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RHIZOPLANE AND RHIZOSPHERE MICROBIAL ACTIVITY IN ECOLOGICALLY CULTIVATED VEGETABLES AND OTHER CROPS

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Abstract

The purpose of the article is to present data from a comparative study of the composition and activity of the microflora from the rhizoplane and rhizosphere in leeks, red beets, chard, spinach, dill, parsley, and common vetch, as well as control sample without vegetation. The experiment was conducted at the training field at the University of Forestry, Sofia, Bulgaria. Microorganisms from the rhizoplane (the surface of the roots) and the rhizosphere, near the roots, were studied. Growing leeks increases the biogenicity of roots and soil to the highest degree. The main share in the composition of the total microflora is occupied by non-spore-forming bacteria in all variants. In the vegetated variants, the catalase value was highest when growing leek, followed by parsley. Cellulase activity is highest in red beets and leeks. The studied microbiological and enzymatic indicators are sensitive indicators of soil fertility and qualitative development of the analyzed crops. The activity of the rhizosphere and rhizoplane microflora depends on the humidity in the root zone, and the quantity of microorganisms is not the only and independent prerequisite for their activity.

Key words: *catalase, cellulase, legume, microflora, spices.*

INTRODUCTION

On the roots surface (rhizoplane) and the root-inhabited soil layer (rhizosphere), an amazing number of microorganisms develop, participating in the cycles of nutrients, the improving soil fertility, quality and yield of agricultural crops. Plant characteristics influence soil fertility, yield, soil organic decomposition processes, soil nutrient cycling and soil organic matter levels (Bray et al., 2012; Cornwell et al., 2008; Hobbie, 2015; Sofo et al., 2020). Key soil processes are governed by interactions between plant roots, C and N content, and microbial metabolism, which drive decomposition reactions, soil fertility, and plant net primary production (Sofo et al., 2020). The complex composition and interaction of root-associated microbes, detailed characterization of microbiomes from three rhizocompartments (rhizosphere, rhizoplane, and root), are critical to plant health and performance (Wang et al., 2021).

In the present study, rhizoplane and rhizosphere microbiological activity was analyzed in organically cultivated vegetables and other crops: leek, dill, parsley, spinach, chard, red beet, common vetch.

Dill (*Anethum graveolens* L.) is the only species of the genus Anethum, family Apiaceae. It is used as an herb, spice and essential oil crop. The plant is annual. Green parts reach 90-160 cm in height, with thin stems and alternate leaves with strongly cut petioles. (Jana & Shekhawat, 2010). The root is spindle-shaped, slightly branched and relatively shallow (Daskalov and Kolev, 1958).

Parsley (*Petroselinum crispum* Mill. Nyman) belongs to the Apiaceae family and is grown in temperate and subtropical climates throughout the world. There are two varieties, leafy - convar. Crispum and root - convar. Tuberosum. All parts of the plant are usable, including leaves, stems and main roots. (Marthe, 2020). Leaf parsley is a biennial plant, which in the first year forms leaves and a strongly branched root, and in the second passes into a generative phase.

Spinach (*Spinacia oleraceae* L.), family Chenopodiaceae, is a long-day plant. The culture is annual for the conditions of Bulgaria, and under unfavorable conditions (drought, waterlogging, other stress or in conditions of a long day), instead of forming a product part (leaves), it quickly passes into a generative phase (Pandey & Kalloo, 1993). It is grown in early spring or late autumn with the greatest success due to its biological characteristics (Murcia et al., 2020). It has a dense root system composed of a central root and lateral branches of various orders. The central root grows rapidly and can reach a depth of 180 cm. Lateral roots develop initially at a depth of 15-26 cm and later up to 90 cm (https://www.nottingham.ac.uk/hiddenhalf/herbs/spinach.aspx). The "Large winter" variety is widespread and preferred in the country. It is very early and highly productive. It forms a large rosette with tender and juicy leaves.

