Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2019, Vol. 3 (3), 411-419
https://doi.org/10.29329/ijiaar.2019.206.7

Original article

Soil Microorganisms Quantitative Dynamic Characterizing the Overall Biological State in Rhizosphere of Tobacco and Tomato Plants Infected by Broomrape [1]

Tsveta Hristeva [a, *], Iliya Denev [b] & Radka Bojinova [a]

[a] Tobacco and Tobacco Products Institute, 4018 Plovdiv, Bulgaria
[b] University of Plovdiv, Department of Plant Physiology and Molecular Biology, 4000 Plovdiv, Bulgaria

Abstract

The quantitative dynamics of soil microorganisms in the rhizosphere of tobacco and tomato plants infected by broomrape /Phelipanche ramosa L./ were investigated. Parallel pot experiments with the two hosts of the parasite - Oriental type of tobacco and tomato were presented. The variants were: by introducing contamination into the soil - seeds by broomrape and control pots - without the seeds by broomrape. Experiments were carried out under controlled conditions. Soil samples for microbiological analyzes were taken from the rhizosphere, in dynamics. The microbiological analyses were executed according to Koh's, in three replications (MPN/g a.d.s), with confidence level 0.05. The surveyed set of soil microorganisms has been comprised two indicators groups characterizing the overall biological state of the microbial communities: Autochthonous (on soil extract agar) and Oligotrophic (on diluted soil extract agar). Diversity indexes Shannon (H) and Simpson (D) and the distribution evenness (EH) in the microbial communities were determined. A statistical analysis has been made. The obtained results showed that the quantities of the two groups of microorganisms begun to change visibly around and after 20th day, compared to the amount at the rhizosphere of uninfected by broomrape plants. There was an increase at the population density of autochthonous microorganisms between 20 and 60 days and reduced significantly after this period. The trend was reversed at oligotrophic microorganisms, but the quantities were higher throughout the study period. These dynamics coincide with the phases of broomrape development - germination, formation of a haustorium (about 10-20th day) and attachment to the root of the host (up to about 40-60 days) and the above-ground phases (80-100 days). The analysis showed that microbial communities formed in rhizospheres in the presence of a broomrape had been an oligotrophic character. The relative share of oligotrophic microorganisms in these microbial communities was over 50% at both host rhizospheres.

Keywords: Branched broomrape, Tobacco, Tomato, Rhizosphere, Autochthonous microorganisms, Oligotrophic microorganisms.

Received: 01 September 2018 * **Accepted:** 10 July 2019 * **DOI:** https://doi.org/10.29329/ijiaar.2019.206.7

* **Corresponding author:**

Tsveta Hristeva, Tobacco and Tobacco Products Institute, 4018 Plovdiv, Bulgaria.
Email: zveta_h@abv.bg

[1] A part of this study was presented at the International Agricultural, Biological and Life Science Conference, Edirne, Turkey, September 2-5, 2018.

INTRODUCTION

One of the main crops which are hosts of the branched broomrape *(Phelipanche ramosa L.)* are tobacco *(Nicotiana tabacum L.)* and tomato *(Solanum lycopersicum L.).* The species is a holoparasite plant of the Orobanchaceae family. The economic damage for these crops are enormous annually (Joel et al., 2007; Parker, 2009; Longo et al., 2010).

In relation to the root-parasite flowering plants the interest about soil microorganisms dates back to a long time, but is mainly directed towards their using as biological means of control (Ammseles et al., 2001; Zonno and Virro, 2002; Sauerborn et al., 2007; Zermane et al., 2007; Vurro M. et al., 2009; Fernández-Aparicio et al., 2010; Chen J. et al., 2016).

