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ANTAGONISTIC POTENTIAL OF PHYLLOPLANE FUNGI OF *SOLANUM LYCOPERSICUM* AGAINST *ALTERNARIA SOLANI*

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ABSTRACT

Fungi are vital components of nearly all ecosystems and affect human health and our economy in many ways. Monitoring fungal biodiversity from various systems including phylloplane is essential. The occurrence of phylloplane fungi on leaf surface of tomato (*Solanum lycopersicum*) was investigated. A total number of 19 fungal species were isolated from surface sterilized leaf segments using dilution plating technique. Among these *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Aspergillus terreus, Curvularia, Fusarium oxysporum, F. moniliformis, Macrophomina phaseolina, Dreschlera, Penicillium citrinum, Penicillium chrysogenium, Rhizopus stolonifer, Trichoderma harizianum, Trichoderma viride , White sterile mycelium, yellow sterile mycelium* Diversity of phylloplane assemblages were also measured. The colony interaction between the *Alternaria solani* and the phylloplane fungi of tomato were assessed following the model of proposed by Dickinson. *Alternaria solani* overgrew the colony of *Aspergillus flavus, A. niger, A. fumigatus* and showed type A interaction while *Trichoderma harizianum, T. viride* inhibited the growth of *A. solani* and produced coiling around the *A. solani* (type B interaction). *Fusarium oxysporam, Fusarium moniliforme, Aspergillus terrus, Penicillium citrinum,* and *Peneicillium chrysogenum* inhibited the growth of *A. solani* but did not produce coiling (type C). *Rhizopus sp and Macrophomina phaseolina* met with *A. solani* colony.

Key-words: Phylloplane fungi, tomato, *Alternaria solani*, antagonistic activity, biocontrol fungi.

INTRODUCTION

Surface of plant leaves have a complex terrestrial microhabitat which is characterized by a the occurrence of variety of microorganism like bacteria, filamentous fungi and yeast. Phylloplane fungi are the mycota growing on the surface of the leaves (Lengard 1980). Phylloplane fungi are classified into two groups, i.e., residents and casual (Norse 1972). Residents can not affect the host but casual land on the leaf surface but can't grow (Leiben 1965).

Numerous investigations have been carried out on the phylloplane mycobiota assemblages of different cultivated or wild plant species (Breeze and Dix, 1981; De Jager *et al*., 2001; Andrew et al., 2002; Bakker et al., 2002; Ososno, 2002; Osono *et al*., 2004; Kishore et al., 2005; Levetin and Dorsey, 2006) in many part of the world. Dynamics of microbial populations on leaves in time and space are a function of growth and death. The surface of leaves contain stimulatory inhibitory substances that regulate the colonization of leaf surface organisms (Fating and Khare, 1978). The phylloplane microflora is subjected to the influence of various environmental factors and physiological changes in the plants (Sinha, 1965). The diversity of phylloplane fungi may be altered by gaseous, particulate atmospheric pollutants (Saunder, 1971, Mowll *et al*., 1985). Organisms that exist in the phylloplane are also influenced by air contaminants (Smith, 1976). Pathogen modifies the surface microflora of leaves which may partially or completely protect the leaf against subsequent infection by the other pathogen. The phylloplane microorganisms are of considerable importance as some of them are antagonists to pathogenic microorganisms infecting the plant species (Blakeman, 1991; El-Said, 2001; Mandhare and Suryawanshi, 2009). Thus, use of phylloplane microorganism has been important for management of foliar diseases. Indigenous fungi suppress the disease by reducing pathogen population and thereby minimize the disease severity. Phylloplane fungi are usually abundant and considerably diverse on the surface of the leaf. The antagonistic potential of several phylloplane fungi of tomato against *Alternaria solani* causing early blight disease of potato, tomato, brinjal and nightshade was examined.

