

THE INFLUENCE OF TOMATO ROOT EXUDATES ON STRUCTURE AND DIVERSITY OF RHIZOSPHERE COMMUNITIES OF BACTERIA AND FUNGI

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Abstract

The paper aimed to present the results of research carried out on soil microbiota as compared to that colonizing rhizosphere of tomato plants (*Solanum lycopersicum* L.), FLAVIOLA variety, for assessing the influence of root exudates on composition and abundance of microbial communities in relationship with benefits for plant nutrition and health conferred by their interaction. Cosmopolitan fungal species from genera *Fusarium*, *Penicillium* and *Humicola*, as well as bacteria from genera *Bacillus*, *Pseudomonas*, associated with Actinomycetes dominated microbial communities from soil. Rhizosphere communities were dominated by fungal species belonging to genera *Aspergillus*, *Penicillium* and *Trichoderma* accompanied by nematophagous fungi from genus *Arthrobotrys*. *Pseudomonas fluorescens*, important for biocontrol of root pathogenic microorganisms, associated with other non-fluorescent species of *Pseudomonas* were the most abundant in rhizosphere. Rhizosphere effect assessed using as indicator the ratio between the value of total counts of microbes in rhizosphere and in soil (R/S) confirmed the stimulation of microbial abundance and diversity by plant root exudates as compared to soil.

Key words: rhizosphere effect, tomato roots, biocontrol agents, microbial communities, root exudates.

INTRODUCTION

Soil is a complex system of habitats providing variable conditions and hosting a microbial biodiversity complex with multiple microbe-microbe, microbe-environment and plant-microbe, interactions (Prasad et al., 2021). Plants rhizosphere is a place different from bulk soil because it is enriched in various metabolic compounds permanently released by roots (Verma et al., 2018). Their composition is variable in time during the development stages of plant and other environment conditions (pH, temperature, light) (Singh et al., 2007). These exudates consist in carbon-based compounds (organic acids, amino acids, sugars, enzymes, mucilage) playing the role of attractants for various species of microorganisms belonging to the groups of bacteria and fungi (Reinhold-Hurek et al., 2015).

Creighton Miller et al. (1986) and Rasmann & Turlings (2016) stated that these root signals are also able to mediate mutualistic interactions in the rhizosphere. Research carried out by Fu et al. (2021) revealed that differences in bacterial and fungal community composition

correlated with specific modifications in composition of root exudates released by tomato plants across different developmental stages. Rhizosphere microbiota, protected by microenvironment conditions from structural aggregates presents a more intense activity, releasing also various metabolic biosynthesized products: enzymes, exopolysaccharides with role in binding soil particles, pigments, compounds with structure similar to humic substances belonging to aromatic group including quinones, anthraquinones, naphthoquinones, flavines, proteins (Matei et al., 2022). ACC deaminase produced by plant-growth promoting rhizobacteria was able to alleviate salinity stress in French bean (*Phaseolus vulgaris* L.) plants (Gupta & Pandey, 2019) and improved drought stress tolerance in grapevine (*Vitis vinifera* L.) (Duan et al., 2021).

A recent study using modern approach revealed that microbial carbon use efficiency (CUE) is a major determinant of soil organic carbon (SOC) storage, supporting the idea that a high microbial CUE promotes SOC storage more than SOC loss. The high efficiency leads to

SOC accumulation by increased synthesis of microbial biomass and by-products but a low efficiency drives to SOC loss by partitioning more carbon towards cellular respiration (Tao et al., 2023).

Thus, plants rhizosphere and their bacteriome and mycobiome play an important role in carbon (C) sequestration and combating the negative effects of climate changes.

Microbial necromass is considered as the main component of SOC sequestration, its contribution to aggregate-C sequestration being of 43.96% with higher values from fungal necromass-C than that of bacterial (Zhang et al., 2023).

Research has been carried out on soil microbiota as compared to that colonizing rhizosphere of tomato plants, for assessing the influence of root exudates on composition and abundance of microbial communities in relationship with benefits for plant nutrition and health conferred by their interaction.

