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Investigation of Phylloplane microflora of Tomato

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Phylloplane denotes to the leaf surface of the plants. Leaf surface environment is dynamic due to the physiochemical properties of leaf surface and activity of phylloplane microflora. The present study aims to understand and explore the potential microbial bio agents on the phylloplane of the tomato to devise efficient management of their diseases by using phylloplane microflora. The study showed that phylloplane microflora associated with diseased leaves of tomato are more in number compared to healthy leaves and are a natural source of eco-friendly bioagents which may control plant pathogens. This investigation confirms that leaf surface mycobiota such as *Trichoderma* species found to be effective antagonists against *Alternaria solani* and *Fusarium* sp. of tomato as it is having mycoparasitic ability.

Introduction

The implication of disease caused by pathogens on the plant parts are usually high and lead to a major loss to the fruit and plant annually. The phyllosphere refers to the entire aerial habitat of plants while phylloplane describes the entire leaf surface. These microbes are either associated with plant surfaces as epiphytes or may reside inside tissues as endophytes [1]. Phylloplane dwellers have been studied as bio protectants and enhancers of growth in host plants[2]. The nature of intra and inter specific competition among leaf surface microorganisms largely determine the success and failure of advancing plant pathogens. The inhibition of phytopathogens by phylloplane colonizers has gained significance and may help in controlling occurrence of disease in plants. The present study aims to understand and explore the potential microbial bio agents on the phylloplane of the tomato as an aid to devise possible management of their diseases by utilization of appropriate phylloplane microflora.

Material and Methods

For isolation, identification and *in vitro* evaluation of isolated microflora of tomato against pathogens of tomato following methodologies were used.

Collection of infected and non-infected leaves: Healthy and diseased leaves of Tomato (cv. GT-2) were collected at the time of flowering. Five plants each for healthy and diseased C were tagged. Three leaves *viz.,* top, middle and bottom from each plant were carefully excised using sterile forceps and were put into sterile Petri plates for transportation to the lab. A composite sample of healthy leaves was prepared by mixing the individual healthy leaves together and same for the diseased samples.

Isolation of phylloplane microflora from healthy and diseased leaves: A modified leaf washing technique was adopted to estimate phylloplane microflora of healthy and infected leaves [3]. Disc of 1 cm diameter were cut randomly from each healthy and diseased leaves with sterile cork borer. Fifty discs each of healthy and infected leaves were placed in 250 ml conical flask containing 100 ml of sterile distilled water and was shaken for 20 minutes to get a homogenous suspension. One ml suspension were pipette out and were transferred into sterile Petri plates, containing Potato Dextrose Agar (PDA) for isolation of fungi, Nutrient Agar for isolation of bacteria and Actinomycetes agar for isolation of Actinomycetes. The suspension was spread uniformly in Petri plates and plates were kept undisturbed at room temperature for 3 -5 days. On incubation different colonies were observed and pure cultures were maintained.

Isolation of pathogen: For isolation of fungal pathogen standard tissue isolation method was adopted. Small pieces of infected plant parts were surface sterilized with 0.1 % $HgCl_2$ for 1 minute followed by rinsing with three subsequent changes of sterile distilled water and were placed aseptically in sterile Petri plates containing PDA. The plates were incubated at room temperature for 7 days. After incubation

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the fungal colonies were observed and maintained in pure culture for further investigation.

Identification and characterization of phylloplane microflora: Microbial isolates was identified based on standard microbial techniques. Isolates was identified based on cultural, morphological and microscopic observation. Bacteria were identified based on standard biochemical test.

***In vitro* evaluation of isolated microflora against pathogens of tomato:** Evaluation of isolated phylloplane microflora against pathogen was done *in vitro* by dual culture method.

Dual culture method [4]: The test organisms and the pathogen were grown on PDA medium each separately and from 8 days old cultures, a 5 mm diameter disc of the test organism (phylloplane microflora) and pathogen were taken. The Petri plates (90mm) were inoculated aseptically with pathogen and test organisms, by placing 5 mm diameter culture blocks at 70 mm apart from each other. Three replications of each treatment were kept and the Petri plates with only pathogen served as control. All the plates were incubated at temperature (28 ± 2^{0}C) and the radial growth of the test organism and pathogen was measured after 7 days. The per cent growth inhibition (PGI) was worked out by using the formula [5].

