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Relationship between Phylloplane Mycroflora Aeromicroflora and Soil Microflora

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Abstract: ***Aeromicrobial studies of microbe population are for understanding the survival of microbes in the soil leaf surface and air.The objective of this study was to isolate and evaluate relationship between phylloplane micro flora aero micro flora and soil micro flora. Qualitatively studies on a total my co flora my co flora 30 fungi belonging to 11 genera with sterile form and pink yeast.***

I. INTRODUCTION

The occurrence of a wide variety of microorganisms in the atmosphere have a direct correlation between surface colonizers and atmospheric spores as the spores from atmosphere settle down on the surface of the plants which play an important role in the leaf infection (or infection on aerial parts) and in the development of disease syndrome. In other words we can say that from open air, leaves remove some of the microbial burden normally carried out by wind as they remain in touch with the environment. Spores trapping by leaves is a natural phenomenon in nature. Spores and other propagules in the air reach the leaves by sedimentation under the influence of gravity, and by rain and splash droplets. Leaf surface commonly known as phylloplane provides a suitable habitat for the growth of antagonistic microorganisms which can compete with the pathogen for nutrients and inhibit pathogen multiplication by secreting antibiotics or toxins (Blakeman, 1982; Yadav et al., 2011). The phyllosphere of plants is a dynamic ecosystem inhabited by specific bacteria, yeasts and fungi. Their activity is related to various interactions between the biotic and abiotic factors of the environment (Behrendt et al., 1997, 1999). Interactions of microorganisms living on the surface of the plants aboveground parts are based on antibiosis, competition and parasitism (Chakraborty et al., 1994, Stromberg et al., 2000) which protect the plants from pathogenic microorganisms and improving their health.

Thus studies of aeromicroflora are necessary to understand the survival of fungi in air, phylloplane and soil. The present study was undertaken to assemble the following information.-

Periodicity of aeromicroflora over Stevia field

To evaluate the relationship between phylloplane microflora between aeromicroflora and soil microflora.

II. MATERIAL AND METHODS

Aeromycoflora of *Stevia* field was obtained by gravity petridish method (Durham1946). Four petri dishes containing Martins Rose Bengal agar medium were exposed for 5-10 minutes at 4 feet height above the ground level at 8 a.m.,1 p.m. and 6 p.m. The plates were transferred to the laboratory and kept for incubation at 25^0 C $\pm 2^0$ C for a period of 7 days and then the plates were examined for the development of fungal colonies. The number of fungal colonies appeared were counted. The numerical expression of the fungal population was obtained by

$$\text{Bacterial / fungal propagules / 100cm}^3 = \frac{\text{Total number of colonies in five plates}}{\text{Volume of Petri dish x 5}} \times 100$$

Percentage of abundance and percentage of frequenvy of each species occurring on different plate was calculated as follows:-

$$\text{Percentage of abundance} = \frac{\text{No. of colonies of individual species in all the plates studied}}{\text{Total no. of colonies of all the species}} \times 100$$

$$\text{Percentage of frequency} = \frac{\text{No. of colonies of individual species in all the plates studies}}{\text{Total no. of observations}} \text{x100}$$

III. RESULTS AND DISCUSSION

A total of 40 fungal comprising zyomycetes ascomycetes and deuteromycetes including sterile forms were isolated during the course of investigation. Seasonal and diurnal fluctuation in the fungal content of air over *Stevia* field in year 2013 are given in table 1.6. the content of air at a particular sampling time varied between 6.2and 35.6 propagules/100cm^3. Minimum number of propagules was trapped on 06/03/013 at 6p.m. and maximum number on 20/06/013 at 1p.m.in the 2nd sampling the fungal content in the air i.e mean of all the twelve petri dishes exposed in a day was highest. The fluctuation in the aeromycoflora can be attributed to prevailing environmental condition. It is clear from the data that temperature, humidity and rainfall play a major role in quantification of aeromycoflora. The maximum number of fungal microflora spore was sampling in 2nd sampling on 26/06/013. Thirty six petri dishes containing Martin's Rose Bangal Agar Medium were exposed. A total of 639 colonies were trapped from the air of stevia field giving an average 10.75 colonies/plate. This is due to rain which provided humidity to the dry weather and helps in stimulating sporulation after the rain cease ordiffusion of phylloplane mycoflora. The lowest mycoflora on 06/03/013 may be due to low temperature and absence of rain. Jogdand (1998) and Gadekar (2014) observed greatest concentration of fungi over others during rainfall while investigating aerospora on Jowar crop.