Chard (*Beta vulgaris* ssp. *cicla* f. *hortensis* Alef.), family Chenopodiaceae, is a leaf chard/beet, spinach beet. Forms a very highly developed leaf rosette. The leaves are over 20 cm long. The main root is thickened in its upper part and forms a branched woody root crop, the lateral roots are stronger and strongly branched than in beetroot (Daskalov and Kolev, 1958).

The leek (*Allium ampeloprasum* L.), family Alliaceae (Amarilidaceae) has a root system typical for the family Alliaceae (Amarilidaceae) - bearded, composed of many unbranched or slightly branched string-like roots, very strongly developed. It is mainly located in the soil layer up to 30 cm deep. Individual roots reach up to 70-75 cm. Roots do not die until late in the fall (Shaban et al., 2014). The "Starozagorski 72" variety is preferred in the country because of the larger product part it forms compared to other varieties (Pidov et al., 1995).

Red beet (*Beta vulgaris* L. var. *esculenta*), family Chenopodiaceae, is a biennial plant that forms a rosette of leaves and a root crop in the first year of sowing, and in the second it goes into a generative phase. The product part can be round, oval or conical in shape depending on the variety and which parts and organs take part in its formation. The product part of the "Bordeaux" variety is formed with the participation of the sub- and supra-coelomic knee and its shape is round (Daskalov and Kolev, 1958). The root system is extensive and well branched (Greenwood et al., 1982). .

Common vetch (*Vicia sativa* L.), family Fabaceae, genus Vicia has a well-developed root system and as a nitrogen-fixing legume, a process localized in small root nodules formed by the symbiosis with *Rhizobium* bacteria, is considered as an improver of soil fertility and a valuable source of protein and minerals for farm animals.

A high bacterial abundance and a lower fungal development were found in dill cultivation (Sofo et al., 2020). Spore-forming bacteria, fluorescent *Pseudomonas* bacteria, diazotrophs, Actinomycetes, microscopic fungi have been isolated from parsley rhizosphere soil (Trzciński et al., 2018). *Rhizobium laguerreae* colonizes the roots of spinach plants, forming microcolonies typical of biofilm initiation, significantly increases several vegetative parameters such as leaf number, size and weight, as well as chlorophyll and nitrogen content, an excellent plant probiotic that increases yield and quality of spinach (Jiménez-Gómez et al., 2018). According to a study by Nunes da Rocha et al. (2013) the rhizosphere is a common habitat for Acidobacteria, subdivision 8 (class Holophagae), with Holophaga 16S rRNA gene counts being more abundant in leek rhizosphere than in bulk soil and grass and potato rhizospheres. These authors propose to monetize strain CHC25 *Candidatus Porrumbacterium oxyphilus* (class Holophagae, phylum Acidobacteria), the first cultured representative with rhizosphere competence. Tubercles are formed on the roots of the common vetch, in which tuberous bacteria live symbiotically, for example from the genus *Rhizobium*. When analyzing the quality of 15 red beet varieties, Nizioł-Łukaszewska and Gawęda (2015) found that the 'Chrobry' variety showed the most favorable quality characteristics - characterized by high antioxidant activity, high content of dry matter, soluble sugars and betalain pigments. Continuous cultivation of sugar beet (*Beta vulgaris* L.) showed lower bacterial diversity and higher fungal diversity in the root-inhabited soil layer compared to intermittent cultivation (Cui et al., 2022).