$$PGI = \frac{100(DC - DT)}{DC}$$

Where,

PGI = Per cent growth inhibition,

DC = Average diameter of mycelial colony of control set

DT = Average diameter of mycelial colony of treated set

Results and Discussion

Collection and isolation of phylloplane microflora from infected and non-infected leaves of tomato as well as isolation of pathogens of tomato: In infected tomato leaves total number of microbial population was higher than that of healthy tomato leaves. Totally eight different fungi, two different bacteria and one Actinomycetes were isolated from phylloplane microflora of healthy and infected tomato leaves. Eight fungi *viz., Alternaria solani*, *Trichoderma* sp., *Aspergillus niger*, *Fusarium* sp., *Penicillium* sp., *Aspergillus flavus*, *Penicillium* sp. and white sterile mycelium were isolated. Two bacteria *viz., Bacillus* sp. and *Pseudomonas* sp. were isolated as phylloplane bacteria. Out of eight different fungi, six fungi were isolated as phylloplane mycoflora i.e. *Trichoderma* sp., *Aspergillus niger*, *Penicillium* sp., *Aspergillus flavus*, *Penicillium* sp. and white sterile mycelium while two fungi i.e. *Alternaria solani* and *Fusarium* sp. were isolated as pathogens of tomato. Total number of microflora isolated from healthy tomato leaves were 2.26 microflora/ per cm^2 and total number of microflora isolated from diseased leaves were 4.39 microflora / per cm^2.

In isolation of pathogen of the tomato, after incubation two different fungal colonies were isolated. Colonies were identified as *Alternaria solani* and *Fusarium* sp. based on Cultural, morphological characteristics and microscopic observations. Leaf spot disease intensity was 58.33 % and Fusarium wilt disease incidence was 20.83% at the time of collection of infected leaves for isolation of phylloplane microflora.

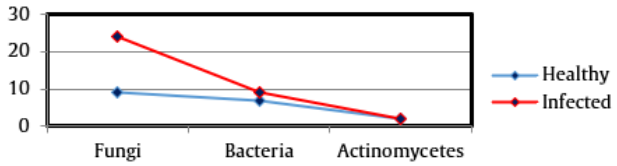

Fig.:1 Total number of colonies of phylloplane microflora isolated from healthy and diseased tomato leaves (GT-2)

Identification and characterization of phylloplane microflora: The morphological and cultural characters of the isolated phylloplane mycroflora were studied and compared with those mentioned in literature. For identification of bacteria biology were performed.

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***In vitro* evaluation of isolated microflora against pathogens of tomato:** In this study, eight phylloplane microflora were evaluated *in vitro* for their antagonistic effect against *Alternaria solani* and *Fusarium* sp. by dual culture method.

The results of phylloplane microflora against *Alternaria solani* presented in Table-5 revealed that the least growth of *Alternaria solani* was recorded in the treatment of *Trichoderma* sp. (9.67 mm) where the *Trichoderma* sp. over grew the small colony of *Alternaria solani*, restricting its further growth. This was significantly superior in its efficacy over the rest. Next best in order of merit was *Aspergillus niger* (16.67 mm) followed by *Penicillium* sp. (29.33 mm). Maximum growth inhibition of *Alternaria* sp. was observed in the treatment of *Trichoderma* sp. (88.85 %) followed by treatment of *Aspergillus niger* (80.77 %).

The results of phylloplane microflora against *Fusarium* sp. presented in Table- 5 revealed that the least growth of *Fusarium* sp. was recorded in the treatment of *Trichoderma* sp. (10.00 mm) where the *Trichoderma* sp. over grew the small colony of *Fusarium* sp., restricting its further growth. This was significantly superior in its efficacy over the rest. Next best in order of merit was *Aspergillus niger* (19.00 mm) followed by *Penicillium* sp. (39.67 mm). Maximum growth inhibition of *Fusarium* sp. was observed in the treatment of *Trichoderma* sp. (88.68 %) followed by treatment of *Aspergillus niger* (78.49 %). Thus, *Trichoderma* sp. proved the most effective in inhibiting mycelial growth of both the pathogens. The rest of the phylloplane microflora were comparatively less effective in inhibiting the mycelial growth of the pathogens of the tomato.

Conclusion

Investigations on phylloplane microflora of tomato showed that from the isolated microflora fungal population was higher as compared to bacteria and Actinomycetes. Among the phylloplane population of healthy and diseased leaves, in most of the cases microflora population was high in diseased leaves as compared to healthy leaves of tomato. In antagonistic activity of phylloplane microflora isolated against pathogens of tomato, *Trichoderma* sp. have found with highest antagonistic property which directly affects and

inhibit the growth of the pathogens of tomato. So it can be concluded that *Trichoderma* sp. has potential to be used as antagonists against foliar and soil borne pathogens of tomato.