The studies on soil microflora as a mandatory bio-ecological element of the root environment that could be influence the host-parasite system relationships have been limited, despite numerous studies and scientific advances at various aspects (Hristeva et al., 2013; 2017; Ranjan et al., 2016). All biochemical processes of communication mainly carried out in the rhizosphere of plants, and its main inhabitants have been the soil microorganisms. Numerous and diverse both in terms of species composition and in terms of functions and relationships with the environment is their community. Complex, bi-directional and based at metabolic processes is the relationship of soil microorganisms with plants. The plants through the emission of chemical compounds which exuded in the rhizosphere have been steered and formed the microbial diversity and density. The microorganisms an environment for the plants development have been produced. A large range of extracellular enzymes in the soil environment are synthesizing and producing by the soil microorganisms. This is related to their vital strategy - the ability to react to chemical signals that serve to inform them of changes in their immediate area. This process is known as "quorum-observation." All kinds of relationships in the rhizosphere are controlled by this process, presumably (Werner, 2001; Dennis et al., 2010; Burns R. et al., 2013; Cavar et al., 2015). The presence of a parasitic plant complicates the interactions and processes in the rhizosphere of the host plants at this aspect, yet. Some authors as early as the early 1980s have been suggested that soil microorganisms should be included as a biotic factor in studying the relationships between parasitic weeds and their hosts (Borg, 1986; Wegman, 1986).

The aim of the present study was to track the population densities some of major trophic groups of soil microorganisms, indicative of the overall biological state of microbial communities, at rhizospheres of tobacco and tomato plants infected by broomrape, depending on the stages of development of the parasite plant, in dynamics.

Materials and Methods

Parallel pot experiments with the two hosts of the parasite *Ph. ramosa* - Oriental type of tobacco variety Plovdiv 7 and tomato - Milyana variety were performed. The variants were: By introducing

contamination into the soil - seeds by broomrape and control pots - without the seeds by broomrape. The experimental pots were a capacity of 5 kg. The alluvial-meadow /Fluvisols – FAO/ soil type has been used with the following agrochemical characteristics: organic matter content (by Turin) -1.82%; total nitrogen content (by Kjeldahl) - 0.076%; available P2O5 – 28.9 mg/kg (by Egner - Reem); available K2O – 514.6 mg/kg (in 2n HCl); soil reaction pH (H_2O) - 7.74 (by potentiometry).

The amount of broomrape seed imported 200 mg/kg of soil (Panchenko, 1975). The broomrape seeds used have been collected from experimental fields with tobacco infected by *Ph. ramosa* (Plovdiv region, Bulgaria). The broomrape seeds were preconditioning in water, 7 days at dark, at temperature $28C^0$. Each variant was set in five reps. The experiments were conducted under controlled conditions in a phytostatic room (with lighting mode - 10 hours night /14 hours day at temperature 28 C°).

Soil samples for microbiological analyzes were taken from the rhizosphere, in dynamics - 0 day; 10; 20; 40, 60, 80, 100, and 120 days, after the start of the experiments. The microbiological analyses were executed according to Koh's method – culture of diluted soil suspensions on specific for each trophic group of microorganisms nutrient media, in three replications. The most probable number technique is used to estimate microbial population sizes per gram absolute dry soil (MPN/g a.d.s), with confidence level $p \leq 0.05$. The surveyed set of soil microorganisms includes two indicator groups characterizing the overall biological state of the microbial communities: Autochthonous microorganisms (on soil extract agar) and Oligotrophic microorganisms (on diluted soil extract agar). Culturing was carried out at 30 °C temperature, for 7 days period (Koleshko, 1991). The data were subjected to single factor analysis of variance (ANOVA). For every trophic group were determined effect of the factor broomrape and its statistical significance. The ratio between the quantities of oligotrophic microorganisms and the quantities of autochthonous microorganisms was calculated and was determined the values of oligotrophic index (OI). Diversity indexes Shannon (H) and Simpson (D) and the distribution evenness (E_H) in the microbial communities were determined (Magurran, 2004). Calculations were made using the following equations: $H = \sum_{i=1}^{S} p_i \ln p_i$; $D = \frac{1}{\sum_{i=1}^{S} p_i^2}$ and $E_H = \frac{H}{\ln S}$