Quintero *et a*l., (2017) reported that increasing humidity induces sporulation and Levetin and Horner (2002) showed that rain drops facilitating the dispersion of these from vegetation. Indiurnal cycle fungi showed afternoon tendency in all the samples except 1st sampling. Second peak was obtained at eveningexcept in the 1st when it was obtained at afternoon. Morning and evening gave minimum count in all except 1st sampling. A total of thirty fungal forms (table -6.2) were trapped from the air during the period of investigation, the dominant species above75% frequency of occurrence were a.alternata, c. cladosporioidies and sterile white mycelia. Fungal species with 50-75% frequency of occurrence were A.tenuissima, A.niger andP.citrinum. Highest occurrence of air mycoflora in month of June might be due to increase in temperature.

The periodicity and occurrence of various fungi in the air over many crop in relation to environmental condition were studied by Afzal *et al*.,2004, Uddin, 2004,2005,2007; Ray *et al* .,2011 Irga And torpy, 2015.

Table-6.1 Seasonal and diurnal variation in the density aeromycoflora per 100 cm^3 over *S.rebaudiana* field

Sampling time	I 06/03/13	II 20/06/13	III 20/09/13
8 a.m.	15.1	25.4	13.63
1 a.m.	7.80	35.6	22.28
6 a.m.	6.20	26.4	14.94

Two-way analysis of table

Source of variation	Sum of square	DF	Mean of Square	F	P-value	F crit
Among means of treatment A	68.3929	1	68.3929	79.07607816	0.071291	161.4476
Among means of treatments B	153.5121	1	153.5121	177.491155*	0.047696	161.4476
Residual	0.8649	1	0.8649			

A=Diurnal cycle B= Time interval;DF= Degree of freedom,* Significant at 0.05 level

Table-1.2 Percentage abundance of aeromycoflora over *S.rebaudiana* field

S.N.	Name of microorganism	sampling no., date & sampling time											
		I			II			III					
		06/03/13			20/06/13			30/09/13					
		8 am	1 pm	6 pm	8 am	1 pm	6 pm	8am	1 pm	6 pm			
1.	Alternariaalternata	5.3	15.4	28.2				31	36.6	13.1	10.4	-	2.5
2.	Alernariatenuissima	21.5	19.8	17.5				-	-	-	-	-	5.4
3.	Aspergillusfumigatus	-	-				-	13.3	4.3	-	-	-	-