MATERIALS AND METHODS

In order to assess the influence of root exudates of plants on composition and abundance of microbial communities an experiment was conducted in Mitscherlich pots with 8 kg soil Typic Chernozem (WRB) from Fundulea (with pH 7.51, humus content 3.18%, N content 0.154%, C/N 14, PAL 38ppm, KAL 163 ppm) with tomato plants grown under control conditions at NRDISSAE, Bucharest, during the summer 2023.

Romanian cultivar FLAVIOLA is characterized by high foliar biomass, small, uniform and very tasty tomato fruits, with a high productivity on various culture substrates or greenhouse technologies (Drăghici & Pele, 2012; Jerca et al., 2016; Jerca et al., 2021; Matei et al., 2023). After 60 days, soil and rhizosphere samples were collected and analysed according to the specific methods of chemical and microbiological analysis described in the manual utilized in soil monitoring system (Dumitru & Manea, 2011).

Thus, microbiological parameters determined for soil and rhizosphere by plating serial dilution on solid agar culture media, were: Total Number of Bacteria-TNB (on Nutrient

agar - NA) and Total Number of Fungi-TNF (on potato-dextrose agar - PDA), taxonomic composition of microbiota, carried out by morphologic criteria (using a MC5.A optic microscope) and specific manuals for bacteria (Bergey & Holt, 1994) and for fungi (Cooke & Godfrey, 1964; Domsch & Gams, 1970; Liu et al., 1992; Dackman, 1992; Watanabe, 2002).

Rhizosphere effect was assessed using as indicator the ratio between the value of total counts of microbes in rhizosphere and in bulk soil (R/S).

Another parameter represented by the global physiological activity of microbiota (expressed as the quantity of CO₂ released by soil, respectively rhizosphere microbial communities) was assessed by substrate induced respiration (SIR) method and bacterial and fungal biomass calculation.

According to this selective inhibition technique (Bailey et al., 2002), soil and rhizosphere microbial community structure was established by interpreting the values of the ratio of fungal to bacterial biomass (F: B).

The species richness or total number of species (S), as well as the relative abundance (A%) of each species in microbial communities from soil and tomato rhizosphere were calculated.

Microbial biodiversity in bulk soil and in rhizosphere of tomato plants was also assessed by calculating the diversity index Simpson (D) and Shannon index (H'), its value taking into account both the richness and evenness E(ε) of the community (Mohan & Ardelean, 1993).

RESULTS AND DISCUSSIONS

The rhizosphere of tomato plants is represented by soil aggregates adhering to the roots, formed by soil particles, bound together with decomposed organic residues, root exudates (rich in phenolics) and exometabolites released by microorganisms (Figure 1), as noticed in other research (Makoil & Ndadikemi, 2007), revealing the composition of exudates binding aggregates in rhizosphere of leguminous plants and their role in providing support for plant roots.

Allard et al. (2016) reported that conditions from *Solanum lycopersicum* (tomato) rhizosphere influenced the composition and structure of robust bacterial communities when

grown in soil amended with various organic and synthetic fertilizers.



Figure 1. Soil aggregates in rhizosphere of tomato plants

Arshad et al. (2023), Jerca et al. (2023) underlined the importance of climatic conditions from the greenhouse on tomato production and fruits quality.

Analysis of results presented in Table 1 reflects the general tendency of increasing the total counts of both groups of microorganisms with one order of magnitude under the influence of root exudates released by tomato plants as compared with microbiota from bulk soil.

Thus, total counts of heterotrophic aerobic bacteria increased from low value of 9.974×10^6 viable cells $\times g^{-1}$ dry soil for bulk soil to high value of 38.372×10^6 viable cells $\times g^{-1}$ dry soil in rhizosphere and from moderate value of 89.448×10^3 colony forming units (c.f.us) $\times g^{-1}$ dry soil to high value of 136.415×10^3 colony forming units $\times g^{-1}$ dry soil for microscopic fungi.