Results and Discussion

The life cycle of development of the broomrape can be conditionally divided into two stages - underground and above-ground. The underground stage includes the following phases: seed germination, rubber formation and root penetration of the host; the formation of tuberculum and the development of root-star "structures" and the beginning of the formation of flower stems "distaff". The over-ground stage includes: - the growth of the flower stems; button; flowering; seeds formation. The duration of the different phases varies depending on the influence of a number of abiotic factors (temperature, humidity, etc.). Usually, the duration from germination to linkage with the host roots is about 20 days; tuberculum formation and active association with the host cell system for about 40-60

days; formation of flower stems and exiting of a parasite plant over the soil surface after 80-100 days. The duration of the whole life cycle of *Ph. ramosa* from germination to forming the seeds is about 90-120 days. The development and occurrence of the individual pheno-phases of *Ph. ramosa* at the present experience corresponds to that known of the literature (Joel et al., 2004).

The results obtained show changes in the quantities of trophic groups of soil microorganisms compared to the amount at the rhizosphere of uninfected by broomrape plants. The changes ware similar in both host rhizospheres - tobacco and tomato, and suggest dependence on the parasite development phase. There was an increase in the population density of Autochthonous microorganisms between 20, 40 and 60 days. The increase reached hundreds of millions cells/g absolutely dry soil around the 60th day. After this period (around 70th-80th day), their quantities sharply declined, with hundreds of millions differences to rhizospheres of uninfected hosts. This dynamics corresponds to the underground and above ground phases of the parasite development. Broomrape under the specific conditions of the experience, appeared about the 58th - 62th day on surface at the tobacco and about 65th-70th days at the tomato, i. e. in the period with the lowest density of autochthonous microorganisms, the parasite has well-formed flower stems and was in button and flowering phases. The density of autochthonous microorganisms was increased during the underground phases of parasite development. The increase started around the 20th day when the haustorium has been penetrated the host roots and reached peak values around the 60th day, when the connection to the host conduction system was active and completed (Fig. 1-2). The influence of broomrape infection on that trophic group of microorganisms at tobacco rhizosphere was 8.01% not statistically proven ($F_{exp} = 2.96 \leq F_{tab} = 4.13$ $p \leq 0.05$). The influence of the factor "broomrape" was 15.79% statistically proven with a 99% probability ($F_{exp.} = 8.63 \geq F_{tab} = 7.44$ $p \leq 0.01$) at tomato.

