Microorganism Complexes in the Soil and in the Root Zone of Plants

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The aim of the investigation was to make comparative studies on the microbial complexes in the soil and the root zone and to assess the scale of microbial colonization of the rhizoplane at early stages of plant development.

Materials and methods

Studies were made on the number and composition of microorganisms in a soddy-podzol cultivated soil and in the rhizoplane of one-two-week-old plants of onion, barley, blue millet, tomatoes and cucumbers, grown in a laboratory in natural light. The water-washed crops from cleaned roots were placed on Capek's medium, Hause-1 and on meat-peptone agar. The biomass of the mycelium and spores of fungi in the root zone were identified by the Hansen method of membrane filters, as modified by DIOMKINA and MIRCHINK /ZVYAGINTSEV, 1980/. The scale of rhizoplane colonization was evaluated by calculating the number of microorganisms, taking into account their dimensions per unit root area. The dimensions of the root surface of two-week-old plants were determined by direct measurements on the roots using a light microscope, as well as by measuring the dimensions and number of root hairs and by subsequent calculations for 1 g of fresh roots.

The removal of the thin layer of soil particles which sticks to the plant roots and impedes observations of microorganisms in the rhizoplane during direct microscopy with a scanning electron microscope was carried out using the following method: the seeds were sown in the soil between two webs of nylon net with a mesh diameter of 150-250 mm.

During the growing period the complex of microorganisms in brown carbonate soil, in the rhizosphere and in the rhizoplane of blue millet, Columb's grass and cotton were studied in the south of Tadjikistan. An analysis of the number, biomass and composition of microorganisms was carried out in the same way as in the laboratory experiment. In addition, the rhizosphere /the soil layer located not more than 5-7 mm from the roots/ was investigated. The extent to which different microorganisms were present in the soil and in the root zone was assessed using the species abundance index /density/, and for micromycetes the frequency of occurrence in space and in

time was used as well. For the assessment of the differences between the micromycetes complexes in the soil, the rhizosphere and the rhizoplane, the similarity coefficient was used, and the frequency of occurrence was taken into account as weil /MIRCHINK, 1982/.

Results

The microbial colonization of the rhizoplane during the early stages of plant development in the laboratory experiment

The rhizoplane effect in two-week-old plants was 10-100 for bacteria, and not more than 1.5-5 for actinomycetes and fungi. The number of colony-forming units /CFU/ for micromycetes in the onion rhizoplane was found to

Table 1
Microbial colonization of the rhizoplane in early stages of plant development /2 weeks/

Method	Onion	Barley	Blue millet	Tomatoes	Cucumbers
Area of the surface 1 g fresh roots, m^2	0.36	0.56	0.43	-	-
Plate counts: /number/1 g fresh roots/[x]					
"Total" bacteria . 10^8	14	11	13	18	25
Actinomycetes . 10^6	19	15	-	12	11
Filamentous fungi . 10^4	0.8	10.5	-	4.6	4.6
Bacteria[xx] /percentage coverage of roots/	0.19	0.10	0.15	-	-
Observations In scanning electron microscope /percentage coverage of basal part of roots/	0.03-0.54	0.03-0.18	0.03-0.18	0.03-0.25	0.03-0.20
Membrane filters method: Biomass of fungi/1 g fresh roots					
Spores, µg/g	0.24	0.86	0.58	-	-
Mycelium, µg/g [xxx]	0.32	1.59	1.1	-	-
µ/g	15.9	79.5	54.6		
Fungal mycelium [xxxx] /percentage coverage of roots/	0.01	0.04	0.03		

[x] Number of bacteria in the soddy-podzol soil - 1.5 . 10^8, actinomycetes - 2.5 . 10^6, fungi - 2.3 . 10^4/1 g

[xx] Cover of the one cell bacterium of the root, 0.5 μm^2

[xxx] Diameter of fungal mycelium - 5 µm

[xxxx] Biomass of fungal spores 0.51 µg/g, mycelium 0.70 µg/g soil

be lower than in the soil /the rhizoplane effect was 0.3-0.5/. The scale of bacterial colonization on the roots of onion, barley and blue millet was 0.19, 0.10 and 0.15%, respectively /Table 1/.

