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QUALITATIVE AND QUANTITATIVE VARIATIONS OF FUNGI IN TOMATO RHIZOSPHERE IN RESPONSE TO PLANT AGE

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Abstract

The variations in rhizospheric viable fungal count in response to plant age of tomato was studied. A gradual increase in rhizospheric fungal count with plant age was found. A sharp increase in rhizospheric fungal number was observed during 45-60 days of plant growth and after this age the tomato plant starts flowering. The dominant members of the fungi isolated from the rhizosphere of tomato plant belongs to the genera *Aspergillus, Penicillium, Trichoderma* and *Fusarium.*

Key words : *Aspergillus*, microbiome, fungal count, rhizosphere.

Introduction

The microbes associated with root has been referred to as "the second genome of the plant" due to its significant impact on plant growth and health (Berg, 2009 and Berendsen *et al*., 2012). The narrow zone of soil surrounding the roots where microbial population are stimulated by root activities is termed as rhizosphere by Hiltner in 1904 (Neumann and Romheld, 2002, 2005). Plant roots exert strong effects on rhizosphere through rhizodeposition (root exudation, release of sloughed off root cells) and by providing suitable ecological niche for microbes (Bais *et al*., 2001 and Hawes *et al*., 2000). Plant roots produce various bioactive substances that attract or inhibit specific microbial groups (Badri *et al*., 2013), providing selective forces that modify the structure of the root-associated microbiome (Berg *et al*., 2009; Hartmann *et al*., 2009 and Bakker *et al*., 2012). The aim of present work was to study the influence of plant growth stages on the population size of culturable fungi associated with tomato rhizosphere so that they can be used to improve crop productivity.

Materials and Methods

Certified seeds of *Lycopersicon esculentum* variety Sania were obtained from IARI, Pusa, New Delhi. Surface sterilized seeds equal in size, shape, weight and colour were sown in earthen pots and allowed to germinate. 10 tomato seedlings were uprooted along with their roots from all earthen pots after different days of germination (15, 30, 45, 60, 75 and 90) and brought to the laboratory in a sterile polythene bag. In the laboratory, the rhizospheric soil was collected from roots of 15-d, 30-d, 45-d, 60-d, 75-d and 90 days old tomato plants under a laminar hood. The rhizospheric soil sample was serially diluted from 10^{-1} to 10^{-5} dilutions and 0.1 mL diluted sample was plated on Czapeks dox agar (CDA) plates. The inoculated petri plates were incubated at 28±2°C for 5 days for fungal growth (Johnson and Curl, 1972).

The fungal colonies developed on CDA plates after incubation were counted with the help of a colony counter. The number of fungi present in soil was then calculated with the help of following formula-

Number of fungal cell per g of soil

$$= \frac{Number\ of\ cfu\ /\ mL \times Dilution\ factor}{Weight\ of\ soil\ (g)}$$

cfu = colony forming unit, dilution factor = 1/ dilution number.

The isolated dominant fungi were identified on the basis of colonial and cellular morphological characteristics. The cellular characteristics were studied by lactophenol cottonblue staining (Aneja, 2009). All the experiments were carried out in triplicates and results are given as mean values ± S.D.

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Table 1 : Influence of tomato (*Lycopersicon esculentum*) plant age on rhizospheric fungal count.

S. no.	Plant age (days)	Fungal count (10^4) (cfu/g of soil) (mean ± S.D.)
1	0	0.15 ± 0.010
2	15	0.29 ± 0.012
3	30	0.35 ± 0.042
4	45	0.54 ± 0.034
5	60	1.24 ± 0.118
6	75	1.98 ± 0.114
7	90	2.10 ± 0.160

Results and Discussion

Table 1 shows the alteration in rhizospheric fungal population of tomato with plant age. The results indicate the gradual increase in rhizospheric fungal count with plant age. A sharp increase in rhizospheric fungal number is observed during 45-60 days of plant growth. The tomato plant starts flowering during 60-70 days and fruiting during 90-100 days of plant growth. The primary substrate for microbial growth along older roots include cellulose and other recalcitrant cell wall materials from sloughed root cortex tissues. These root exudates (such as cellulose,

