Studies on the rhizosphere mycoflora of Abelmoschus esculentus Moench. I. Influence of Varieties and Age of the Plant

By

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Introduction

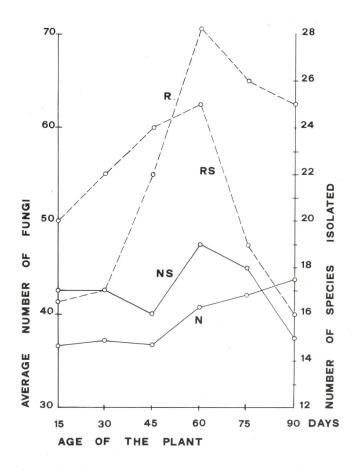
Age of the plant has an important bearing on rhizosphere population as it alters the underground flora, and the stage of maturity controls the magnitude of the rhizosphere effect and the degree of response by specific microorganisms. Mention may be made to the references of Starkey (1929 a, b, c; 1931 a, b; 1938); Timonin (1940, 1941); Katznelson (1946); Contois (1953); Agnihothrudu (1953); Chesters and Parkinson (1959); Ivarson and Katznelson (1960); Rao (1962); Tiwari and Mehrotra (1968); Youssef and Mankarios (1968); Gujrati (1968) and Mishra and Kamal (1972).

Different plant species or varieties often establish somewhat different subterranean flora. Effect of plant varieties on rhizosphere mycoflora has been studied by Lochhead, Timonin and West (1940); Agnihothrudu (1954); Buxton (1957 a) and Shrivastava and Saxena (1968).

Most of the quantitative work on the rhizosphere effect has been done with bacteria, fungi and actinomycetes. A wide variety of plants have been studied and positive effects were obtained. The representative contributions from various parts of the world on the overall effect of plant roots need be noted: Ishizawa et al. (1957); Ramchandra-Reddy (1959); Maliszewska and Moreau (1959); Edward, Shrivastava and Naim (1960); Strzelczyk (1961 a); Rouatt and Katznelson (1961); Bollen (1961); Rangaswami and Vasantharajan (1962 a).

Isolations from dilution plates also show qualitative differences between nonrhizosphere and rhizosphere soil as observed by Parkinson (1958); Peterson (1958); Ebben (1959); Catska, Macura and Vagnerova (1960); Papavizas and Davey (1961); Goos and Timonin (1962).

Okra (*Abelmoschus esculentus* Moench.) is an important vegetable crop grown thoughout the country. Beside its vegetable use it has been reported to have many medicinal properties (Nadkarni, 1927); as well as industrial importance in paper and gur industry (Mehta, 1959). There-



Graph 1: Rhizospheric and non-rhizospheric fungi from soil grown with Abelmoschus esculentus variety "Pusasavani".

Scale on left side: average number of fungi per g of soil (in thousands); scale on right side: number of species isolated.

N = nuber of fungi per g of non-rhizospheric soil; NS = number of species isolated from non-rhizospheric soil; R = number of fungi per g of rhizospheric soil; RS = number of species isolated from rhizospheric soil.

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fore in view of its importance, four varieties of *Abelmoschus esculentus*, i. e. 'Pusasavani', 'Satdhari', 'Meghdoot' and 'Deshi' were selected to see the effect of age of different varieties on rhizosphere mycoflora. For this purpose, quantitative and qualitative studies of nonrhizosphere and rhizosphere soil of each variety was done at fortnightly intervals.

Materials and Methods

The inoculation of the nonrhizosphere and rhizosphere soil from natural plots was done at fortnightly intervals. The roots of the four varieties of *Abelmoschus esculentus* were dug separately with a sterile trowel and adhering soil particles were removed off by slight tapping. The root system was then cut off with a sterile scissor and transferred aseptically into 250 ml Erlenmayer flask containing 100 ml sterile distilled water. The flask was shaken thoroughly and the suspension was designated as rhizosphere soil solution.

Nonrhizosphere soil samples from natural plots were taken from a distance of nearly one foot from the root system of plants. 10 g of soil was weighed and three grades of dilution viz., 1:100, 1:1000 and 1:10000 were prepared in sterile distilled water. The rhizosphere soil solution of each variety was shaken well and one ml aliquot was pipetted in 20 sterilized Petriplates (5 replicates for each variety). Fifteen Petriplates were prepared for the nonrhizosphere soil (5 for each dilution) and one ml of the soil solution was pipetted into each plate using separate sterilized pipettes for each dilution. About 20 mls of sterilized melted*) Peptone dextrose agar medium with rosebengal and streptomycin (Martin, 1950); was poured in Petridishes.

