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Investigations into rhizosphere microflora. IV. Fungal association in different root regions of some rainy-season crops*

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Abstract:

Non-rhizosphere, rhizosphere and rhizoplane microflora of the crown and distal regions of *Echinochloa crusgalli* (L.) Beauv. and *Paspalum scrobiculatum* L. were studied from seedling stage to the harvest.

The variation in bacterial and fungal flora in relation to host species, stage of development and zone of the rhizosphere were studied.

The differences between fungal and bacterial flora are described. The relation between rhizosphere microflora and roots exudates is described.

INTRODUCTION

Considerable research is in progress in different parts of the World on various aspects of the rhizosphere microbial complex. The intensive biological activity in the vicinity of roots known as "rhizosphere effect", has been studied in different plants, environmental canditions and with regard to various chemical, physical and physiological factors. In spite of this, some of the aspects still require further investigation. No detailed information is available on the rhizosphere microflora of *Echinochloa crusgalli* (L.) Beauv. and *Paspalum scrobiculatum* L. Therefore it was considered worthwhile to elaborate the non-rhizosphere, rhizosphere and rhizoplane microflora of these two rainy-season crops in relation to their root exudation and physico-chemical characters of the soil at various stages of growth and to find out whether there exists any succession of fungi from non-rhizosphere to rhizoplane, and, consequently, what is the role of the rhizosphere.

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MATERIALS AND METHOD

Echinochloa crusgalli and Paspalum scrobiculatum are grown in the suburbs of Gorakhpur as important rainy-season crops. The crop fields are situated in a low-lying area and remain sufficiently moist owing to frequent rains during the rainy-season from the 3rd week of June to mid October. The soil of this area is young alluvial soil known as "Kachhar soil" which is a mixture of "matiar" and "domat" soils having a sufficient amount of clay. The whole root system of the two plant species was sampled fortnightly from seedling stage to the harvest. Further sampling, however, was not possible as the fields were ploughed immediately after the harvest.

The assessment of the rhizosphere microflora (fungi and bacteria), non-rhizosphere and rhizoplane mycoflora was done by the method described earlier (Starkey 1929). Moisture content was determined by the method suggested by Piper (1944), and pH with an electric pH-meter. Quantitative data of microflora were statistically analysed by analysis of variance.

Amino acids present in root exudate and root extract of the two plants were determined by the method described below:

Root exudate. Seeds of *E. crusgalli* and *P. scrobiculatum* were surface-sterilized with 0.1% HgCl₂ solution and washed thoroughly with sterilized distilled water to remove any trace of chemicals and microbes. The seeds were then soaked in sterilized distilled water for 12 hours and transferred to 500-ml conical flasks containing sand. The sand was first treated with nitric acid, thoroughly washed with several changes of distilled water to make it acid-free and then autoclaved for one hour at 15 pounds per square inch pressure. Twenty seeds were placed in each flask and six replicates were run for each plant seed. All the flasks were plugged with sterilized cotton to avoid contamination and kept moist for the germination and growth of seedlings. The plants were removed carefully after 15 days and rinsed with sterilized distilled water. The sand was also washed several times and filtered. The filtrate in each case was then concentrated to 10 ml by evaporating at 60°C in water bath.

Root extract. Roots of the two plants were collected at the stage of maximum vegetative growth and the post-flowering stage and washed thoroughly with distilled water to remove the attached soil particles. Root (2g) of each plant was crushed in 10 ml of 80% alcohol and then filtered to remove the root debris.

Amino acids of the root exudates and extracts were detected by unidirectional chromatography. The solvent used was a mixture of n-butanol, glacial acetic acid and distilled water (4:1:5). Ninhydrin $(0.2^{9})_{0}$ in acetone) was used as spraying agent for the development of clear

spots. The chromatogram was air-dried at room temperature and finally at 100° C for 5 minutes. Identification of amino acids was done by running the known amino acids as markers.

Total free amino acids per plant in the case of root exudate and per 1g of root in the case of root extract were determined by the method described by Peach and Tracey (1955).

RESULTS

The findings of present investigation are compiled in tables 1-4 and figures 1 and 2.