Promoting organic farming can build an ecologically, nutritionally, and economically sound nation in the near future (Wang et al., 2021). Wang et al. (2021) determined carbon and enzyme activity of microbial biomass in a six-field crop rotation (tomato, cucumber, celery, dill, cauliflower, and eggplant) using

three management practices: low-input, conventional, and organic systems. These authors found that soil microbial biomass and enzyme activities (urease, catalase, protease) in the organic farming system were significantly higher in samples collected in December compared to those obtained from soil that had been cultivated with conventional practices with low input resources. Certain red beetroot clones produce abundant levels of the peroxidase enzyme with good thermal stability (Rudrappa et al., 2005).
The aim of the research is to analyze the effect of the biological cultivation of leaf and root agricultural crops on the occurrence and activity of beneficial groups of microorganisms in the microbiomes of three rhizocompartments of the root-inhabited soil layer (rhizosphere, rhizoplane and root). The literature review on the subject showed little research in the study area for the crops studied in the present material: dill, parsley, spinach, chard, leek, common vetch, red beet, especially for chard and common vetch.

MATERIALS AND METHODS

The experiment was carried out at the training field at the University of Forestry - Sofia, Bulgaria, during the vegetation period of the studied crops in 2022. Microorganisms from the rhizoplane (on the surface of the roots) and rhizosphere were studied, and soil particles near the roots were covered. The roots, together with the particles stuck to them, were ground and analyzed for microbiological and enzymatic parameters.
The studied variants are the following:
- V0 – control, soil without vegetation;
- V1 – dill (*Anethum graveolens* L., family Apiaceae, variety "Mesten");
- V2 – parsley (*Petroselinum crispum* Mill., family Apiaceae, variety "Festival");
- V3 – spinach (*Spinacia oleraceae* L., family Chenopodiacea, variety "Large winter");
- V4 – chard (*Beta vulgaris* ssp. *cicla* f. *hortensis* Alef. family Chenopodiaceae, variety “Mageneta sunset”);
- V5 – leek (*Allium ampeloprasum* L., family Alliaceae (Amarilidaceae), variety "Starozagorski 72");
- V6 – common vetch (*Vicia sativa* L., family Fabaceae, genus *Vicia*, variety “Tempo”)
- V7 – red beet (*Beta vulgaris* L. var. *esculenta*, family Chenopodiaceae, variety "Bordeaux").
The distribution of crops by product part is presented in Table 1.

Table 1. Distribution of crops by product part

Leaf crops	Leaf-stem crops	Mixed (leaf-stem and foliar crops)	Root crops	Fruit crops
Petroselinum crispum Mill.	*Allium ampeloprasum* L.	*Anethum graveolens* L.	*Beta vulgaris* L. var. *esculenta*	*Vicia sativa* L.
Beta vulgaris ssp. *cicla* f. *hortensis* Alef.				
Spinacia oleraceae L				

Microbiological analyzes include the determination of heterotrophic, aerobic, mesophilic groups of microorganisms: non-spore-forming bacteria, spore-forming bacteria (bacilli), actinomycetes, micromycetes (mold fungi), and bacteria assimilating mineral nitrogen. The method of limiting dilutions and subsequent inoculations on specific solid nutrient media (meat-peptone agar, Actinomycete isolation agar, Chapek-Dox agar – supplier: “Ridacom”, Bulgaria) was used (Mishustin and Emtsev, 1989; Gushterov et al., 1977). The colony-forming units for each group of investigated microorganisms were listed and the results were recalculated for 1 g of absolutely dry substrate. Total microflora and mineralization coefficient (MC) were calculated according to the formula: bacteria assimilating mineral nitrogen/(non-spore-forming bacteria + bacilli) (Mishustin and Runov, 1957; Malcheva and Naskova, 2018).
Catalase activity of the rhizoplane and rhizosphere microorganisms was determined by the titration manganese-metric method (Khaziev, 1976).
Cellulase activity of the rhizoplane and rhizosphere microorganisms was determined dynamically over a period of 60 days, and during 15 days the percentage of degraded area of the cellulose filter sheets (50/10 mm) placed on the surface of the roots and the soil adhering to them in petri dishes was calculated. A 60%

marginal field moisture content is maintained (Khaziev, 1976).
Moisture content of the samples was determined on a moisture balance, model DBS 60-3, KERN, Germany.
Statistical processing of the data from the microbiological indicators included the calculation of the mean of three replicates and the coefficient of variation (CV). A correlation analysis was applied for some of the investigated indicators. Data were statistically processed using Excel 2010.