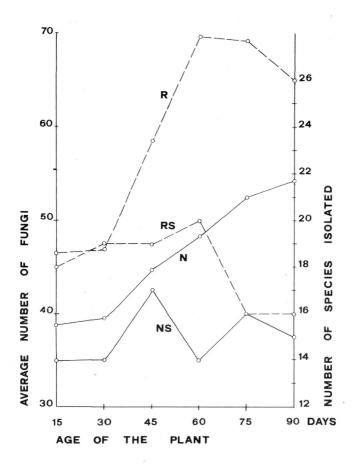
The plates were incubated at room temperature and the colonies developing were identified. Number of colonies of each species as well as total number of colonies in each plate were recorded. Number of fungi per g of soil in nonrhizosphere and rhizosphere soil were recorded by the method described by Saksena (1955).

Observations

Quantitative and qualitative studies of nonrhizosphere and rhizosphere soil of four varieties of *Abelmoschus esculentus* in relation to age of the plants was undertaken and obervations are summarized as follows:

It has been observed from the quantitative study of rhizosphere soil of four varieties that the number of mycoflora per gram of soil was higher than the nonrhizosphere soil, so a positive rhizosphere effect was observed in all the four cultivars (Graphs 1–4).

^{*)} Dextrose, 10 g; Peptone, 5 g; KH2PO4, 1 g; MgSO4, 7H2O, 0,5 g; agar agar, 20 g; distilled water, 1 litre; 1:30000 Rosebengal and $30 \,\mu g/m$ l streptomycin (a combination of streptomycin sulphate and dihydro-streptomycin sulphate at equal levels).



Graph 2: Rhizospheric and non-rhizospheric fungi from soil grown with Abelmoschus esculentus variety "Satdhari".

Scale on left side: average number of fungi per g of soil (in thousands); scale on right side: number of species isolated.

N = number of fungi per g of non-rhizospheric soil; NS = number of species isolated from non-rhizospheric soil; R = number of fungi per g of rhizospheric soil; RS = number of species isolated from rhizospheric soil.

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From the qualitative study of rhizosphere soil of four cultivars, it has been observed that the total number of species were more than the nonrhizosphere in all the four varieties. So in this case also a positive rhizosphere effect was observed (Graphs 1–4 and Table 1).

Regarding the effect of age of the plant on rhizosphere mycoflora of four varieties, it has been observed that the rhizosphere mycoflora increases with the increase in age of the plant in all the four varieties upto flowering and fruiting stage. After this a decline in number of fungi per gram of soil as well as in total number of fungal species was observed in all the four cultivars (Graphs 1–4).

A significant difference in the quantitative and qualitative results have been observed among the four varieties (Graphs 1–4 and Table 1).

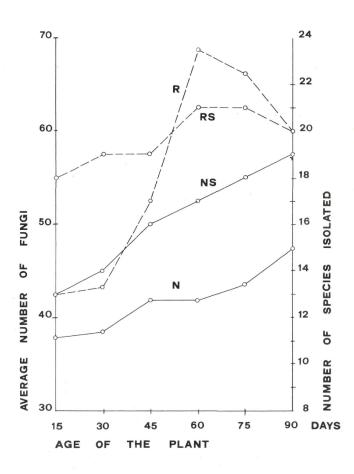
Trichoderma sp. a prominent antagonist was totally absent in "Deshi" variety but was present in other three varieties (Table 1). Colonization of roots by pathogenic species of *Rhizoctonia bataticola*, *Sclerotium rolfsii*, *Ozonium taxanum*, *Macrophomina phaseoli*, *Fusarium* sp. and *Verticilium* sp. were recored in early stages of plant growth, when the plants were 15 to 45 days old in all the four varieties, but it was slightly more in "Deshi" variety (Table 1).

From the qualitative study it has been observed that Chaetomium spirale, Emericellopsis sp., Thielavia sepedonium, Acrophialophora fusispora, Chaetomella raphigera, Chrysosporium luteum, Cylindrocladium sp., Drechslera austrialiensis, Myrothecium verrucaria, Paecilomyces persicinus, Periconia sp., Phoma humicola, Scopulariopsis brevicaulis and Verticillium effusum were only present in rhizosphere soil (Table 1).

Discussion

A careful study of the results obtained during the quantitative and qualitative studies of four varieties of *Abelmoschus esculentus* ('Pusasavani', 'Satdhari', 'Meghdoot' and 'Deshi') revealed that the rhizosphere mycoflora was higher than nonrhizosphere (Graphs 1–4). It seems that increase in rhizosphere microflora was being governed by various factors like root exudates (Containing sugars, aminoacids, organic acids, vitamins, growth substances), sloughed off root epidermal tissues serving as energy source, as observed by a number of workers such as Rovira (1956 a), Sadasivan (1960), Sadasivan and Subramanian (1960), Schroth and Hildebrand (1964), Rovira (1965 a, b), and Youssef and Mankarios (1968).