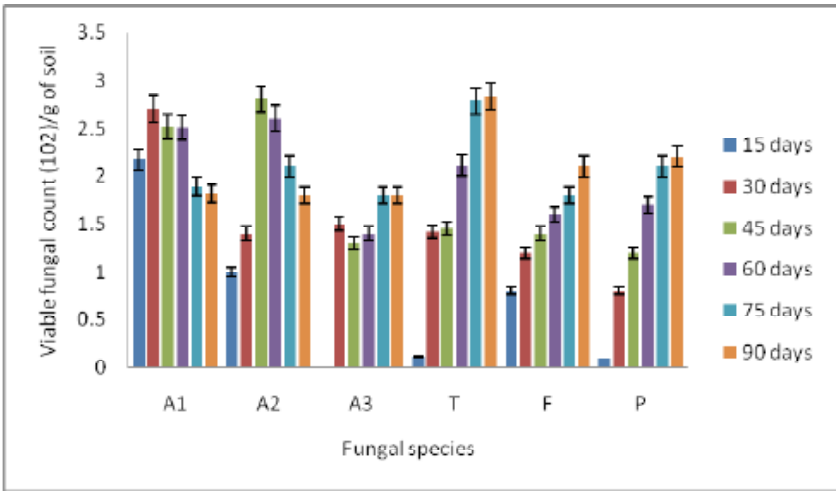

Fig. 1 : Influence of tomato (*Lycopersicon esculentum*) plant age on viable count of dominant rhizospheric fungal species (A1-*Aspergillus niger*, A2-*Aspergillus flavus*, A3- *Aspergillus terreus*, T- *Trichoderma viridae*, F-*Fusarium oxysporium* and P-*Penicillium chrysogenum*).

Table 2 : The cultural and microscopic characteristics of dominant fungi isolated from rhizosphere of tomato plant.

Fungal isolate no.	Cultural characteristics	Reverse side of colony	Microscopic characteristics	Identified fungal species
1.	Colonies powdery with white mycelium becoming black on development of conidia	hyaline	Septate hyphae,conidiophore long and thick, Vesicles spherical, biseriate, globose, rough conidia covering entire vesicle	*Aspergillus niger*
2.	Colonies powdery and yellow green in colour	hyaline	Septate hyphae,conidiophore long and thick, vesicles elongated with primary and secondary phialides, globose, smooth conidia covering ¾ vesicle	*Aspergillus flavus*
3.	Colonies velvety and orange brown in colour	Yellow brown	Septate hyphae,conidiophore long,smooth and thin,vesicles subglobose, biseriate, globose , smooth conidia covering ½ vesicle	*Aspergillus terreus*
4.	Colonies compact and white in colour	hyaline	Highly brached conidiophore, subglobose phialides, ovoid conidia in small terminal clusters	*Trichoderma viridae*
5.	Colonies cottony, initially white becoming pink on maturity	hyaline	Septate, branched hyphae, short, branched conidiophore bearing a whorl of phialides, mutiseptate sickle shaped macroconidias	*Fusarium oxysporium*
6.	Fluffy colonies velvety with bluish green colour	Reddish brown	Non-septate hyphae,conidiophores smooth, branched and relatively short, brush like ending in phialides, conidia smooth and ellipsoidal	*Penicillium chrysogenum*

chitin and lignin) cannot diffuse to longer distance and thus accumulate in rhizosphere with age (Kumar *et al*., 2006). Several researchers demonstrated that the developmental stage and physiological state of plant strongly influenced the rhizosphere effect possibly through differences in the quality and quantity of rhizodeposits (Nardi *et al.,* 2000; Jones *et al*., 2004 and Bais *et al*., 2006). Oyeyiola (2009) reported that the rhizosphere effect increased progressively with increase in tomato plant age until the 6th week after seed sowing and then declined.

The isolated dominant rhizospheric fungi were identified as- *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus, Trichoderma viridae*, *Fusarium oxysporium* and *Penicillium chrysogenum* by cultural and morphological characteristics (table 2). Influence of tomato plant age on number of few rhizospheric fungal species is presented in fig. 1. The *Aspergillus* species dominate in early stage of growth and *Trichoderma* species in last stage of vegetative growth.

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