Different growth stages are abbreviated as follows:

Cr - crown rhizosphere

D — distal rhizosphere

Crc — cortical rhizoplane of crown region

Crs — stelar rhizoplane of distal region

Ds — stelar rhizoplane of distal region

Distribution of mycoflora in the root regions of Echinochloa crusgalli

In all 69 fungal species were isolated from the non-rhizosphere 61 from rhizosphere and 37 rhizoplane regions. The non-rhizosphere region harboured 10 Phycomycetes, 7 Ascomycetes, 49 Deuteromycetes and 3 mycelia sterilia.

9 Phycomycetes, 5 Ascamycetes, 44 Deuteromycetes and 3 mycelia sterilia were isolted from the rhizosphere region. The rhizoplane region included 5, 1, 27 and 4 species of the above groups, respectively. 12 specific fungal species, viz., Absidia ramosa, Mucor mucedo, Chaetomium globosum, Aspergillus japonicus, Penicillium brefeldianum, P. luteum, P. frequentas, Penicillium sp., Gliocladium roseum, Stachybotrys atra, Helminthosporium sativum and Helminthosporium sp. were isolated from this plant.

The dominant fungal species in this crop varied according to the sampling period. *Rhizopus nigricans* and *Aspergillus nidulans* were isolated throughout plant life from the non-rhizosphere region.

Phycomycetous forms were present infrequently in the rhizoplane of the crown and distal region with the exception of *Rhizopus nigricans* which was dominant in the distal region. Amongst five Ascomycetes of the rhizosphere region, only *Aspergillus nidulans* was dominant in both the rhizospheres. The two rhizosphere regions were mostly dominanted by representatives of Deuteromycetes like *Aspergillus flavus*, *A. niger*, *A. terreus*, *A. versicolor*, *Paecilomyces fusisporus*, *Curvularia lunata*, *Fusarium nivale* and *Fusarium* sp.

The common dominants associated with different rhizoplane regions were *Mucor luteus*, *Aspergillus flavus*, *A. niger*, *Penicillium* sp. 1, *Curvularia lunata*, *Fusarium* spp. and White sterile colonies I. The fungal species dominant in different regions were *Syncephlastrum racemosum*

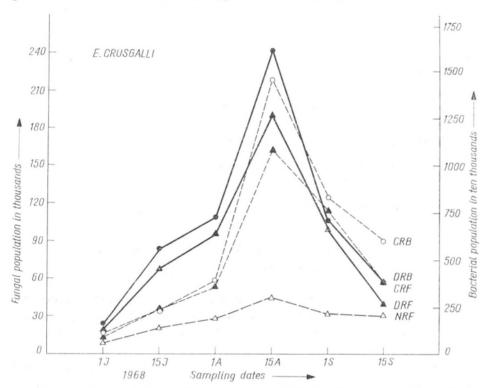


Fig. 1. Microbial population in the root regions of *Echinochloa crusgalli* at different stages of growth.

CRB — crown rhizosphere bacteria; DRB — distal rhizisphere bacteria; CRF — crown rhizosphere fungi; DRF — distal rhizosphere fungi; NRF — non-rhizosphere fungi

in Crc; Aspergillus sydowi and yellow starile colonies in Crs; Rhizopus nigricans, Mucor racemosus and Trichoderma viride in Dc; and Mucor racemosus, Aspergillus sydowi and Black sterile colonies I in the Ds region. Representatives of Ascomycetes showed a low frequency of occurrence. The difference in dominants was pronounced during post-flowering stages. No specific fungal species was isolated from the non-rhizosphere, rhizosphere and rhizoplane regions.

Distribution of mycoflora in root regions of Paspalum scrobiculatum

This plant species harboured 73, 71 and 33 fungal species in the non-rhizosphere, rhizosphere and rhizoplane regions, respectively. Non-rhizosphere was represented by 9 Phycomycetes, 7 Ascomycetes, 51 Deutero-

mycetes and six mycelia sterilia. From the rhizosphere 9, 5, 53 and 4, and 7, 2, 21 and 3 from rhizoplane species of Phycomycetes, Ascomycetes, Deuteromycetes and mycelia sterilia, respectively were isolated.