MATERIALS AND METHODS

***Sample collection and cultural conditions*:**

For the isolation of fungi, leaf samples of tomato were collected and immediately brought to the laboratory in sterile polythene bags. Leaf fragments of 1cm size were cut with the help of a sterilized cork-borer and shaken in flask containing 200ml of sterilized distilled water. 0.2ml of suspension of microorganism was transferred into Petri dishes containing potato dextrose agar with Penicillium and streptomycin. The inoculum was spreaded uniformly and incubated at room temperature (30ºC) for 4 to 5 days. The fungal colonies were counted. Using the data of

colony forming units (CFU). For dual culture essay 5mm diameter inoculums disc of *A. solani* was placed near the edge of Petri dish containing PDA and similar inoculum disc of test organism were placed at opposite end. Plates were incubated at 28ºC. Colony diameter of test organism and pathogen were measured and type of interaction were also recorded.

***Measurement of species diversity*:**

A number of diversity indices have been proposed to measure diversity (Magurran, 2004). Diversity indices represent a useful means for quantifying community diversity and have been instrumental in revealing the microorganism diversity associated with the phylloplane communities (Thomas and Shattock, 1986; Joshi, 2008). Several diversity indices were employed to compare treatment effects. Various diversity measures estimate different aspect of community structure. The general species diversity of the fungal communities was measured by the generally accepted Shannon–Wiener information theory function:

$$H' = - \sum P_i \log P_i \qquad i=1 \ldots S$$

Where H' is the general species diversity and P_i the proportion of total number of CFU for fungal species belonging to the i*th* species and S equals the total number of species in the assemblage (Shannon and Weaver, 1963). . The general diversity incorporates two components of diversity: species richness, which expresses the number of species S as a function (ratio) of the total number of individuals N; and equitability that measures the evenness of allotment of individuals among the species (Magurran, 2004). The equitability component of diversity and its variance were measured in accordance with Pielou (1975):

$$J' = H' / H'_{max} = H' / \log S$$

The equitability index $J'$is the ratio between observed H' and maximal diversity $H'_{max.}$:

The species richness was ascertained using the index developed by Menhinick (1964), as follows:

$$d = S/ \sqrt{N}$$

Dominance concentration (complement of diversity) was measured by using Simpson's index (Southwood and Henderson, 2000) as:

$$D = \sum \{[n_i \ (n_i -1)] / \ [N(N-1)] \} \qquad i = 1 \ldots S$$

in which n_i number of CFU for a fungus

For the computation of diversity indices and the dominance concentration, a program package was developed by one of us (S.S.S.) in C++ and is available from the senior author at a nominal cost.

Table 1. Phylloplane fungi of tomato from two collection sites.

Fungal species	Number of colonies	
	Urdu University	Karachi University
Aspergillus flavus	14	11
A. fumigatus	14	17
A.niger	12	21
A.terreus	16	6
Fusarium moniliforme	7	3
Fusarium oxysporum	9	7
Macrophomina phaseolina	16	19
Penicillium citrinum	10	3
Penicillium chrysogenum	26	12
Curvularia sp.	0	7
Dreschlera sp.	5	0
Rhizopus sp.	9	15
Rhizoctonia solani	1	6
Trichoderma harizianum	7	4
Trichoderma viride	11	6
White sterile mycelium	2	1
yellow sterile mycelium	2	2
Black sterile mycelium	1	0

Table 2. Effect of phylloplane fungi of tomato on *in vitro* growth of *Alternaria solani.*

Fungal species	Day of Incubation	Colony diameter of		Types of Interaction
		Test fungi (mm)	Pathogen (mm)	
Aspergillus flavus	4	35	61	A
A. fumigatus	4	43	45	A
A.niger	4	46	35	A
A.terreus	5	62	43	C
Fusarium moniliforme	5	52	36	C
Fusarium oxysporum	4	35	50	D
Macrophomina phaseolina	3	45	41	D
Penicillium citrinum	4	53	37	C
Peneicillium chrysogenum	4	52	30	C
Rhizopus sp.	4	37	51	D
Trichoderma harizianum	5	55	35	B
Trichoderma viride	4	53	37	B

A= overgrowth; B= coiling of test fungus; C= growth inhibition but no coiling; D= Inhibition but colnies of both test and pathogens met.