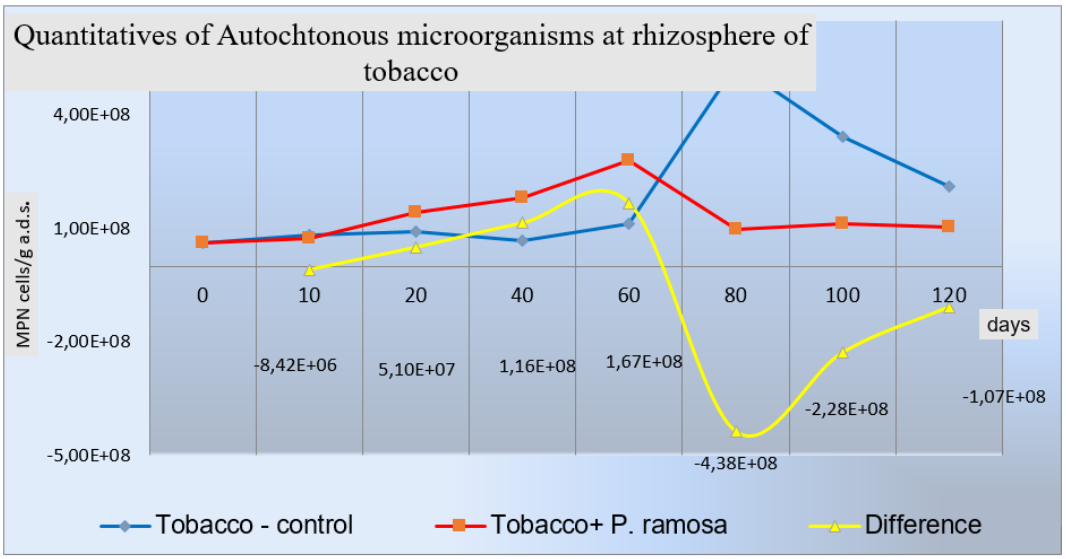

Figure 1. Quantitatives of Autochtonous microorganisms at rhizosphere of tobacco

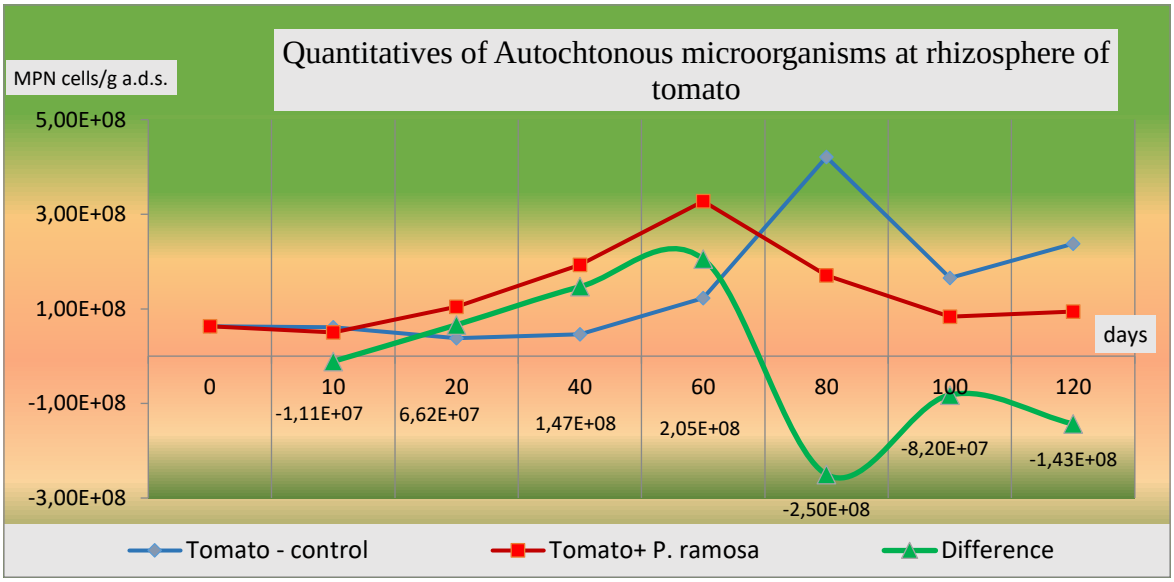

Figure 2. Quantitatives of Autochtonous microorganisms at rhizosphere of tomato

Population density of the other tested trophic group - oligotrophic microorganisms was higher in the rhizosphere of broomrape infected plants throughout the study period. The differences with the control variants were particularly strong after the 60th - 80th day, which coincides with the blossoming of the blue wrist and the formation of seeds. They have been in the order of hundreds of millions/g a.d.s. (Fig. 3 and 4). The influence of broomrape factor at tobacco host was 11.87% and 10.97% at host-tomato, statistically proven at confidence level $p \leq 0.05$. (Fexp tobacco = 4.58 and tomato Fexp = 4.19 ≥ Ftab = 4.13 $p \leq 0.05$).