4.	A. niger	6.8	-	-	1.03	-	1.2	-	1.2	-
5.	Botrytis cinerea	-	-	-	-	1.50	-	-	-	-
6.	Cladosporiumclodosporioides	37.9	13.3	17.3	-	17.6	7.2	25.9	28.5	15.8
7.	Curvularialunata	-	-	-	-	-	-	-	3.5	-
8.	Diplococciumspicatum	-	-	-	-	-	16.2	-	-	-
9.	Dreschleratrigonis		-	-	-	-	-	-	5.7	-	-
10.	Fudariumsporotrichioides	-	-	-	22.9	10.4	13.5	-	-	-
11.	Geotrichumcandidum	-	-	-	39.2	45.3	29.3	-	-	-
12.	Mucorhiemalis	-	-	-	-	1.48	-	5.3	8.9	-
13.	Penicilliumcitrinum		-	-	13.1	1.03	-	-	-	7.1	9.2
14.	P. cyclopium		-	-	8.6	-	-	-	-	-	-
15.	P. expansum		3.4	10	-	-	-	-	-	-	-
16.	P. Purpurogenum	3.4	-	17.3	-	-	-	-	-	-
17.	P. notatum		-	-	-	-	-	-	5.7	-	-
18.	P. oxalicum		-	-	-	-	-	-	1.9	4.7	-
19.	penicillium sp.	-	-	-	-	-	9.0	-	-	-
20.	Rhizopusoryzae	-	-	-	-	-	-	3.8	-	-
21.	Scopulariopsisbrevicaulis	-	-	-	-	5.2	-	-	-	-
22.	Scytalidiumlignicola	6.8	-	-	-	-	-		-	8.8
23.	Scytalidium sp.	-	-	-	-	-	-	-	-	7.68
24.	Sporobolomycesroseus	-	-	-	-	-	-	7.7	5.9	2.3
25.	Sporothrix sp.	-	-	-	-	-	-	1.9	3.6	3.5
26.	Syncephalastrumracemosum	-	-	-	10.3	13.3	8.1	-	-	-
27.	Trichodermahamatum	-	-	-	10.4	5.0	7.8	-	-	-
28.	Trichophyton sp.	-	-	-	-	-	-	-	-	3.5

29.	Sterile Brown mycelia	1.7	6.6	4.3	-	-	-	-	-
30.	Sterile white mycelia	8.6	20.0	20.6	3.1	-	-	15.3	2.4

Table 6.3 Percentage contribution & percentage frequency of aeromycoflore over *S.* rebaudiana field

S.N.	Name of microorganisms	%contribution	%frequency
1.	Alternariaalternata	12.52	88.89
2.	Alernariatenuissima	6.89	44.45
3.	Aspergillus fumigates	0.79	22.22
4.	A. niger	1.26	44.45
5.	Botrytis cinerea	0.32	11.11
6.	Cladosporiumclodosporioides	16.90	88.89
7.	Curvularialunata	0.47	11.11
8.	Diplococciumspicatum	1.41	11.11
9.	Dreschleratrigonis	0.47	11.11
10.	Fudariumsporotrichioides	7.98	33.33
11.	Geotrichumcandidum	20.50	33.33
12.	Mucorhiemalis	2.04	33.33
13.	Penicilliumcitrinum	2.35	44.44
14.	P. cyclopium	0.31	11.11
15.	P. expansum	0.78	22.22
16.	P. Purpurogenum	0.94	22.22
17.	P. notatum	0.47	11.11
18.	P. oxalicum	0.78	22.22
19.	penicillium sp.	1.57	11.11
20.	Rhizopusoryzae	0.31	11.11
21.	Scopulariopsisbrevicaulis	1.09	11.11
22.	Scytalidiumlignicola	1.25	22.22
23.	Scytalidium sp.	0.47	11.11
24.	Sporobolomycesroseus	1.57	33.33
25.	Sporothrix sp.	0.94	33.33
26.	Syncephalastrumracemosum	5.79	33.33
27.	Trichodermahamatum	4.07	33.33
28.	Trichophyton sp.	0.31	11.11
29.	Sterile Brown mycelia	0.63	33.33
30.	Sterile white mycelia	4.85	77.78

Table 6.4 Value of colony forming units (cfu/g) for fungi in soil at 10^{-3} dilution

Sampling no. and date		
I	II	III
06/03/13	20/06/13	30/09/13
0.54	1.3	0.733

Table 6.5 Percentage occurrence of fungal species in soil (10^{-3} dilution) obtained by soil dilution plating technique