In the rhizoplane the biomass of the mycelium was considerably higher than in the soil, except for onions, and the biomass of the spores was about the same. The mycelium coverage of the roots of two-week-old plants did not exceed 0.03-0.05% for barley, 0.02-0.05% for blue millet and 0.01-0.02% for onion /Table 1/.

Observations made using a scanning electron microscope have shown the uneven distribution of the microorganisms on the root surface in two-week--old plants. The bacterium coverage of the upper /basal/ parts of the roots averaged some tenths or hundredths of a percent /0.03-0.054/ and was higher in onions than in tomatoes, cucumbers and blue millet /Table 1/. The colonization by bacteria of separate parts of the rhizoplane reached 4.5-9% in onion, 1-4% in blue millet, 1.8-2.7% in cucumbers, and 1.3-1.8% in barley. The bacterium coverage of the middle part of the root did not exceed 0.01%. On the ends and apical parts of the roots no microorganisms were found. In the rhizoplane of the plants, microscopic fungi and actinomycetes were observed less frequently than bacterial cells.

An analysis of the composition of microorganisms in the rhizoplane has shown the predominance of Pseudomonas and of other non-spore forming species of bacteria in the early stages of plant development. In the rhizoplane of onions and tomatoes a fairly high quantity of bacteria of the genus Arthrobacter was observed as well, while Bacillus was found in barley and blue millet. In all plants studied, actinomycetes of the genus Streptomyces, section Albus, series Albus, sec. Cinereus ser. Achromogenes and sec. Helvolo-Flavus ser. Helvolus were isolated from the surface of the roots. Considerable differences were established in the relative numbers of different groups of Streptomyces in the soil and in the rhizoplane. In the rhizoplane there was a decrease in the Penicillium abundance compared with that in the soil. In the rhizoplane of blue millet the prevailing fungi were Fusarium, Mucorales, Trichoderma hamatum and Gliocladium, in that of barley T. hamatum, Aspergillus niger and Mucorales, in that of onion Penicillium sec. Biverticillata, Trichoderma aureoviride and A. niger, in tomatoes Mucorales, Fusarium, T. hamatum and A. niger, and in cucumbers Penicillium ser. funiculosum, T. hamatum, Fusarium solani, Mucorales and A. niger.

Comparative study of the microbial complex in the soil and in the root zone of plants in a field experiment

Determinations of the microorganism number in brown carbonate soil and in the root zone of blue millet, Columb's grass and cotton have shown that in the rhizosphere there is no significant increase in fungal CFU, a slight increase in actinomycetes and a marked increase in bacteria /to not less than 5 times the original value/. The increase in the number of CFU of microorganisms in the rhizoplane was more significant: the rhizoplane effect ranged from 10-20 for bacteria, and was about 5 for fungi and actinomycetes.

In the rhizosphere the biomass of mycelium increased 2-3 fold compared with that in the soil, while the biomass of spores remained at the previous level or somewhat decreased. This led to an increase in the proportion of mycelium in the total fungal biomass of up to 50-75%. In the rhizoplane a further 5-30% increase in the proportion of mycelium in the fungal biomass was observed /Fig. 1/.

In the rhizosphere the species diversity of the bacteria on the growing medium increased: a decrease was observed in the abundance of bacteria belong-

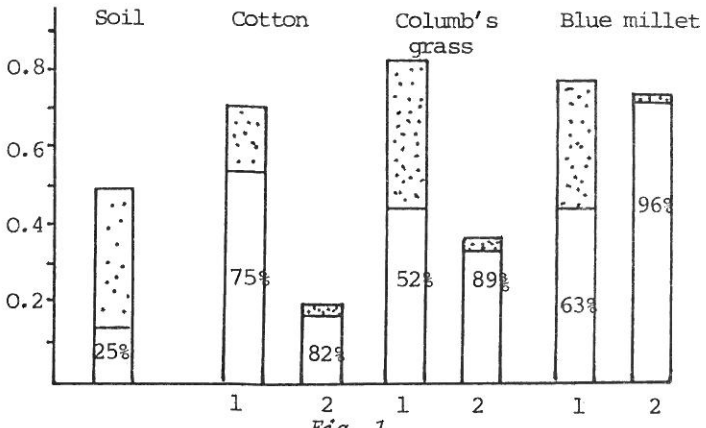

Fig. 1

Biomass of the mycelium and spores of fungi in the brown carbonate soil and the root zone of plants. 1. Rhizosphere; 2. Rhizoplane