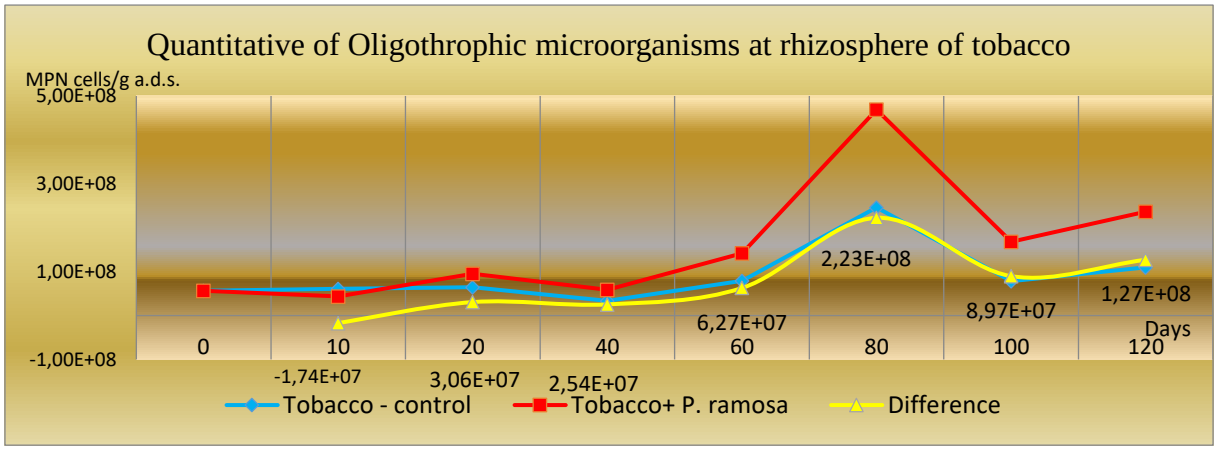

Figure 3. Quantitatives of Oligothrophic microorganisms at rhizosphere of tobacco

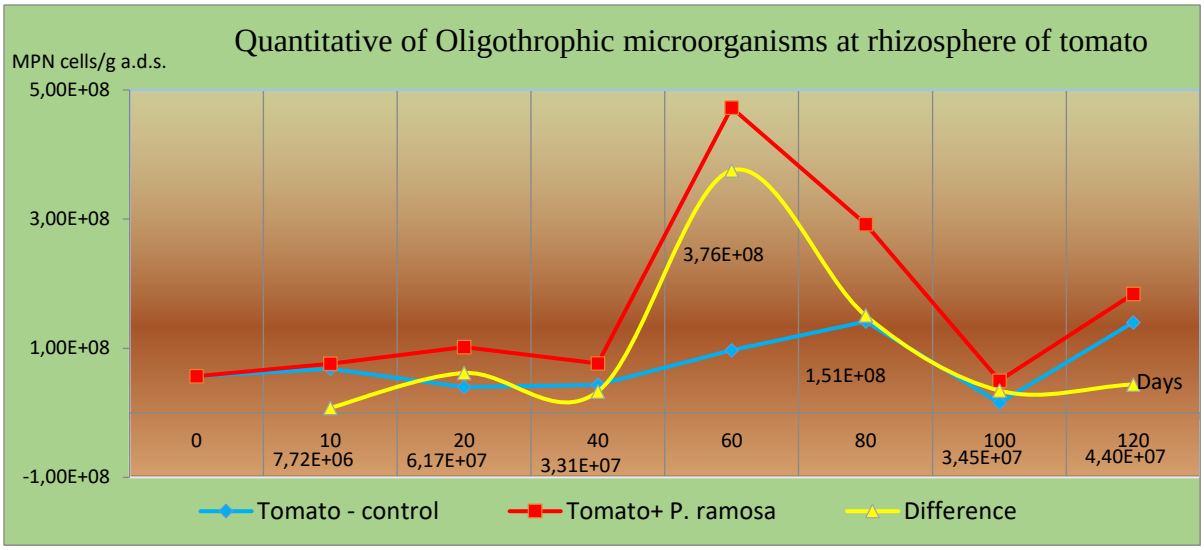

Figure 4. Quantitatives of Oligothrophic microorganisms at rhizosphere of tomato

The analysis of reported quantitative changes in microbial communities showed that microbial communities formed in rhizospheres in the presence of a broomrape have an oligotrophic character. The relative share of oligotrophic microorganisms in these microbial communities was over 50% (Fig. 5).

