. 1, 2 and 3). It goes increasing with the age of the plant.

A diversity index provides information about the community composition and structure (Simmons, 1970). The Simpson's diversity index (D), Shannon's diversity index (H) and the Evenness (E_H) were calculated for all the treatments at 41days, 76 days and 111 days of sowing. The results are tabulated in Table 4.

The Simpson's diversity index (D) indicates high diversity in Non Rhizosphere region. Least diversity was indicated in rhizosphere region of all the treatments as compared to CFU and control. The values of Shannon's diversity index (H) indicated higher diversity in the rhizosphere population of all the Treatments as compared to CFU and control. Evenness (E_H) of the species was found to be high as compared to non Rhizosphere, and control.

CONCLUSION

The detail study on the mycoflora of the rhizosphere and non rhizosphere of spinach revealed distinct rhizosphere effect. The present information will help as ground work for the study on role of rhizosphere fungi in growth of spinach.

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