Table 1: Total no. of microbes/cm² of healthy and diseased leaves of tomato

	Fungi	Bacteria	Actinomycetes
Healthy	1.27	0.71	0.28
Diseased	3.11	1.13	0.14

Table 2: Microbial frequency (%) from each of the healthy and diseased sample of tomato

Microflora	Healthy	Diseased
Alternaria solani	0	14.29
***Trichoderma* sp.**	0	05.71
Aspergillus niger	11.11	08.57
***Fusarium* sp.**	05.56	11.43
***Penicillium* sp.**	11.11	0
white sterile mycelium	05.56	05.71
Aspergillus flavus	05.56	08.57
***Penicillium* sp.**	11.11	14.29
***Bacillus* sp.**	16.67	14.29
***Pseudomonas* sp.**	22.22	11.43
Actinomycetes	11.11	05.71

Table 3 : Cultural characteristics of isolated phylloplane microflora from tomato

Sr. No.	Name of the phylloplane microflora	Cultural characteristics of microflora
1	*Aspergillus niger*	Colonies are initially white, quickly becoming back with conidial production. Reverse is pale yellow and generate radial pattern
2	*Aspergillus flavus*	Colonies are fast growing, green with white margin
3	*Alternaria solani*	Colonies are black to dark grey colored zonation
4	*Trichoderma* sp.	Colonies are initially white which turn into greenish in colour with concentric rings and granular.
5	*Penicillium* sp.	rapid growth, dark green color, granular powdery colony and the back side of colony was pale yellow in color
6	*Penicillium* sp.	olive green in color and the back side of colony was off white in color
7	*Fusarium* sp.	Colonies are usually fast growing, white cottony mycelium.
8	White sterile mycelium	Colonies are fast growing, white in colour and cover entire Petri plate in 4 days
9	*Bacillus* sp.	Colonies are flat or slightly convex with irregular edges
10	*Pseudomonas* sp.	Colonies are small, rough, strongly cohesive
11	Actinomycetes	The colonies are powdery mass over the surface of culture media, often these are pigmented when the aerial spores are produced.

Table 4 : Morphological characteristics of isolated phylloplane microflora from tomato

Sr. No.	Name of the phylloplane microflora	Morphological characteristics of microflora
1	*Aspergillus niger*	Conidial heads up to 3 mm x 15 to 20 µm in diameter, conidia 3.5 to 5 µm
2	*Aspergillus flavus*	Conidial head 250 - 350 µm in diameter, Conidia 4.7 to 6.5 µm
3	*Alternaria solani*	Conidia measured with beak 20.47 x 50.14 µm
4	*Trichoderma* sp.	Conidia measured 4.0 x 3.5 µm with flask shaped phialides
5	*Penicillium* sp.	Mycelium 2.5 µm to 4.5 µm, single celled conidia 3.5 µm to 5 µm in diameter
6	*Penicillium* sp.	Mycelium about 1.5 µm to 5 µm in diameter, single celled conidia 3.5 µm to 7.5 µm
7	*Fusarium* sp.	Conidiophores 9 - 17 µm, Micro conidia (8 - 14 X 1.3 – 4.5 µm, Macro conidia (38-79 X 2.5 – 5.5 µm
9	White sterile mycelium	Width of the mycelium ranged from 5.4 to 10.6 µm.

Table 5: *In vitro* evaluation of isolated phylloplane microflora against *Alternaria solani* and *Fusarium* sp. of tomato

Sr. No.	Name of the Phylloplane microflora	*Alternaria solani*		*Fusarium* sp.	
		Average mycelium growth (mm)	Growth inhibition (%)	Average mycelium growth (mm)	Growth inhibition (%)
1	*Aspergillus niger*	16.67	80.77	19.00	78.49
2	*Aspergillus flavus*	41.00	52.69	40.00	54.72
3	*Trichoderma* sp.	09.67	88.85	10.00	88.68
4	*Penicillium* sp.	48.33	44.23	49.00	44.53
5	*Penicillium* sp.	29.33	66.16	39.67	55.09
6	*Actinomycetes*	80.33	7.31	76.33	13.58
7	*Bacillus* sp.	66.00	23.85	70.00	20.75
8	*Pseudomonas* sp.	65.00	25.00	59.33	32.83
9	Control	86.67		88.33	
	S.Em.	0.94		0.92	
	CD	2.79		2.71	

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