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Changes at the Rhizosphere Microbiota of the Sunflower – *Orobanche cumana* Wallr Pathosystem

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A B S T R A C T

The influence of two factors - "broomrape" and "soil type" on quantities of seven trophic groups of soil microorganisms (Autochthonous; Oligotrophic; Actinomycetes; Microscopic fungi; Ammonifying; Assimilating mineral nitrogen; Aerobic nitrogen-fixing bacteria of the genus *Azotobacter*) were determined. Soil samples for microbiological analysis were taken from the rhizosphere zone of plants in sunflower crops from two soil types (Haplic Vertisols and Chromic Cambisols), in phenological phases of sunflower and of broomrape - "flowering". Reduction the quantities on all investigated groups of microorganisms in the system (sunflower + broomrape) were observed. The average population density as a whole in the microbial communities with presence of broomrape decreased by over 65% compared with the density in the microbial communities at uninfected plants, in both soil types. The power of influence of the factor "broomrape" was less than that of factor "soil type", but distinctly and statistically proven. The presence of broomrape had the strongest impact to the group of microscopic fungi (50% of total variance). Structural changes in the distribution between the different trophic groups of microorganisms in the microbial communities have been established - an increase the shares of oligotrophic microorganisms, of assimilating mineral nitrogen microorganisms and of microscopic fungi.

Introduction

Sunflower broomrape */Orobanche cumana* Wallr./ is one of a major pests in agriculture worldwide with expanding area of distribution (Jose *et al*., 2015). The broomrape is a limiting factor for the production on sunflower in the regions of the Mediterranean, Eastern Europe and Southeast Asia (Ma and Jan, 2014; Molinero-Ruiz *et al*., 2015). The parasite has spread to new areas in recent years, including in regions of North Africa (Amri *et al*., 2012). The yearly losses of sunflower seeds are average 50% when grown sensitive varieties. Yield losses close to 100% under high infestations (Jestin *et al*., 2014)

The main way to control of broomrape attacks has been the development of resistant sunflower cultivars based on vertical resistance mechanisms (Velasco *et al*., 2007). Sunflower broomrape has great capacity of

dispersion and mutation. A serious obstacle to limiting the spread and control at broomrape is the fact that the parasite formed very rapidly new and becoming more virulent physiological races, which overcomes the resistance and tolerance of the selection sunflower hybrids (Molinero-Ruiz *et al*., 2015; Antonova, 2014; Batchvarova, 2014). The causes and mechanisms of their occurrence are not yet fully understood. It is supposed to the virulence could be the result of release of auxins and pectinolytic enzymes during the process of germination of the seed by *O. cumana* (Slavov *et al*., 2004). The intensity of the production of these enzymes by the different races is proportional to the virulence of each race (Véronési *et al*., 2005).

The achievements in research on various aspects concerning the parasitic species of Orobanchaceae are many, but also the questions unanswered. The relationships in plant systems: host / broomrape are extremely complex and specific. Multitude diverse factors (abiotic and biotic) have influence on them (Wegmann, 1986). One of the directions of research has been focused to soil microorganisms. Several microbiological species have been tested as bioagents for control of economic important broomrapes, including of *O. cumana* Wallr. Among them, various fungi such as *Fusarium, Alternaria* and *Myrothecium verrucaria*; bacteria of genus *Pseudomonas* have been used successfully against *Orobanche, Phelipanche* and *Striga* (Andolfi *et al*., 2005; Sauerborn J. *et al*., 2007; Zermane *et al*., 2007; Sun, 2011). Considerable progress has been registered in the field of arbuscular mycorrhizal fungi (AMF) research in connection with relationships in the parasitic system and also potential for use them about biological control. These fungi live in all kinds of terrestrial ecosystems and associate with the vast majority of land plants, forming the most widespread type of symbiosis. It has been shown that the substances (phytohormones from the family of strigolactones), which induce germination of the seeds of the majority of Orobanchaceae species have the same effect on the spores of AMF (Akiyama *et al*., 2006). A reduction of infection by broomrape after mycorrhizal colonization of the host and inhibition of the germination of the seeds of several broomrapes have been reported (Bouwmeester *et al*., 2007). This effect attributed to a reduced synthesis and exudation of germination stimulants by mycorrhizal plants (López-Ráez, 2011). Some degree of reduction germination of *O. cumana* Wallr., seeds in the presence of AMF and their exudates have been found. Besides a lower production of germination stimulants was also marked antagonism between the AMF and the parasite (Louarn, 2012). The most recent research has shown that not only strigolactones stimulate the germination of seeds of broomrapes, but a several substances of other classes metabolites have been identified (Cordozo *et al*., 2011; Fernandez-Aparicio *et al*., 2011; Raupp *et al*., 2013). Although sunflower produces strigolactones, dehydrocostus lactone has been proposed as a major germination stimulant (Yoneyama *et al*., 2011; Joel *et al*., 2011; Ueno *et al*., 2014). The studies on the specifics of the various parasitic systems, on the root exudates in biochemical aspect are continuously deepen and enrich with new facts. Extensive information on signals from different of sources, including microorganisms, and their effect on the parasitic species can be found (Umehara *et al*., 2008). About chemical inducers, plant inducers, microbial inducers leading to suicidal germination of the parasite in the absence of host (Christeva *et al*., 1998; Lachia *et al*., 2014); about inhibitors of the germination process (Zermane *et al*., 2007; Chen *et al*., 2016); about microbial toxins (Andolfi *et al*., 2005; Vurro *et al*., 2009; Louarn, 2011; Sun, 2011) have been reported.
It is well known, that in the rhizosphere