Table 1. The total counts of bacteria, fungi in soil and rhizosphere microbiota and R/S index

Origin of microbiota	TNB ($\times 10^6$ viable cells $\times g^{-1}$ d.s.)	R/S	TNF ($\times 10^3$ cfus $\times g^{-1}$ d.s.)	R/S
Soil microbiota	9.974	3.847	89.448	1.525
Rhizosphere microbiota	38.372		136.415	

The value of R/S ratio (3.847) for bacteria, higher than the value calculated for fungi (1.525) indicates that the composition of root exudates released by tomato plants was very appropriate and stimulated the development of a numerous bacterial community and to a less extend for the group of fungi.

The species *Bacillus megaterium* (A=22.2%) was dominant in composition of soil bacteriome, accompanied by other species of the genus *Pseudomonas*, *Bacillus*, *Arthrobacter* and actinomycetes from Series Albus, Fuscus and Luteus (Figure 2).

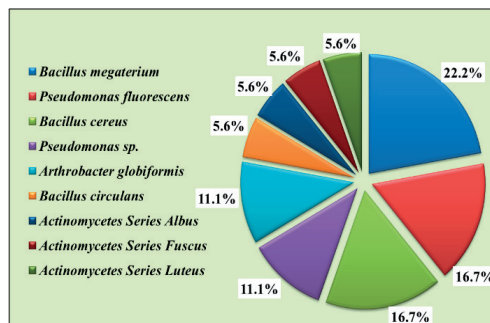


Figure 2. Composition and species relative abundance in soil bacteriome

A higher number of species (17) influenced by the presence of tomato root exudates has developed in rhizosphere conditions (Figure 3).

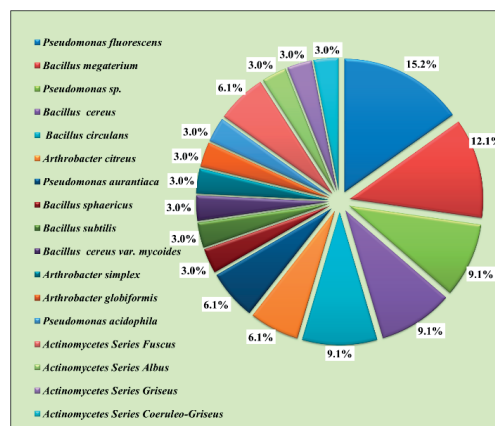


Figure 3. Composition and species relative abundance in rhizosphere bacteriome

The most abundant species was *Pseudomonas fluorescens* (A=15.2%), recognized for the role of biocontrol agent of plant pathogens, accompanied by a larger number of species from genera *Pseudomonas*, *Bacillus*, *Arthrobacter* and actinomycetes from Series Fuscus, Albus, Griseus and Coeruleo-Griseus. Fungal community (mycobiome) from soil consists of 9 species, dominated by *Penicillium aurantiogriseum* (A=21.4%), accompanied by

other 3 cosmopolitan species from genera *Aspergillus*, *Fusarium* and *Humicola* (each with A=14.3%) and 5 species (A=7.1% each) with high cellulolytic capacities, too (Figure 4).

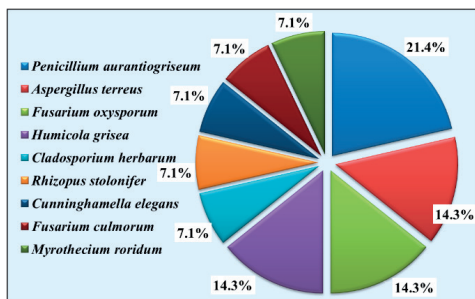


Figure 4. Composition and species relative abundance in soil microbiome

Conditions created in rhizosphere by exudates excreted by tomato roots sustained a more complex mycobiome consisting of 13 species (Figure 5), with 5 of them twice more abundant (each with A=11.11%) than the other 8 species (each with A=5.6%). The five codominant species were antagonists of phytopathogens (*Trichoderma viride*), strong cellulose decomposers from genera *Penicillium* and *Aspergillus* and nematophagous fungus *Arthrobotrys arthrobotryoides*.