Graphs (1-4) also reveal that the rhizosphere mycoflora of four varieties of *Abelmoschus esculentus* increases with the increase in age of the plants. Minimum number was observed when the plants were only 15 days old and maximum at flowering and fruiting stage when the plants were 60 days old. After that a decline in number was observed. It seems that increase of rhizosphere mycoflora with the age of the plant was stimulated by various factors like increased exudation, decomposition of



Graph 3: Rhizospheric and non-rhizospheric fungi from soil grown with Abelmoschus esculentus variety "Meghdoot".

Scale on left side: avergae number of fungi per g of soil (in thousands); scale on right side: number of species isolated.

N = number of fungi per g of non-rhizospheric soil; NS = number of species isolated from non-rhizospheric soil; R = number of fungi per g of rhizospheric soil; RS = number of species isolated from rhizospheric soil. moribund root hairs, epidermal cells and cortex, accumulation of cell material in the form of cast-off root cap cells around the root region as observed by Rovira (1956 a), Rogers, Pearson and Pierre (1942), Vagnerova, Macura and Catska (1960 a, b), Strzelczyk (1961 a), Venkatesan (1962), Tiwari and Mehrotra (1968).

It appears from the Graphs 1–4 and Table 1 that there are marked quantitative and qualitative differences among the four varietes. "Pusasavani" has maximum number of fungi per gram of soil as well as total number of species isolated during the season. The minimum number was observed in 'Deshi' variety. 'Satdhari' and 'Meghdoot' varieties have slight variation both quantitatively and qualitatively. It seems that different varieties have different selective action on the fungal population which probably depend on the intrinsic characters of the species in question such as exudates and root sloughing as observed by Tiwari and Mehrotra (1968).

Trichoderma sp. was totally absent in the 'Deshi' variety but was present in other three varieties. The same reason holds good for this variation as well.

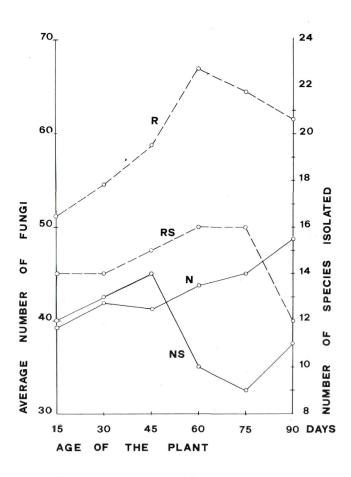
Colonization of roots by pathogenic species of *Rhizoctonia bataticola*, *Sclerotium rolfsii*, *Ozonium taxanum*, *Macrophomina phaseoli*, *Fusarium* sp. and *Verticillium* was found in early stages of plant growth, when the plants were 15–45 days old. It seems that fungal population varies with age and kind of plant as observed by Peterson (1959), Catska et al. (1960), Rouatt, Peterson et al. (1963). Root may also stimulate the growth of certain root pathogenic organisms as indicated by Meshkov and Khodokova (1954), Barton (1957), Buxton (1960–62).

It appears from the Table 1 that Chaetomium spiralae, Emericellopsis sp., Thielavia sepedonium, Acrophialophora fusispora, Chaetomella raphigera, Chrysosporium luteum, Cylindrocladium sp. Drechslera australiensis, Myrothecium verrucaria, Paecilomyces persicinus, Periconia sp., Phoma humicola, Scopulariopsis brevicaulis and Verticillium effusum were only present in rhizosphere soil. It seems that roots stimulate germination of spores of both root and soil inhabiting fungi as observed by Garrett (1956), Jackson (1957–60), Tolle and Rippel-Baldes (1958), Buxton (1957 a, b), and Schroth, Toussoun and Snyder (1963).

Summary

The rhizosphere mycoflora of four varieties of *Abelmoschus esculentus* ('Pusasavani', 'Satdhari', 'Meghdoot' and 'Deshi') was studied in relation to age of the plant. It was observed that the rhizosphere mycoflora was higher than nonrhizosphere in all the four cultivars.

Moreover the rhizosphere mycoflora increases with the increase in age of the plant upto the flowering and fruiting stage and after that a continuous decline was observed. Root pathogenic fungi were found colonizing during the early stages of plant growth in all the four varieties.



Graph 4. Rhizospheric and non-rhizosperic fungi from soil grown with Abelmoschus esculentus variety "Deshi".

Scale on left side: average number of fungi per g of soil (in thousands); scale on right side: number of species isolated.

N = number of fungi per g of non-rhizospheric soil; NS = number of species isolated from non-rhizospheric soil; R = number of fungi per g of rhizospheric soil; RS = number of species isolated from rhizospheric soil.