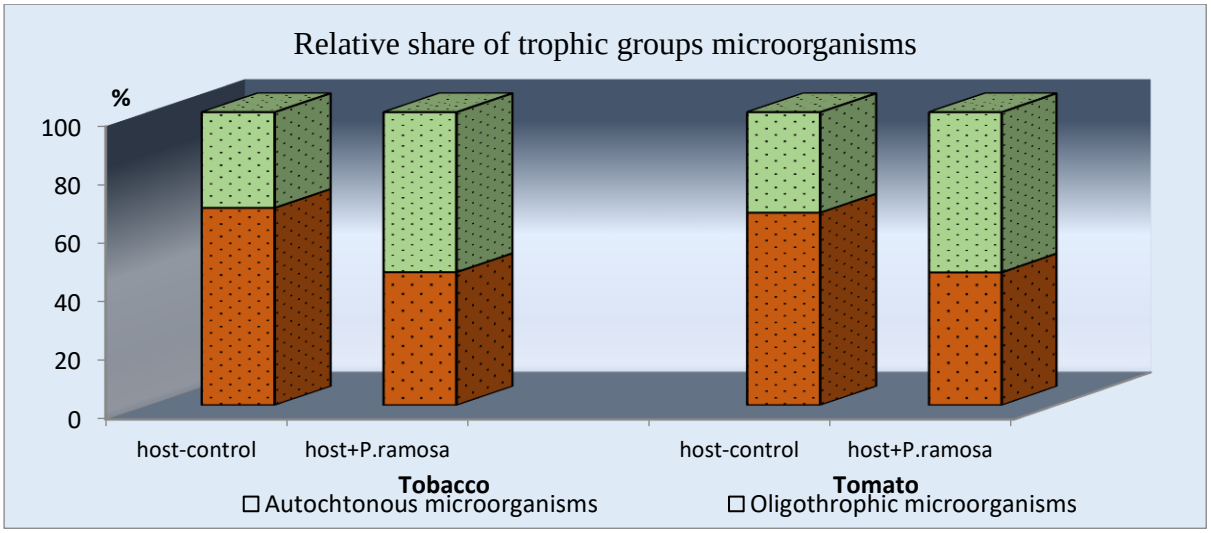

Figure 5. Relative share (%) of trophic groups microorganisms in microbial communities at rhizosphere the host plants

The two trophic groups of microorganisms are organotrophic and according to their metabolism and functions can be referred to the so-called K-strategists. They are characterized by low growth rates, but with constant representation in soil microbial communities. Whereas autochthonous microorganisms evolve independently of the amount of nutrients in the environment, oligotrophic develop intensively at low concentrations of carbon compounds, only.

The homeostatic microbial communities there a balance between the amounts of the two groups of microorganisms, which in the presence of a broomrape were disturbed. The high values of the oligotrophic index markedly changed after the 60th day, i. e. around and after the appearance of the broomrape over the surface of the soil have been showed this also (Table 1).

Table 1. Values of the oligotrophic index at dynamics - days after the start of the experiments

Variants / hosts	0^{th}	10^{th}	20^{th}	40^{th}	60^{th}	80^{th}	100^{th}	120^{th}
Tobacco - control	0.896	0.744	0.709	0.497	0.697	0.460	0.229	0.520
Tobacco + P. ramosa	0.896	0.590	0.670	0.321	0.505	4.826	1.480	2.286
Tomato - control	0.896	1.116	1.054	0.935	0.793	0.336	0.093	0.588
Tomato + P. ramosa	0.896	1.516	0.977	0.396	1.443	1.710	0.598	1.946

The calculated values of the diversity indices in the microbial communities formed in the presence of a broomrape at both hosts have been lowed. More pronounced decrease was in the rhizosphere of the tobacco – host (Table 2). The indices used are statistically informative, providing an opportunity to describe common community properties and comparison, including at trophic level (Morris et al., 2014). Despite differences in values, the results cannot be interpreted as significant deviations from control variants. Biodiversity in microbial communities in presence of broomrape was preserved and they can be classified as moderate.