RESULTS AND DISCUSSIONS

The composition and mineralization activity of the microorganisms from the rhizoplane and rhizosphere are presented in Table 2.

Table 2. Quantity ($\log_{10}$ cfu/g), composition, and mineralization activity of rhizoplane and rhizosphere microorganisms

Variants	Total microflora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-mycetes	Bacteria assimilating mineral nitrogen	Minerali-zation coefficient
V0 Control (without vegetation)	6.28	6.04	4.88	5.84	4.73	6.67	4.063
V1 Dill	6.75	6.71	5.44	4.85	4.85	6.50	0.585
V2 Parsley	5.46	5.28	4.85	4.04	4.22	6.27	7.104
V3 Spinach	6.54	6.42	5.79	5.07	4.89	6.51	0.985
V4 Chard	6.52	6.25	6.17	4.08	4.86	6.11	0.401
V5 Leek	6.86	6.79	6.01	4.32	4.76	6.62	0.584
V6 Common vetch	6.64	6.54	5.46	5.42	5.53	6.53	0.894
V7 Red beet	5.93	5.61	5.57	4.70	4.23	6.54	4.453

*CV up to 10% for all variants (low dispersion).

The biogenicity of the samples compared to the control (V0) increases when growing leeks (V5 - 3.8 times), dill (V1 - 2.9 times), common vetch (V6 - 2.3 times), spinach (V3 - 1.8 times) and chard (V4 - 1.7 times). Cultivation of parsley (V2) and red beet (V7) did not increase the total microflora compared to the control, without vegetation. In the variants with vegetation, biogenicity decreases in the order: leek (V5) > dill (V1) > common vetch (V6) > spinach (V3) > chard (V4) > red beet (V7) > parsley (V2). However, the mineralization activity is highest in the two variants with the lowest biogenicity - in the cultivation of parsley (V2) and red beet (V7), higher than in the control (V0). In the remaining variants, the values of the mineralization coefficient are lower than in the control. The rate of decomposition of organic matter in the variants with vegetation decreases in the order: V2 (parsley) > V7 (red beet) > V3 (spinach) > V6 (common vetch) > V1 (dill) > V5 (leek) > V4 (chard). Therefore, the activity of microorganisms does not depend solely on their quantity, which was also established in other studies (Yankova et al., 2016, Malcheva, 2021). One of the main factors for their development is soil moisture in the root layer. The moisture content of the tested samples is presented in Figure 1.

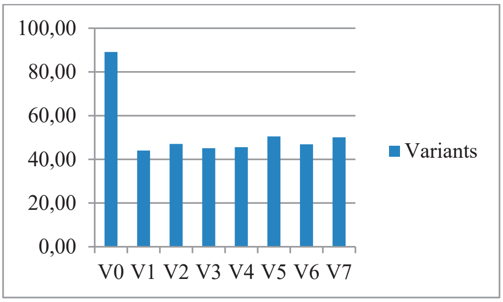

Figure 1. Rhizosphere and rhizoplane soil moisture (%)

The soil moisture in the control is about 2 times higher compared to the other variants. The moisture values of the roots with adhering soil in the vegetated variants are close, with the highest moisture in leek (V5) and red beet (V7) and the lowest in dill (V1).