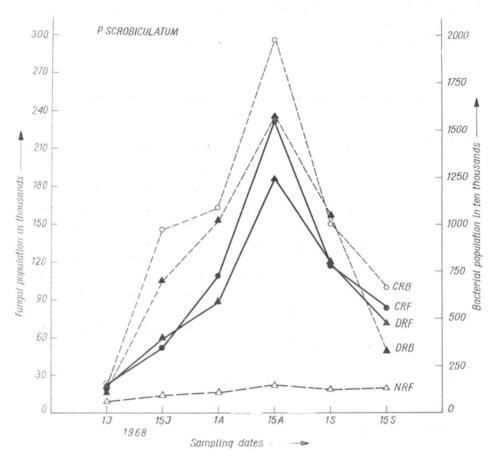


Fig. 2. Microbial population in the root regions of Paspalum scrobiculatum at different stages of growth.

Explanations as in fig. 1.

Fifteen fungal species of restricted occurence, viz., Absidia spinosa, Rhizopus arrhizus, Mucor baineri, Chaetomium indicum, Gelasinospora sp., Monilia acremonium, Aspergillus clavatus, A. fischeri, Penicilium oxalicum, P. rockfortii, Stysanus medius, Fusarium chlamydosporum, F. culmorum, Epicoccum nigrum and White sterile colonies.

The non-rhizosphere region was dominated by Aspergillus nidulans, A. flavus, A. niger, A. terreus, A. versicolor, Paecilomyces fusisporus and Fusarium nivale. Phycomycetes appeared with low frequency.

The dominants in the rhizosphere of the crown and distal region were nearly the same. Common dominants in the two rhizosphere

regions, i.e. crown and distal were Aspergillus nidulans, Paecilomyces fusisporus, Curvularia lutea, Fusarium nivale and White sterile colonies I. Aspergillus flavus, A. niger and Penicillium nigricans were dominant in the distal region. Other species, though isolated frequently, were of low percentual occurrence. Fusarium culmorum was isolated as a specific fungus in the rhizosphere of the crown region.

Table 1

Number of fungal species and Mn. no. of Cols. per plate in different root regions of two plant species

Plants' age in days	* NR	Cr	D	Crc	Crs	Dc	Ds
15	**18/17	15/13	15/13	6/7 (4)/(3)	2/3 (1)/(2)	4/5 (2)/(2)	2/2 (1)/(1)
30	29/31	20/14	19/9	7/4 (4)/(3)	6/5 (4)/(2)	5/4 (4)/(3)	6/5 (3)/(2)
45	30/33	21/25	20/25	9/8 (5)/(4)	5/7 (3)/(3)	7/7 (4)/(4)	7/6 (4)/(2)
60	39/37	34/35	30/33	7/7 (4)/(4)	9/11 (2)/(4)	8/7 (3)/(4)	6/5 (2)/(3)
75	32/24	30/22	24/19	7/6 (4)/(5)	6/4 (2)/(2)	6/7 (4)/(5)	5/5 (3)/(3)
90	31/31	29/24	27/20	9/6 (5)/(5)	9/6 (4)/(4)	8/8 (4)/(6)	8/7 (3)/(4)

Digits in bracket stand for Mn. no. of Cols, per plate.

* NR - Non-rhizosphere

Crs - Stelar rhizoplane of of Crown region

Cr - Crown rhizosphere

Dc - Cortical rhizoplane of distal region

D — Distal rhizosphere

Ds — Stelar rhizoplane of distal region

Crc - Cortical rhizoplane of Crown region

The common dominants in the different rhizoplane regions were Rhizopus nigricans, Aspergillus flavus, A. sydowi, Curvularia lunata, Fusarium nivale and White sterile colonies I. Phycomycetous sterile colonies were dominant in Crc; Aspergillus candidus, A. niger in Crs; A. niger in Dc; and A. niger together with Penicillium decubens in Ds regions. Black sterile colonies II (producing black hard bodies) and black sterile colonies I appeared as dominants in Dc and Ds regions respectively during post-flowering stages.

Both quantitatively and qualitatively the microflora was always least in the young stage and highest during maximum vegetative growth, a decrease followed during post-flowering stage in both the crops. In the non-rhizosphere region the trend was the same, but here the population was always small (Fig. 1 and 2).