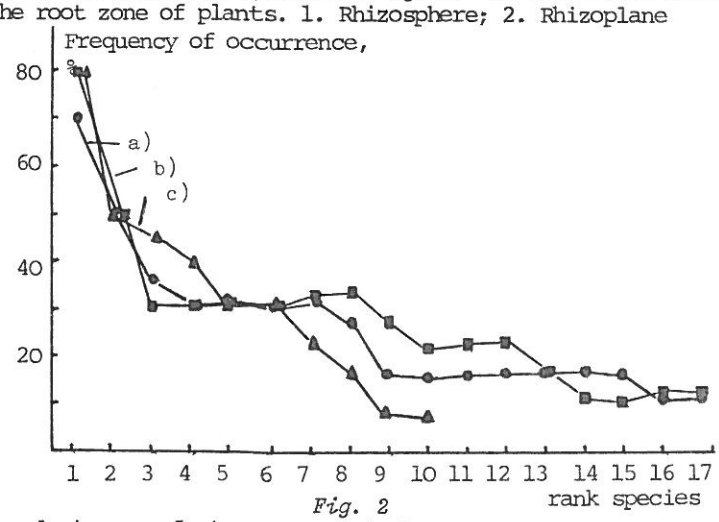

Fig. 2

Relative predominance of micromycetes in brown carbonate soil /a/, the rhizosphere /b/ and rhizoplane /c/ for blue millet

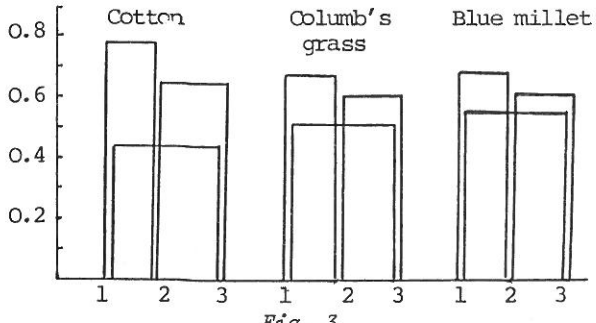

Fig. 3

Coefficient of similarity between complexes of micromycetes in the brown carbonate soil /1/, rhizosphere /2/ and rhizoplane /3/

ing to the genera Arthrobacter and Bacillus and an increase in Rhodococcus and Streptomyces sec. Roseus and sec. Helvolo-Flavus. In the rhizoplane, however there was a decrease in the number of species present in the microbial complex, while bacteria of the genera Pseudomonas and Rhodococcus and actinomycetes of the genus Streptomyces sec. Cinereus and Albus were predominant.

The species abundance of fungi showed a tendency to increase while the frequency of occurrence in the rhizosphere and the soil levelled out. In the rhizoplane, on the other hand, a sharp decrease /by 1.5-2 times/ in the species diversity of micromycetes was found compared with that in the soil /Fig. 2/. Calculations on the Sierensen similarity coefficient have shown that the micromycetes complexes in the soil and in the plant rhizosphere have fairly close values /0.66-0.77/, while those in the soil and the rhizoplane differ considerably /0.43-0.55/ /Fig. 3/. Fungi of the genus Fusarium were found to be predominant in the rhizoplane of cereals, and there was a constant presence of micromycetes of the order Mucorales, family

Table 2

Complexes of the typical species of microscopic fungi in brown carbonate soil and the root zone of plants