In the vegetated variants, non-spore-forming bacteria take the main share, followed by bacilli, which play a major role in the initial stages of decomposition of organic matter. In the red beet (V7) and chard varieties (V4), the percentage of non-sporing bacteria and bacilli is more evenly distributed: 48% non-sporing bacteria and 44% bacilli in red beet (V7); 53% non-sporing bacteria and 44% bacilli in chard (V4). While in the other variants, the percentage of non-sporing bacteria varies from 66% in parsley (V2) to 93% in dill (V1). Less represented are actinomycetes and mold fungi, which participate more actively in the final stages of organic destruction. In the control (V0), the quantity of actinomycetes was higher than that of bacilli. The percentage distribution of the groups of microorganisms in the composition of the total microflora in the control (V0) is as follows: non-spore-forming bacteria 57%, bacilli 4%, actinomycetes 36%, micromycetes 3%. The quantity of bacteria is higher than that of fungi when growing agricultural crops (Yankova et al., 2016; Plamenov et al., 2016; Malcheva et al., 2018; Malcheva et al., 2019; Malcheva, 2021). The highest percentage in the composition of the total microflora when growing vegetables is occupied by the ammonifying bacteria (non-spore-forming bacteria and bacilli), and the least represented are micromycetes and actinomycetes (Malcheva et al., 2020). A high bacterial abundance and a lower fungal development were found in dill cultivation (Sofo et al., 2020). Spore-forming bacteria, fluorescent *Pseudomonas* bacteria, diazotrophs, Actinomycetes, microscopic fungi have been isolated from parsley rhizosphere soil (Trzciński et al., 2018). *Rhizobium laguerreae* colonizes the roots of spinach plants (Jiménez-Gómez et al., 2018), and according to a study by Nunes da Rocha et al. (2013) the rhizosphere is a common habitat for Acidobacteria. Tubercles are formed on the roots of the common vetch, in which tuberous bacteria live symbiotically, for example from the genus *Rhizobium*. However, continuous cultivation of sugar beet (*Beta vulgaris* L.) showed lower bacterial diversity and higher fungal diversity in the root-inhabited soil layer compared to intermittent cultivation (Cui et al., 2022). Fungi are more closely related to environmental factors than bacteria (Cui et al., 2022).

The catalase activity of rhizoplane and rhizosphere microorganisms is presented in Figure 2.

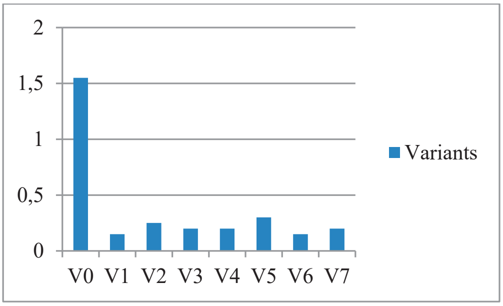

Figure 2. Catalase activity of the rhizoplane and rhizosphere microflora (ml O_2/30 min)

The results showed that catalase activity was significantly lower in the vegetated variants compared to the non-vegetated control. In the variants with vegetation, the value of the catalase enzyme is highest when growing leeks (V5), followed by parsley (V2), and the lowest is with dill (V1) and common vetch (V6). In leek (V5) cultivation, catalase activity correlates with the total quantity of microorganisms. While with parsley (V2) no dependence was established with a higher quantity of microorganisms, but with the highest mineralization activity. Therefore, catalase activity does not depend independently on the quantity of microorganisms. Influence is exerted by a complex of factors: type of soil and root system, soil temperature and humidity, pH, type of vegetation and other factors. When growing rapeseed, a lower catalase activity was also found in the variants with vegetation compared to the control without vegetation (Malcheva et al., 2019).

The cellulase activity of rhizoplane and rhizosphere microorganisms is presented in Figure 3.

Cellulose degradation is fastest in red beets (V7) and leeks (V5) – 100% degraded area on the 45th day.