^{**} Numerators stand for E. crusgalli and denominator for P. scrobiculatum

Table 2

Amino acids in root extract and root exudate of two plant species (+=Present, --=Absent)

Amino acids	E. crusgalli				P. scrobiculatum			
	Ex	Y	M	PF	Ex	Y	M	PF
Alanine	+	+	+	+	+	+	+	+
Glycine	+	+	+	+	+	+	+	+
Hydroxyproline	+	+	+	+	+	+	+	+
L-leucine	+	+	+	+	+	+	+	+
Proline	_	+	+	+	+	+	+	_
Threonine	+	+	+	+	_	+	+	+
Tryptophan	+	+	+	+	+	+	+	+
Valine	_	+	+	+	+	+	+	+
Unidentified spot		+	+	_		+	+	+
Number of spots	6	9	9	8	7	9	9	8
* Total free amino acids	200	1080	1140	880	220	1000	1000	920

Ex - Root exudate of 15 days old plants

Y,M and PF - Root extracts of 30,60 and 90 days old plants respectively

Chromatograms of root exudates of two crops during young stage and of root extracts of plants at three different growth stages exhibited some quantitative and qualitative variations. Qualitatively the amino acids present in root extracts were more numerous in the young stage and less in the post-flowering stage. Root exudates gave a relatively lesser number of spots than the root extracts during the young stage. Quantitative estimation of total free amino acids of the root exudates and extracts of the two plant species gives a different picture. The total free amino acids in the root extracts were most abundant during maximum vegetative growth, a decline followed in young and post-flowering stages (Table 2).

Table 3

Physico-chemical analysis of soil (Average of three replicates)

	E. cru	sgalli	P. scrobiculatum		
Sampling dates	Moisture content %	рН	Moisture content %	pН	
1.7.1968	20.88	6.9	21.71	7.1	
15.7.1968	18.00	7.7	20.43	7.1	
1.8.1968	20.76	7.5	20.65	7.3	
15.8.1968	21.50	7.5	20.50	7.1	
1.9.1968	19.60	7.6	18.04	7.4	
15.9.1968	16.81	7.7	17.50	7.6	

^{*} Quantity of total free amino acids in mug/plant for root exudates and mug/g of root for root extracts

Table 4

Calculated values of F for rhizosphere microbial variation with regards to age and treatments

Microflora	E. c	rusgalli	P. scrobiculatum		
Wicionora	Treatment	Age	Treatment	Age	
Fungi	8.14**	6.02**	9.90**	4.40*	
Bacteria	3.67	31.99**	4.71	30.22**	

^{**} Signifificant value at 1% level

The differences in the micro-population in different root regions of the two plant species at different stages of growth were found to be statistically highly significant. However, the differences in bacterial population of the two regions were insignificant (Table 4).

DISCUSSION

Various workers (Starkey 1926; Katznelson 1946; Chersters and Parkinson 1959; Neal, Bollen and Zak 1964; Rovira 1965; Mishra 1964) have stressed the importance of plant age when determining the micro-population of the rhizosphere. The maximum rhizosphere microflora at the time of maximum vegetative growth has been widely reported. The increase in the population at this stage could be ascribed to the maximum root exudation during this period, which directly stimulates the microflora.

Generally the rhizosphere microflora of the two crops was higher in the crown region and lower in the distal one. The crown region is an assemblage of old and new roots in all the plants under investigation. This region is characteristic in providing nutrients to the organism both in the form of exudation of metabolites mostly by younger roots and organic debris by the older ones wherein death and decay are continuous processes. These two combined factors resulted in the growth and development of heterogeneous types of microbes, some of them dependent upon dead organic tissues and others favoured by root exudates. The total surface area of the crown region is also relatively large what provides more space for the colonization of various microbes. Moreover, the upper horizon, where the microflora was reported by various workers to be more abundant (Cobb 1932; Saksena 1955; Mishra 1964) provides ample opportunity for the colonization of various microbes in the root region of this area. The distal region with lesser root surface area in the lower horizon of the soil with deficient oxygen and nutrient supply proved less favourable for microbial growth and harboured less microflora. Thornton (1956) attributed the decrease in the micro-population

^{*} Significant value at 5% level

with increasing soil depth to the lower inorganic matter content. The root system of the distal region, being comparatively young, controls the microflora primarily through exudate, consequently only selective organisms find a suitable ecological niche to grow and multiply.