Species	Frequency of occurrence, %						
	Soil	Cotton		Columbus grass		Blue millet	
		1	2	1	2	1	2
Alternaria alternata		20	20	25		30	10
Aspergillus carneus						10	
A. flavus	15	25	20				30
A. fumigatus	15	20		10		10	
A. niger	50	40	10	50	40	20	45
A. terreus	30	40	50			30	10
A. terreus var. aureus		10					
A. ochraceus	15	25				30	10
A. versicolor		10	10				
A. ustus	35	30		35		10	
A. wentii	15	10		20		10	
Acremonium	10	10			15		20
Dematiaceae /Gonatobotrym, Papularia, Stachybotrys/	15	20	40	20	25	30	25
Cladosporium cladosporiodes	70	35	20	75	90	80	80
Cylindrocarpon			10		10		10
Fusarium gibbosum		15	15	50	50	50	30
F. solani var. coeruleum	15	10	10	90	100	10	15
Gliocladium roseum		15	20	15	15		
Mucorales /Mucor, Rhizopus/	30	30	20	30	35	25	20
Paecylomyces /P. varioti, P. lilacinus/	10	20					
Phialophora melenii				35			
Penicillium canescens	25			30		50	
P. chrysogenum	15	20	40		10	30	40
P. cep. Funiculosum	30	25	30	15		20	
P. nigricans	10	15				20	
Trichoderma /T. koningi, T. harzianum/	30	20	10	35	40	30	50
Verticillium			15				

1 - Rhizosphere, 2 - Rhizoplane

Dematiaceae /Cladosporium cladosporioides, Alternaria alternata, Gonatobotryum/, Aspergillus niger, A. flavus, A. terreus, Trichoderma koningi, T. harzianum, of various species of Penicillium /Table 2/. In the rhizoplane of cotton there was no really dominant species /like Fusarium in cereals/, but the fungi typical of the root zone of this plant were identified as Aspergillus terreus, A. flavus, Trichoderma, Alternaria alternata, Penicillium ser. funiculosum, P. chrysogenum, Mucor, and Gliocladium roseum. In the micromycetes complex in the plant rhizoplane fewer fungi belonging to the genera Penicillium and Aspergillus were present than in the soil, while there was a greater incidence of the genera Fusarium, family Dematiaceae and of various species of other genera /Trichoderma, Mucor, Gliocladium/.

Conclusions

The present study confirms earlier findings concerning the increase in the number of bacteria and the predominance of non-sporous bacteria in the root zone in comparison with the soil. A noticeable increase in CFU for mycelian organisms was only observed in the rhizoplane; in the rhizosphere the increase in their number was only a tendency.

The total biomass of fungi in the rhizosphere did not exceed its content in the soil or was slightly higher. However, there was a change in the biomorphological structure of fungal populations, involving an increase in the proportion of mycelium in the fungal biomass in the root zone, which indicates a more active functioning of the micromycetes in the rhizosphere and rhizoplane, compared with the soil.

The scale of microbial colonization of the roots in early stages of plant development was assessed. The bacterial colonization of the roots, assessed by measuring the area of root surface covered by cells, exceeds the fungal colonization and averages some tenths of a percent/. In contrast to previously published data, the measurements indicate a lower degree of bacterial colonization of the rhizoplane in early stages of plant development.

The microbial complexes of the soil and the rhizosphere differ significantly in composition and in structure, while significantly less differences were found between the complexes of the soil and the rhizosphere. The slight increase in the species diversity in the fungal complex of the rhizosphere gives place to a sharp decrease in species abundance in the rhizoplane.

A definite group of saprotrophic micromycetes was revealed, which is located in the rhizoplane, mainly in the form of mycelium. These investigations need further elaboration and the facts established should be taken into consideration in studies on the inoculation of plants with microorganisms.

References

MIRCHINK, T. G., OZERSKAYA, S. M. and MARFENINA, O. E., 1982. Biologicheskie nauk. 11. 61-66.

ZVAGINTSEV, D. G., 1980. Metoduh pochvennoi microbiologii i biokhimii. MGU. Moscow.