Table 2. Values of the diversity indices - days after the start of the experiments

Variants / hosts / diversity indices	0^{th} day	10^{th} day	20^{th} day	40^{th} day	60^{th} day	80^{th} day	100^{th} day	120^{th} day
Tobacco - control								
Shannon (H) index	0.312	0.320	0.373	0.319	0.324	0.341	0.400	0.365
Evenness (E_H)	0.174	0.178	0.208	0.178	0.181	0.190	0.223	0.204
Simpson (D) index	5.982	5.750	4.524	5.757	5.630	5.170	4.061	4.681
Tobacco + *P. ramosa*								
Shannon (H) index	0.312	0.166	0.165	0.170	0.170	0.306	0.227	0.258
Evenness (E_H)	0.174	0.093	0.092	0.095	0.095	0.171	0.126	0.144
Simpson (D) index	5.982	5.439	5.444	5.167	5.195	2.825	3.766	3.336
Tomato - control								
Shannon (H) index	0.312	0.313	0.386	0.388	0.419	0.167	0.173	0.340
Evenness (E_H)	0.174	0.175	0.215	0.216	0.234	0.093	0.096	0.190
Simpson (D) index	5.982	5.953	4.396	4.294	4.045	5.387	5.054	5.196
Tomato + *P. ramosa*								
Shannon (H) index	0.312	0.162	0.183	0.205	0.221	0.180	0.463	0.178
Evenness (E_H)	0.174	0.091	0.102	0.114	0.123	0.101	0.259	0.099
Simpson (D) index	5.982	5.607	4.661	4.019	3.764	4.764	3.361	4.912

Conclusion

The results to population densities of autochthonous and oligotrophic microorganisms at the tobacco and tomato rhizospheres by the presence of a broomrape (*Ph. ramosa*) have been show clearly changes. Quantitative changes were tied up to the phases of broomrape development. Biodiversity in microbial communities was preserved and classified as moderate.

Conclusions from past research have been confirmed that microbial communities formed in the rhizosphere of infected by broomrape plants changed their structural organization and were of oligotrophic nature.

Acknowledgements

This study was supported by the National Science Fund of Bulgaria, Ministry of Education and Science - Project: DN 06/9 (Fundamental Research Competition – 2016)

REFERENCES

Amsellem, Z., S. Barghouthi, B. Cohen, Y. Goldwasser, J. Gressel, L. Hornok, Z. Kerenyi, Y. Kleifeld, O. Klein and J. Kroschel (2001). Recent advances in the biocontrol of Orobanche (broomrape) species, J. BioControl, 46, 2, 211-228.

Beals, M., L. Gross and S. Harrell (1999), Diversity Indices: Simpson's D and E.

Burns, R. G., J. L. De Forest, J. Marxsenc, R. L. Sinsabaughd, M. R. Strombergere, M. D. Wallensteinf, M. N. Weintraubg and A. Zoppinih (2013). Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol. Biothech., 59, 216-234.

Cavar, S, B. Zwanenburg and P. Tarkowski (2015). Strigolactones: occurrence, structure, and biological activity in the rhizosphere. Phytochem Rev., 14, 691–711.

Chen, J., Q. H. Xue, C. S. McErlean, J. H. Zhi, Y. Q. Ma, X. T. Jia, M. Zhang and X. X.Ye (2016). Biocontrol potential of the antagonistic microorganism *Streptomyces enissocaesilis* against *Orobanche cumana*. J. BioControl, 1, 1-11

Dennis, P. G., A. J. Miller and P. R. Hirsch (2010). Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol. Ecol., 72, 313–327.