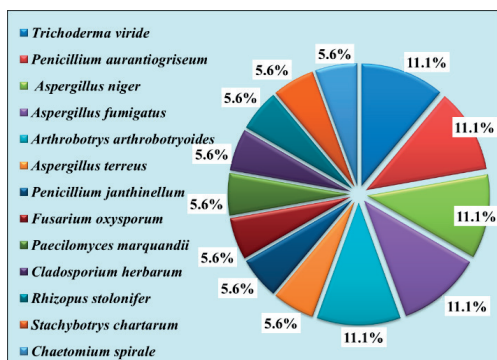


Figure 5. Composition and species relative abundance in rhizosphere microbiome

Figure 6 illustrates the capacity of these fungal species to produce exometabolites represented by surface yellow or uncoloured exudates (from species of *Penicillium* and *Aspergillus*) and red pigments diffused into the medium as in the case of interactions between species from

genera *Trichoderma*, *Penicillium* and *Fusarium*.



Figure 6. Fungal exudates and pigments produced by species from rhizosphere microbiome

Results from present research are in concordance with those reported and reviewed by Abdul-Rahman et al. (2021) who found that root exudates accumulated by “rhizodeposition” in rhizosphere ecosystem improved conditions for increasing the density and biomass accumulation of favourable bacteria, as well as higher rhizosphere

respiration rates compared with surface soils. The prevalence of pseudomonads, bacilli and actinomycetes in the rhizosphere of tomato plants complies with the results of other studies on tomato rhizosphere, soil and roots evidencing the presence of Proteobacteria, Actinobacteria, symbiotic and free living diazotrophic microbes, mycorrhizal fungi and phosphate solubilizing microorganisms in composition of rhizosphere microbiome, with importance in biogeochemical cycles of nitrogen, carbon, phosphorus and sulphur (Kalayu, 2019; Abdul Rahman et al., 2021; Trivedi et al., 2021; Anzalone et al., 2022; Naumova et al., 2022).

Alawiye & Babalola (2019) remarked the high biodiversity in bacterial communities from typical rhizosphere of plants and discussed the interactions and their importance for both microbes and plants.

The higher number of fungi in rhizosphere compared to soil has a beneficial effect on C sequestration in their cell walls and enhance C stabilisation by facilitating soil aggregation.

Other researchers (Six et al., 2006) stressed the importance of both groups of microorganisms, interdependent each other, and their role in the main processes from soil and rhizosphere in agroecosystems.

The rhizosphere effect was evidenced by higher values of diversity indices registered for both bacterial and fungal communities as compared with those characterizing the communities from bulk soil (Table 2).

Table 2. Bacterial and fungal biodiversity in soil and rhizosphere microbiota

Origin of microbiota	Bacterial Diversity	Fungal Diversity
Soil microbiota	S=9 Simpson D=0.858 Shannon H'=2.062bit E(ε)=0.837	S=9 Simpson D=0.867 Shannon H'=2.107bit E(ε)=0.829
Rhizosphere microbiota	S=17 Simpson D=0.918 Shannon H'=2.659bit E(ε)=0.853	S=13 Simpson D=0.904 Shannon H'=2.505bit E(ε)=0.847

The communities from rhizosphere were richer in species and more homogenous concerning the distribution of “individuals” on species, as reflected by the increased values of E(ε)=0.853 for bacteria and E(ε)=0.847 for fungi, comparatively with lower values for soil microbiota. These data confirmed the more

homogenous values of abundance (A%) of species from rhizosphere communities previously illustrated.