 Table 1. Incidence of fungi, in the nonrhizosphere and rhizosphere of the four varieties of Abelmoschus esculentus. Figs. 15, 30, 45, 60, 75 & 90 indicate the age of plant in days.

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(Berkeley and Ravenel)																										
Thaxter	+	+	-	+ ·	- +	-	+	+	+		+			+	+	+	-	+	-		+	+				
Cunninghamella bertholletiae																										
Stadel																					+					
Mucor hiemalis Wehmer																					+					
Mucor mucedo (Linne) Brefeld Rhizopus arrhizus Fischer																					+					
Rhizopus nigricans Ehrenberg																					+					
Rhizopus oryzae Went and	+		+		- 1		+		+				1	4-	+	4-	_	+	T		+	T	T		4	7 7
Gerlings	-1-				1		1	1				_					1	-		_	_		_			
Syncephalastrum racemosum	-	T				T	T	T			-			T			T									
(Cohn) Schroeter	+	+		+	_ +		+	+		+	-			+	+	+			+	_	+	+	_	+		+ -
Zygorhynchus exponens Burgeff																					_					
Arachniotus sp.																					+					
Chaetomium bostrychodes Zopf																					_					
Chaetomium funicola Cooke																										
Chaetomium indicum Corda																					+					
Chaetomium spirale Zopf																					_					
Emericella sp.																					+					
Emericellopsis sp.																					_					
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Neocosmospora vasinfecta Smith	-	_	-	1				1		1		1						i.	-	1	_	_	_	_		

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Abbott) Emmons	+		+	+	+		+	+	+			+ -		_	+	+	+			+ -		+			- +		+
Acrophialophora fusispora																											
(Saksena) Samson)	_	+	_	_		_	_	-	_	_		_		-			-	_			-		_				
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Aspergillus luchuensis Inui	+	_	_	+	_	_	+	+	_	_	+			+	+	_	+				-	+	_			~	+
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(Fresenius) Thom and Church		_	_	_	+	_	+	_	_	_		_	_	+	_	_		_	+		_				- +		_
Aspergillus sydowii (Bainier &																											
Sartory) Thom and Church	+		+	_	+	+	_	+	_	_	_	+		_	+	_	_	+	+		_	+			- +	- +	+
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Botryodiplodia theobromae																											
Patouillard	_	_	_					+	+		+		+ -			_	_		_		-		_				
Cephalosporium curtipes																											
Saccardo	+	_	+	_	+		_	_	-	_	_			_	+	+	_					+	+				
Chaetomella raphigera Swift	_		÷	_	_	_				_	_	+ .		+	_	_			<u> </u>							_	
Chrysosporium luteum (cost)																											
Carmichael	_		_	_	_	_	_		_	+	_	_	+ -	_	_	_			_	- +						_	
Cladosporium epiphyllum																											
Persoon	+	+	_	_	-	+		+	+		+			+	+	_	+			+ -		+	_			+	_
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(Persoon) Link	+	_	_	+	+	_	+	+	-	_	_		+ -	_	+	_	_	+	+	- +		+	_	+ -	- +		
Curvularia geniculata (Tracy &																											
Earle) Boedijn	+		+	_	_	+	_	+	_	_	_			_	+	_	_	+	_		_	+			- +		_
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Curvularia pallescens Boedijn																											
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Fusarium equiseti (Corda)																										
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(Frag & Cif.) Booth	+			+	+	_	+	_	_		_			+	_	+			_	_	+	-	_	+ -		
Fusarium solani (Mart.) Sacc.			+	-	_	_	_		+	_	_			+	+	_	-			_	_		_	+ -		
Hormiscium stilbosporum																										
(Corda) Saccardo								+	_			-		- +	_	_	+	_		+		-	_			
Humicola brevis (Gilman &																										
Abbott) Gilman					_			_	_	_				+	_	+		+		_	+				+ -	- +
Macrophomina phaseoli																,										
(Maublanc) Ashby		_		+				_	+	_					+	_		_	_	_	_	+	_			
Memnoniella echinata (Rivolta)																										
Galloway	-	_		_	_	_		_	_	_				- +		+		_					_			
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Ozonium taxanum Neal &																										
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Papularia sphaerosperma (Persoon) von Hohnel Penicillium granulatum Bainier Penicillium humicola Oudemans Penicillium sp. Periconia sp.	++++	- + -	 +	- + + -	+++++		- +	 + +		++++-		- + - +	+	 + - - + + -	+++++++		 + - - +	+	+ - - '+ + - - +	
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O'connor, and Cummins	-	$\cdot^{+1} - \cdot$	- + -		—			+ -		_					_			_		2
Phoma humicola Gilman & Abbott		- + -					-		-				_							
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Graphs 1-4: Non-rhizosphere and rhizosphere fungal population of the four varieties of Abelmoschus esculentus.

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