S.N.	Name of microorganisms	Sampling no. and sampling date		
		I	II	III

		06/03/13		20/06/13		30/09/13	
1.	Aspergillusflavus	18.72		11.52		-	
2.	A. fumigatus		37.83		10.64		-
3.	A niger	2.54		7.98		-	
4.	A terreus		1.5		6.65		-
5.	Aspergillus sp.	-		2.66		-	
6.	Cladosporiumcladosporioides		3.75		7.8		5.15
7.	Curvularialunata	-		-		9.27	
8.	Emericellanidulans		-		2.66		-
9.	Fusariumsporotrichioides	-		6.65		3.09	
10.	Geotrichumcandidum	-		-		3.03	
11.	Gliocladium sp.	-		-		5.15	
12.	Histoplasma sp.	-		-		26.78	
13.	Microsporum sp.	-		-		7.21	
14.	Mucorhiemalis	-		2.66		-	
15.	Penicilliumcitrinum		-		7.68		-
16.	P. cyclopium		29.32		7.98		-
17.	P. oxalicum		-		-		4.12
18.	P. purpurogenum	6.76		-		-	
19.	Rhizomusorvzae	-		18.62		-	
20.	Scytalidiumlignicola	-		-		8.24	
21.	Sporobolomycesroseus	-		-		11.33	
22.	Stachybotryschartarum	-		-		2.06	
23.	Trichodermahamatum	-		-		9.27	
24.	Sterile White mycelia	-		1.83		5.15	

Table 6.6 Percentage contribution and percentage frequency of soil fungi is S.rebaudiana field

S.N.	Name of microorganisms	%contribution		%frequency	
1.	Aspergillusflavus	11.79		66.67	
2.	A. fumigatus		19.19		66.67
3.	A niger	3.37		66.67	
4.	A terreus		2.36		66.67
5.	Aspergillus sp.	0.67		33.33	
6.	Cladosporiumcladosporioides	5.39		100	
7.	Curvularialunata	3.03		33.33	
8.	Emericellanidulans		0.67		33.33
9.	Fusariumsporotrichioides	2.69		66.67	
10.	Geotrichumcandidum	1.01		33.33	
11.	Gliocladium sp.	1.68		33.33	
12.	Histoplasma sp.	8.76		33.33	
13.	Microsporum sp.	2.36		33.33	
14.	Mucorhiemalis	0.67		33.33	
15.	Penicilliumcitrinum	2.02		33.33	
16.	P. cyclopium	15.15		66.67	
17.	P. oxalicum	1.35		33.33	
18.	P. purpurogenum	3.03		33.33	
19.	Rhizomusorvzae	2.69		33.33	
20.	Scytalidiumlignicola	2.69		33.33	
21.	Sporobolomycesroseus	3.70		33.33	

22.	Stachybotryschartarum	0.67	33.33
23.	Trichodermahamatum	3.03	33.33
24.	Sterile White mycelia	2.02	66.67

Table 6.7 Percentage abundance of phylloplanemycoflora of S. rebaudianaas obtained by dilution plate method

S.N.	Name of microorganism	Sampling no., sampling date and agr of plant (Day)								
		I			II			III		
		06/03/13			20/06/13			30/09/13		
		56			162			264		
				Y	M	Y	M	Y	M	
1.	Alternariatenuissima	-	-	-	-	10.85	5.75			
2.	Aspergillusflavus	10.12	-	-	-	15.6	12.08			
3.	A. fumigatus		-	25.91	-	25.47	-	-		
4.	A. ochraceus		-	-	-	-	-	19.71		
5.	Aspergillus sp.	-	-	-	5.7	-	-			
6.	Cladosporiumcladosporioides	9.78	25.80	9.5	-	12.54	-			
7.	Emericellanidulans		-	-	14.75	-	-	-		
8.	Mucorhiemalis	-	5	-	5.58	21.3	23.39			
9.	Penicilliumcyclopium	23.39	9.36	24.65	34.90	-	-			
10.	P. expansum		30.26	-	-	-	-	-		
11.	P. notatum		-	-	-	-	12.5	2.08		
12.	P.purpurogenum	14.67	17.55	25.54	14.2	-	-			
13.	Pericomiella sp.	9.78	5.85	12.25	-	-	-			
14.	Scytalidiumlignicola	2.05	3.51	-	-	-	8.85			
15.	Scytalidium sp.	-	-	14.75	10.49	-	-			
16.	Sporobolomycesroseus	-	-	-	13.32	20	16.4			
17.	Stachybtryschartarum	-	-	-	-	-	5.7			
18.	Trichodermahamatum	-	-	-	-	-	5.75			
19.	Seterile White mycelia	-	7.02	-	-	7.5	-			