Similarly, results of Naumova et al. (2022) on bacterial species richness revealed values with an order of magnitude higher in the rhizosphere than in the root endosphere and were explained by the higher versatility of environmental niches in the rhizosphere. The results in the present research are in concordance with a very recent study (Alahmad et al., 2024) concerning the composition of microbial communities and interactions under 4 pearl millet (PM) lines with various potentials of soil aggregating. The rhizosheath, or root-adhering soil (RAS), defined as the cohesive soil layer firmly adhering to plant roots (with role in facilitating uptake of water and nutrients) presented distinct microbial community as compared with those colonizing root tissue (RT) and bulk soil (BS), for both bacteria and fungi. Bacterial α diversity indices (richness and evenness) were not significantly different among PM lines within compartments, but fungal diversity from RT presented much higher evenness compared to BS and RAS.

Bacterial and fungal communities from rhizosphere of tomato plants in present experiment accumulated higher biomass C than the communities from soil (Table 3). F: B ratio calculated for soil microbiota was 0.928 and 0.814 for rhizosphere microbiota. This indicated bacterial-dominated microbial community structure for both soil and rhizosphere.

Table 3. Bacterial biomass, fungal biomass and F: B ratio in soil and rhizosphere microbiota

Origin of microbiota	Bacterial biomass (µg C/g sol)	Fungal biomass (µg C/g sol)	F: B ratio
Soil microbiota	428.192	397.478	0.928
Rhizosphere microbiota	614.958	501.032	0.814

According to Wang et al. (2019), the values of F:B biomass ratios <1 reflect a bacterial-dominated microbial community. The authors argue the importance of this indicator of microbial community structure and its significance in soil ecology. Bacterial versus fungal dominance is clearly related to plant productivity. Also, a higher F: B ratio indicate

a fungal-dominated microbial community and a high sustainability in agro-ecosystems.

Bailey et al., (2002) reported the value of 0.85 for F: B ratio in agricultural soil cultivated with corn and 13.5 for restored prairie soil, the last high value due to increased fungal activity being associated with increased soil C.

Results of a recent study in France (Djemiel et al., 2023) proved the usefulness of F: B ratio as an indicator of soil status, the values determined ranging from 0.24 to 12.5 being influenced by soil characteristics (especially the pH, organic C content, C:N ratio) and land management. 5.5% of soils with ratios <1 have been anthropized soils (under grasslands and various agricultural crops).

Rhizosphere microorganisms play an important role by plant growth promoting under abiotic stress conditions.

Chaudhary et al. (2018) found the beneficial role of *Penicillium* in increasing plants resistance to various abiotic stresses. Thus, various effects of several microbial species from rhizosphere were reported on cultivated tomato plants.

In drought conditions, *Pseudomonas* sp. improved antioxidant enzymes activity, increased tomato biomass accumulation, altered ABA and indole-3-acetic acid (IAA) content (Brilli et al., 2019). *Trichoderma* sp. promoted tomato plant growth and its tolerance to water deficit (through increased N and P uptake), increased shoot dry weight and stomatal conductance (Khoshmanzar et al., 2020).

Yan & Khan (2021) attributed the biological control of bacterial wilt of tomato plants to the metabolites with inhibitory role released by fungus *Trichoderma harzianum* and Mukerjee et al. (2022) evidenced the mycoparasitism as responsible for *Trichoderma*-mediated suppression of plant pathogenic agents.

Trichoderma harzianum induced an increased synthesis of secondary metabolites and defence-related enzymes by tomato (*Solanum lycopersicum*) plants as mechanisms for the control of root-knot nematodes (Yan et al., 2021). Results reviewed by Yao et al. (2023) confirmed the role of *Trichoderma* in biological control of fungal pathogens and nematodes by competition, antagonism, antibiosis and mycoparasitism and in promoting plant growth.

Yoo et al. (2021) reported that tomato plants presented increased tolerance to salinity stress induced by 2 strains of bacteria belonging to the genus *Bacillus* that increased proline accumulation, antioxidant enzymes activity, chlorophyll and carotenoid content and prevented the damage of plant cell membrane.