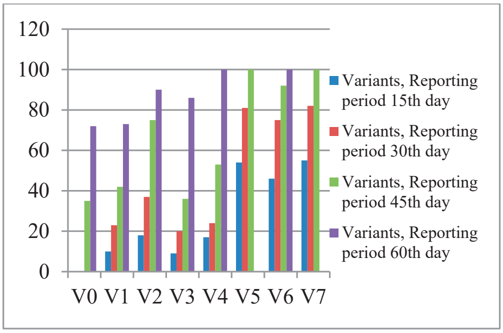

Figure 3. Cellulase activity of the rhizoplane and rhizosphere microflora (% degraded area)

Up to 100% degradation of the cellulose was also found in two other variants – chard (V4) and common vetch (V6), but on the 60th day of reporting. For a period of two months, the percentage of decomposed area for dill (V1) is 73%, for spinach (V3) 86% and for parsley (V2) 90%. Cellulase activity was higher in all variants with vegetation compared to the control (V0 no vegetation), with the cellulase value in dill (V1) being close to that of the control.
The correlation dependences between some of the studied indicators are presented in Table 3.

Table 3. Correlational dependencies

Indicator	Total microflora	Humidity	Catalase	Cellulase	MC
Total microflora	1				
Humidity	-0.25404	1			
Catalase	-0.24256	0.99322	1		
Cellulase	0.053794	-0.51287	-0.57085	1	
MC	-0.83879	0.310997	0.297103	-0.12264	1

A very high positive correlation was established between the humidity of roots and soil, and the catalase activity of the rhizoplane and rhizosphere microflora. The cellulase activity of microorganisms from the rhizoplane and rhizosphere is weakly positively dependent on the total quantity of microorganisms. A moderate correlation was found between humidity and mineralization coefficient values, as well as between the rate of decomposition of organic matter (MC) and catalase activity. In general, the results show that the activity of the rhizosphere and rhizoplane microflora depends on the humidity in the root zone, and the quantity of microorganisms is not the only and independent prerequisite for their activity.

CONCLUSIONS

Organic cultivation of leeks (V5), dill (V1), common vetch (V6), spinach (V3) and chard (V4) increases biogenicity on the roots and the root-inhabiting soil layer closest to the roots, to the highest extent in leeks (V5) and to the lowest extent in chard (V4). While the values of the total microflora in parsley (V2) and red beet (V7) are lower compared to the value of this indicator in the control (V0), without vegetation. However, the mineralization activity is highest in the two variants with the lowest biogenicity - in the cultivation of parsley (V2) and red beet (V7), higher than in the control (V0). A moderate correlation was found between humidity and mineralization coefficient values. The activity of microorganisms in the root layer does not depend solely and independently on their quantity.
The main share in the composition of the total microflora is occupied by non-spore-forming bacteria in all variants. In the vegetated variants (V1-V7), regrouping was found relative to the control (V0). In the control (V0), the actinomycetes, and in the variants with vegetation (V1-V7), the bacilli occupy the second place in the composition of the total microflora. In the red beet (V7) and chard (V4) varieties, the percentage of non-sporing bacteria and bacilli was more evenly distributed compared to the other varieties. Mold fungi and actinomycetes are least represented in cultivated crops. Non-spore-forming bacteria and bacilli are most actively involved in the initial stages of organic matter decomposition. While actinomycetes and mold fungi are more active decomposers of organic matter in the final stages of organic matter degradation in the variants with the studied agricultural crops.
Catalase activity was significantly lower in the vegetated variants (V1-V7) compared to the non-vegetated control (V0). In the variants with vegetation (V1-V7), the catalase value is the highest when growing leeks (V5), followed by

parsley (V2), and the lowest is with dill (V1) and common vetch (V6). A complex of factors, including the type of vegetation and the presence of plant-derived catalase, are likely to be influential. Catalase is highly dependent on moisture in the root layer and is moderately dependent on the rate of mineralization of organic matter (MC).

Cellulase activity was highest in red beet (V7) and leek (V5), followed by chard (V4) and common vetch (V6), and lowest in dill (V1) and the control (V0). The results show that the activity of the enzyme depends on the type of vegetation. Correlation analysis showed that cellulase depends weakly positively on the total microflora on the roots and the nearest root-inhabiting soil layer.

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