Chesters and Parkinson (1959) isolated 30—40 fungal species from the crown rhizosphere and 25—30 species from the tip region using the soil plate method. Some workers (Rovira 1956; Parkinson, Taylor and Pearson 1963) used direct microscopy to show that root tips of Cabbage and Dwarf beans were almost devoid of fungal hyphae while the abundance of these increased towards the crown of the root. Mishra and Srivastava (1970) also reported that both the fungal and the bacterial population in the rhizosphere of certain plants decreases with increase in soil depth.

In the present work, frequently a lesser number of micro-organisms were isolated when the plants were 15 days old. This is due to the limited root surface with a small amount of root exudates available for the growth and multiplication of micro-organisms.

Non-rhizosphere mycoflora which is primarily governed by soil conditions like moisture content, pH, soil composition etc. also fluctuated with the age of the plants (Table 3). It indicates that non-rhizosphere mycoflora is also controlled by roots, but the effect is indirect, through the addition of root debris. The plants' root in the soil by decomposition and release of various organic metabolites raises the organic status of the soil which is further enriched by the addition of microbial cellular substances after their death.

The rhizosphere microflora of the two plants was always more abundant than that of non-rhizosphere soil during all the stages of plant growth (Figs 1 and 2). However, the number of fungal species was always found to be higher in the non-rhizosphere region than in the rhizosphere and least in rhizoplane. Similar results have also been reported by various workers (Netti 1955; Rivière 1959; Papavizas and Davy 1961; Saksena 1969; Gujarati 1965; Mishra and Srivastava 1969). The non-rhizosphere region is characteristically provided with diverse types of nutrients, therefore it harbours miscellaneous fungal species. The conditions are of somewhat special type as regards nutrition in the rhizosphere region where only root exudation and sloughed-off root materials primarily govern the microflora, and, consequently, only specific forms suited to the micro-environment grow and multiply there. In the rhizoplane region the degree of nutritional specificity is further increased and only selective forms best suited to this region are favoured. The number of species is thus reduced in the rhizoplane region.

According to Garett (1954), in the course of fungal colonization, Phycomycetes were the pioneers followed by Ascomycetes, Deutero-

mycetes and Basidiomycetes. In the present work, Phycomycetes occurred more frequently when the roots were younger, and decreased with the age of the plants.

Rhizoplane fungi of the crown and distal regions also differed somewhat from the pattern exhibited by the rhizosphere of the two regions. A remarkable succession in the appearance of fungi in different root regions was observed during the present study. Fusaria and sterile mycelia which were numerous in the non-rhizosphere region in young stages of the plants, gradually migrated to the rhizosphere region with the advancing age of the plants and finally settled in the rhizoplane region. The rhizoplane was badly infected with Fusarium spp. during harvesting stage. Previous studies (Taylor and Parkinson 1961, 1965; Wastie 1961) also showed that Fusarium spp. have a high degree of saprophytic ability. Generally the number of species and the quantity of fungi (mean number of colonies per plate) were always higher in the cortical region and the lowest in the stellar region. Roots of somewhat older plants were found to have epidermal and cortical cell lavers partly damaged, and, consequently, harboured more micro-fungi, thus favouring a more profuse mycoflora in the subsequent stages. Stelar tissues exhibited a lesser number of fungi than the cortical portion which may be due to the prevalence of lignin tissues in the former and pectin and cellulose ones in the latter

There was not much difference in the root region microflora of *E. crusgalli* and *P. scrobiculatum* owing to the similarity in edaphic and climatic conditions of their habitat. More fungal species were isolated from the non-rhizosphere and rhizosphere regions of *P. scrobiculatum* than from those of *E. crusgalli*, while fungal species in the rhizoplane region showed a reverse trend (Table 1). The two plant species were dominated, almost throughout, by *Aspergilli*. *Fusaria* and sterile colonies predominated the root region during the ripeness harvesting stage. *Penicillia* were scarse at the beginning and increased gradually. Cladosporia, which were dominant and occurred frequently in the root regions of winter crops (Mishra and Srivastava 1969) were mostly absent in present crops except in the last sampling period when they occurred with low frequency.

Certain edaphic factors, viz., pH and moisture content of soil seem to be of primary importance for the non-rhizosphere mycoflora. pH of the soil exhibited a drift towards neutrality or alkalinity.