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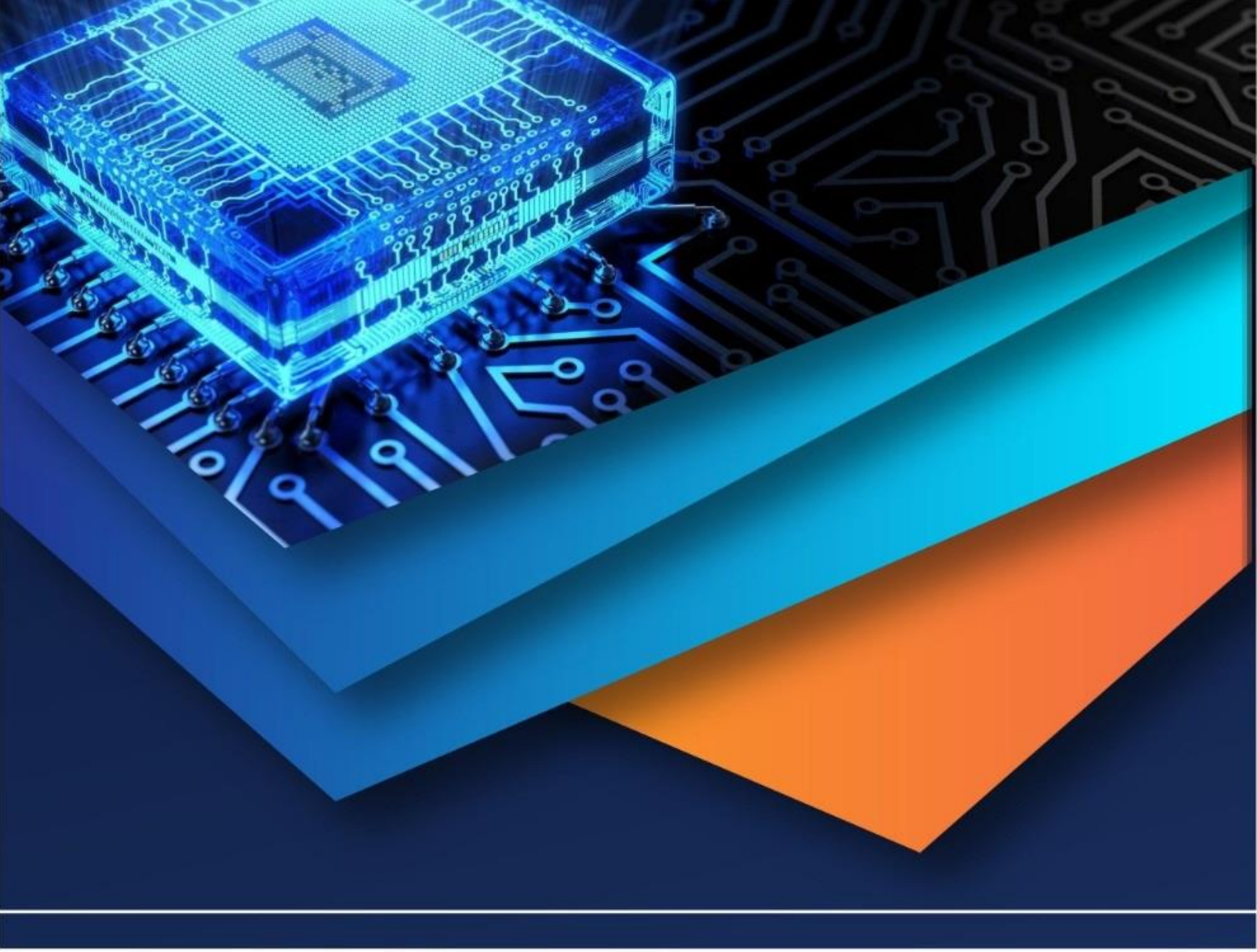

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