Other authors (Calderón et al., 2014) evidenced the role of 2-hexyl, 5-propyl resorcinol produced by a strain of *Pseudomonas chlororaphis* in the multitrophic interactions from rhizosphere of avocado during the biocontrol processes.

Recent results (Boiu-Sicuia et al., 2023) from a study carried out for the evaluation of antifungal potential of 6 bacterial strains belonging to *Bacillus pumilus*, *B. subtilis* and *B. velezensis* isolated from various plants revealed the broad and strong antifungal effect of both living cells, bacterial cell-free supernatants and volatile active compounds on various species of grape spoilage fungi (from genera *Aspergillus*, *Penicillium*, *Botrytis*) and grapevine trunk disease fungi (from genera *Fusarium*, *Diplodia*, *Clonostachys*, *Neofusicoccum*, *Stereum* and *Eutypa*).

Some strains were able to produce fungal growth inhibition by synthesis of lytic enzymes (chitinase, cellulase, protease), difficidin, fengycin, iturins, macrolactin and mycosubtilin. Also, plant beneficial effects were found (e.g. phosphate and phytate solubilization, phytohormone synthesis).

Diazotrophic/N-scavenging bacteria isolated from the soils and rhizospheres of two species of *Solanum* presented plant growth-promoting activity (Zuluaga et al., 2020).

The symbiosis between vesicular-arbuscular mycorrhiza (VAM) in some Nigerian soils and three cultivated plants improved the growth of cowpea (*Vigna unguiculata*), tomato (*Lycopersicon esculentum*) and maize (*Zea mays*) (Sanni, 1976).

Research on the interactions between VAM fungi and rhizosphere microbiota revealed, as compared with non-mycorrhizal gramineous plants, changes in frequency of certain species, such as decrease of % of phytopathogens from genera *Fusarium*, *Helminthosporium*, *Alternaria*, *Pythium* and increasing the representation of beneficial cellulolytic species from genera *Cladosporium*, *Scopulariopsis*,

nematophagous fungi and *Trichoderma*, the last being involved in pathogens inhibition by volatile compounds released (Şesan et al., 2010; Matei, 2011), similarly to results reported by Pescador et al. (2022).

One of the first volatile compound discovered was 6-pentyl- α -pyrone, characterized by a distinct coconut odour, specific to *Trichoderma viride* which inhibited the oospore formation in *Phytophthora cinnamomi* and conidial germination in *Botrytis cinerea* (Dennis & Webster, 1971).

Actinomycetes are well-known for their capability to synthesize bioactive molecules called antibiotics, metabolites with antimicrobial role (Augustine et al., 2008), sort into major structural classes: amino glycosides, ansamycins, anthracyclines, b-lactam, macrolides and tetracyclins, more than 60% being produced by *Streptomyces griseus*. From 12,000 antibiotics discovered in the last 60 years, actinomycetes yielded around 70 %, the rest of 30% being produced by filamentous fungi and non-actinomycete bacteria (Rasche et al., 2006; Singh et al., 2012).

Numerous studies reported the ubiquitous presence of *Trichoderma* spp. in both soil and plant rhizosphere (Matei & Matei, 2002; Matei & Matei, 2010; Sharma & Singh, 2014) in all climatic conditions (Ghorbanpour et al., 2018) and discussed its complex interaction with plants and other microorganisms from rhizosphere or soil (Tyskiewicz et al., 2022). Alexandru et al. (2013) reported the beneficial effect of some *Trichoderma* species on photosynthesis intensity and synthesis of pigments in tomatoes.

El-Maraghy et al. (2021) evidenced the importance of the large variety of mechanisms utilized by plant growth promoting fungi, including *Trichoderma*. These fungi are also capable to synthesize siderophores of hydroxamate-type, (e.g. ferrichromes, coprogens, and fusarinines) and to suppress phytopathogenic agents by depletion of iron sources or by volatile compounds released (Harman et al., 2004; Matarese et al., 2012; Joo & Hussein, 2022).