Table 3.Fungal diversity of phylloplane fungi of tomato.

Diversity Measures	Tomato	
	Urdu University	Karachi University
Diversity H	2.592	2.582
Equitability J	0.915	0.911
Richness d	1.335	1.352
Simpson's Index D	0.796	0.866

RESULTS AND DISCUSSION

The phylloplane fungi recorded at Federal Urdu University and University of Karachi sites between which the distance is 4.5Km. At Federal Urdu University 17 species were recorded of which the dominant species were *Penicillium chrysogenum*, *Macrophomina phaseolina*, *Aspergillus terreus*, *A. flavus*, and *A. fumigatus*. At Karachi University farm (Institute of Environmental Studies) 16 species were recorded *Aspergillus niger*, *Macrophomina phaseolina*, *Aspergillus fumigatus* and *Rhizopus* sp. were found to be abundant as indicated by their high CFUs.

The diversity of tomato phylloplane mycobiota is given in Table 1 at two sites. The general diversity (H) was closely similar at the two sites. Likewise the two components of diversity, namely species richness (d) and equitability (J) also gave similar values. Dominance (D) as expected was found to be inversely related to general diversity and species richness.

The colony interaction between the *Alternaria solani* and the phylloplane fungi of tomato were assessed. *Alternaria solani* overgrew the colony of species of *Aspergillus* and showed type A interaction while *Trichoderma harzianum*, *T. viride* inhibited the growth of *A. solani* and produced coiling around the *A. solani* (type B interaction). *Fusarium oxysporam*, *Fusarium moniliforme*, *Aspergillus terreus*, *Penicillium citrinum*, and *Penicillium chrysogenum* inhibited the growth of *A. solani* but did not produce coiling (type C). *Rhizopus* sp. *and Macrophomina phaseolina* met with *A. solani* and showed (type D interaction).

The best method to control diseases is through integrated pest management wherein biological control component would be significant. *Trichoderma*, which is known to play an important role in the biological control of soil-borne diseases, has been recorded to inhibit the leaf pathogens also. Competition for nutrients and space, the production of antibiotics and hyperparasitism all play important roles in the antagonism of pathogens by *Trichoderma* (Mukerji and Garg, 1988) Using antagonistic microorganism to control pathogen is very effected method. A successful disease-control program could involve just a single practice, but the long-term reduction of disease losses generally requires the application of several control measures. The best way to ensure success of a disease-management program is to use integrated disease-control measures. *Trichoderma* species are used as potential biocontrol agents in the integrated biological control of plant pathogens, *Trichoderma* used as a biocontrol agent againts major diseases. Its vary effected for post harvest disease (Reeny *et al.*, 2010). *Trichoderma* spp could inhibit ten kind of pathogen in different degree of infestation. *Trichoderma harizianum*, *T. viride* inhibited the growth of *A. solani* and produced coiling around the *A. solani*. Same results found by Ning *et al* (2008) who stated

that *Trichoderma* T-115 could parasitize *A solani.* The inhibition against *A. solani* was best for all up to 70%. *Trichoderma harizianum*, *T. viride* significantly reduced the colony diameters of *A. solani* as compared to other phylloplane fungi of tomato. *T. harzianum* was found to be the most effective in reducing the colony diameters of *A. solani* but these initially did not differ significantly from the other antagonists. These antagonists overgrew *A. solani*, resulting in greatly restricted growth of the latter. Foliar spray of *T.viride* was very effective in reducing the disease severity of *A. solani* (Kishore 2008). *Penicillium citrinum,* and *Penicillium chrysogenum* also inhibited the growth of *Alternaria solani*. *Aspergillus terreus* also inhibits *A. solani* but because it produces aflatoxin it can not be used as a control agent. It is concluded that *Trichoderma* spp. provide an effective control of *A. solani* and can be used as biocontrol agent under field conditions.

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