Role of root exudation/excretion

In the opinion of most authors, the most important factors for stimulation of the rhizosphere micro-organisms are the excretion of organic substances and sloughing-off of root hairs and epidermal cells. The present findings on the nature and amount of amino acids present in root exudates and extracts can easily be correlated with the variation of the rhizosphere microflora.

Rovira (1956) obtained more amino acids from pea and less from oat during 21 days of growth. He reported that this factor together with root debris plays an important role in stimulating the rhizosphere microflora. Rhizosphere micro-organisms showed a direct correlation with the amount of amino acids, but on the other hand, the quality of amino acids which varied to a lesser degree at the different developmental stages possibly played a lesser role than the quantity.

Amino acids, important constituent of root exudates (Rivière 1959), which break the dormancy of fungal spores in the soil (Jackson 1960) consequently harbour a higher microflora in the rhizosphere. Tandon and Bilgrami (1957) reported good growth of soil microbes when a mixture of various amino acids was available while, on the other hand, there is evidence trought by bioassay techniques that growth factors (Lochhead and Thexton 1952) and vitamins (Soluchanova 1962) exuded by plant roots are utilized by soil micro-organisms present in situ. Recently Rangaswami and Balasubramanian (1967) supplied direct evidence of the utilization of root exudates by rhizosphere fungi. All the above described findings support the here presented relation between root excretion and rhizosphere microflora, where the former together with plants' age and other edaphic factors is considered to be the cause of increase in latter.

Although only the amino acids of the root exudates and extracts at various stages of growth were considered in detail in the present work, it is possible to explain to some extent the cause of variation in the rhizosphere microflora with the age and type of the plants in the light of qualitative and quantitative variation of amino acids content. However, besides the amino acids other substances of the root exudates and sloughed-off root cells, which also influence the nature of the rhizosphere micro-organisms, if studied in detail, will throw further light on this aspect of the microbial complex.

SUMMARY

Non-rhizosphere, rhizosphere and rhizoplane microflora of the crown and distal regions of two rainy-season crops, viz., *Echinochloa crusgalli* (L.) Beauv. and *Paspalum scrobiculatum* L. were studied from seedling stage to the harvest. The microflora (fungi and bacteria) of the two plant species was generally found to be lesser during young and post-flowering stages and was at its peak during maximum vegetative growth. The crown rhizosphere always harboured more microbes (both quantitatively and qualitatively) than the distal one. Non-rhizosphere fungi exhibited a somewhat similar trend. The number of species isolated

was maximum in the non-rhizosphere, followed by rhizosphere and rhizoplane regions. Rhizoplane fungi showed quite a different pattern: the species of fungi were more numerous in the young stage and decresed with the age of the plants. In this region only some selective root-infecting forms, viz., Fusarium spp. and Black sterile colonies settled on the root. The quantity of fungi in this region exhibited a reverse trend, the maximum occurring during root-decay stages. Cortical rhizoplane always harboured more mycoflora than that of the stelar region. The rhizosphere microflora exhibited a direct correlation with the amount of amino acids present in root exudate and root extract. Soil moisture content and pH showed a poor correlation with the microflora of the rhizosphere and rhizoplane, while they influenced the non-rhizosphere fungi to some extent. Certain specific and dominant fungal species in various root regions were also recorded.

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Author's address: Dr. V. B. Srivastava D/204, Township, Virbhadra Rishikesh, U.P. India Badania nad mikroflorą rhizosfery. IV. Związki z grzybami różnych obszarów korzenia niektórych roślin z terenów o klimacie deszczowym

Streszczenie

Przedmiotem przeprowadzonych badań była mikroflora rhizosfery i środowiska otaczającego korzenie, zarówno w obszarze szyjki korzeniowej, jak i innych części korzenia, dwóch gatunków roślin — *Echinochloa crus-galli* (L.) Beauv. i *Paspalum scrobiculatum* z terenów o klimacie okresowo deszczowym. Badaniami objęto rośliny w okresie siewki do stadium dojrzałości.

Zbadano florę bakteryjną oraz grzybową i stwierdzono wyraźne różnice między nimi w zależności od gatunku roślin, stadium jej rozwoju i obszaru rhizosfery.

Wykazano również ścisłą zależność między mikroflorą rhizosfery a wydalinami korzeniowymi.