Fernandez-Aparicio, M., K. Yoneyama and D. Rubiales (2011). The role of strigolactones in host specificity of *Orobanche* and *Phelipanche* seed germination. Seed Sci. Res., 21, 55–61.

Hristeva, Ts., T. Dekalska and I. Denev (2013). Structural and functional biodiversity of microbial communities in rhizospheres of plants infected with broomrapes (Orobanchaceae). J. Biotech. Biotech. Equip., 27 (5).

Hristeva, Ts. and I. Denev. (2017). Changes at the Rhizosphere Microbiota of the Sunflower - Orobanche cumana Wall Pathosystem. Int. J. Curr. Microbiol. App. Sci., 6(1), 733-746.

Joel, D.M., J. Hershenhorn, H. Eizenberg, R. Aly, G. Ejeta, P.J. Rich, J. K. Ransom, J. Sauerborn and D. Rubiales (2007). Biology and management of weedy root parasites. Hortic. Rev., 33, 267–349.

Morris, E. K., T. Caruso, F. Buscot, M. Fischer, Ch. Hancock, T.Maier, T. Meiners, C. Müller, E. Obermaier, D. Prati, St. Socher, I. Sonnemann, N. Wäschke, T. Wubet, S. Wurst and M. Rillig (2014). Choosing

and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories Ecol Evol., 4(18), 3514–3524.

Koleshko, O. I. (1991). Ecology of soil microorganisms Handbook, University Press., Minsk: 19-136.

Longo, A. M. G., A. Lo Monaco and G. Mauromicale (2010). The effect of *Phelipanche ramosa* infection on the quality of tomato fruit. Weed Res., 50, 58–66.

Magurran, A. E. (2004). Meausuring Biological Diversity. Blackwell.

Parker, C. (2009). Observations on the current status of Orobanche and Striga problems worldwide. Pest Manag. Sci., 65, 453–459.

Panchenko, A.Y. (1975). Accelerated method of assessing breeding materials for resistance in broomrape. Her. Agric. Sci., 2, 107- 115.

Ranjan, A., Y. Ichihashi, M. Farhi, K. Zumstein, B. Townsley, T. David, R. Vera, J. M. García, M. J. Pozo and J. A. López-Ráez (2016). Expression of molecular markers associated to defense signaling pathways and strigolactone biosynthesis during the early interaction tomato Phelipanche ramosa. Physiol. Mol. Plant Pathol., 94, 100–107.

Sauerborn, J., D. Muller-Stover, J. Hershenhorn (2007). The role of biological control in managing parasitic weeds. Crop Prot., 26, 246–254.

Zermane, N., T. Souissi, J. Kroschel and R. Sikora (2007). Biocontrol of broomrape (Orobanche crenata Forsk. and Orobanche foetida Poir.) by Pseudomonas fluorescens isolate Bf7-9 from faba bean rhizosphere. J. Biocontrol Sci.Tech., 17 (5). 483-497.

Zonno, M. C. and M. Vurro (2002). Inhibition of germination of Orobanche ramosa seeds by Fusarium toxins. Phytoparasitica, 30, 519-524.

Vurro, M., A. Boari, A. Evidente, A. Andolfi and N. Zermane (2009). Natural metabolites for parasitic weed management. Pest Manag. Sci., 65, 566-571.

Werner D., (2001). The organic signals between plants and microorganisms. In: Pinton R., Varanini Z., Nannipieri (eds.). Rhizosphere biochemistry and organic substances in the soil-plant interface. Marcel Dekker, New York: 197-222.

Wegmann K. (1986). Biochemistry of osmoregulation and possible biochemical reasons of resistance against Orobanche. In: S. J. ter Borg (ed.) roc. of a Workshop in Wageningen, The Netherlands: 42-50.