Similarity indices between soil and tomato rhizosphere communities in present experiment had the value SI=61.53% for bacteria and SI=45.45% for fungi.

Previous results (Matei et al., 2022) evidenced that specific composition of root exudates from tomato and cucumber plants grown on similar substrates influenced the composition of rhizosphere communities of micro-mycetes, with only 52.17% shared species, half of them being nematophagous species *Arthrobotrys oligospora*, *Monacrosporium cionopagum* and *Harposporium anguillulae*.

Other studies (Hanson & Howell, 2004; Matei et al., 2010; Pršić & Ongena, 2020) evidenced the role of microbial elicitors produced by rhizosphere bacteria (*Pseudomonas fluorescens*) and fungi (*Trichoderma viride*, *T. harzianum*) in triggering plant immunity.

Kravchenko et al. (2003) noticed the effect of root exometabolites of tomato plants on the growth and antifungal activity of plant growth-promoting *Pseudomonas* strains and showed that the antifungal activity of plant growth-promoting rhizobacteria in the plant rhizosphere may depend on the sugar and organic acid (malic acid, citric, succinic acid and fumaric acid) composition of root exudates. Our results also agree with the results of Rini & Sulochana (2007), confirming the antifungal effect of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato plants.

Results reviewed by Santoyo et al. (2012) and Boruah & Kumar (2002) evidenced the presence of edaphic species from genera *Pseudomonas* and *Bacillus* acting as biocontrol and plant growth promoting agents and explained their mechanism of action.

Veliz et al. (2017) evidenced the role of chitinase-producing bacteria in biocontrol activity.

The secondary metabolite Peptaibol Trichogin GA IV released by *Trichoderma longibrachiatum* was able to present antimicrobial activity against phytopathogenic Gram (-) bacteria from genera *Xanthomonas*, *Pseudomonas*, *Ralstonia*, *Agrobacterium*, *Erwinia* and Gram (+) *Bacillus subtilis* of cauliflower and other crucifers (Caracciolo et al., 2023).

Preece & Penuelas (2016) noticed that rhizodeposition modulates composition and structure of soil microbial communities with beneficial consequences for ecosystem resilience under drought climatic conditions.

A rich and dynamic microbiota characterized as low functional diversified may present difficulties in adapting to unfavourable environmental conditions, but rhizosphere microbiota, with high taxonomic and functional diversity is more resilient to climatic changes. Complex interactions root-soil-microbiome in rhizosphere are important for crop production (Zhang et al., 2017), many beneficial microbes from rhizosphere being selected for use as bio-fertilizers in improving plant biomass accumulation (Thenmozhi et al., 2010). Al-Surhanee (2022) reported improved induced resistance of tomato plants to *Fusarium* attack by using eco-friendly agents represented by *Trichoderma* and salicylic acid.

Other researchers noticed that better understanding of the biotic and abiotic factors that influence the rhizosphere microbiome composition and biomass will be important factors for selecting the most beneficial microbiome structure for enhancing agricultural productivity and ecosystem functions (Wu et al., 2023).

CONCLUSIONS

The composition of root exudates released by tomato plants was very appropriate for development of a numerous bacterial community and, to a less extend, for the group of fungi, compared to bulk soil.

The rhizosphere effect was evidenced by higher values of diversity indices registered for both bacterial and fungal communities as compared with those characterizing the communities from soil.

The ratio F:B reflected the main processes such as: nutrient cycles, decomposition, C-sequestration, self-regulation in ecosystem.

The lower value of F: B ratio in tomato rhizosphere denoted a higher importance of bacteria-dominated microbiota, with more dynamic turn -over of readily decomposable substrate and elevated microbiological activity as compared to that developed in soil.

Root exudates from rhizosphere stimulated the development of beneficial species of bacteria and fungi which can be further isolated and utilized as biological control agents, biofertilizers and organic matter decomposers in biotechnologies for plant protection, yield

increasing and increasing C-sequestration for limiting the effects of climatic changes.

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