biochemical processes associated with metabolism, with the nutrient cycles are continuously performed. Typical microbial communities with dynamic structures and ecologo-trophic links are formed in the different plants rhizospheres. Micro-biocenoses characterized by strictly regulated interdependent relations that maintain the state of the homeostasis (Werner, 2001; Allison *et al*., 2007). In this regard, the participation of soil microorganisms in ongoing processes in the rhizosphere at plants infected by broomrapes is not well defined. Initial information about trends towards reduction of the quantity of the microorganisms in the rhizosphere of several systems by different hosts-parasites has been reported (Hristeva *et al*., 2013). Studies on the ecology of the rhizosphere microbiota could help clarify various general issues related to the parasitic plants of Orobanchaceae. The specificity of every distinct parasite-host system requires careful examination. Revealing the role of microorganisms in relations in particular sunflower - *O. cumana* Wallr. would be enrichment of knowledge to the germination mechanisms; to the reasons for invasion of new virulent races in various geographic regions, etc. The rhizosphere microorganisms may offer new prospects for control, also.

The aim of this study was to establish whether are occurred quantitative changes in population density of key trophic groups of soil microorganisms (transforming carbon and nitrogen compounds) in microbial communities formed in the rhizosphere of sunflower plants infected by broomrape and analyzed their state and structure.

Materials and Methods

The objects of the study were microbial communities formed on infected by broomrape sunflower plants. Sunflower crops from field experiments have been used. They were grown on two soil types - Haplic Vertisols and Chromic Cambisols with the following agro-chemical parameters:

Haplic Vertisols - with organic matter content (by Turin) - 2.3%; total nitrogen content (by Kjeldahl) - 0.1%; movable phosphorus P_2O_5 - 6.2 mg/100g soil (by Egner-Reem); movable potassium K_2O - 29.2 mg/100g soil (in 2n HCl); soil reaction pH_{H_2O} - 7.5 (by potentiometry).

Chromic Cambisols - with organic matter content (by Turin) -1.51%; total nitrogen content (by Kjeldahl) - 0.08%; movable phosphorus P_2O_5 – 2.75 mg/100g soil (by Egner - Reem); movable potassium K_2O - 27.5 mg/100g soil (in 2n HCl); soil reaction pH_{H_2O} - 7.2 (by potentiometry).

Soil samples for microbiological analyses were taken from the rhizosphere zone of plants-hosts, in depth (0-30 cm) at phenological phases of sunflower and of broomrape - "flowering". Soil samples from the rhizosphere of uninfected sunflower plants located in close proximity to the infected plants were collected as a control. The soil samples were taken in four replications. Each analyzed soil sample was average prepared by rhizosphere of five plants. For this purpose, the plants were subtracted with the roots and the soil around them.

The microbiological analyses have been carried out on each of the soil samples. They were performed according to Koch's method, by cultivating diluted soil suspensions on specific nutrient media for every trophic group of microorganisms. Analyses were carried out in three replications. Culturing was carried out at 30 °C temperature. The microorganisms of the examined trophic groups are mesophilic (Koleshko, 1991).
The numbers of the following groups of

microorganisms have determined: Autochthonous microorganisms – on soil extract agar, for ten days were incubated; Oligotrophic microorganisms – on tenfold diluted soil extract agar, for ten days were incubated; Actinomycetes – on starch–ammonium agar, for seven days were incubated; Microscopic fungi – on Chapek agar, for seven days were incubated; Ammonifying microorganisms – on meat–peptone stock agar, for five days were incubated; Mineral nitrogen assimilating microorganisms – on starch–ammonium agar, for seven days were incubated; Aerobic nitrogen-fixing bacteria of the genus *Azotobacter* – on Ashby agar, for seven days were incubated.

The microbial population sizes was estimated as colony forming units per g absolutely dry soil (CFU/g a.d.s.), with confidence level 0.05.

The data were subjected to two-factor analysis of variance. For each trophic group microorganisms and for overall population density in microbial communities the effects (ηx^2) of the factors: soil type (A) and broomrape (B) and the level of their statistical significance (p) according to the Fisher's test (F) were determined. Statistical evaluation of the specific differences between infected by broomrape and uninfected plants, for each trophic group of microorganisms, according to the Student's test (t) were determined (Plochinskiy, 1980).

The relative shares (%) of each of trophic groups microorganisms of overall population density in microbial communities were calculated.

Results and Discussion

The analyzed indicative microbiological indices depict a set of organotrophic microorganisms, which can be conditionally divided into: soil carbon converting and soil nitrogen converting microorganisms. These groups are highly descriptive for the quantitative and qualitative state of the microbial communities as a complex, their ability to perform the basic transformations of biogenic elements in the soil, and their impact on plants nutrition.

Data of present research as generally poorer to microbial population at Chromic Cambisols than these at Haplic Vertisols were found. It's normally and is the result of a low content of organic matter and nutrients in Chromic Cambisols. Despite of the soil type, the results demonstrated trends towards decrease in population density at all examined groups of microorganisms in the presence of broomrape.

Ecological-trophic unification of microorganisms related to transformation of carbon compounds in soil includes the following trophic groups: Autochthonous microorganisms, Oligotrophic micro-organisms, Microscopic fungi and Actinomycetes (Table 1). Numbers of autochthonous and oligotrophic micro-organisms are considered representative for overall biological status at the soil. The registered reduction in density of autochthonous microorganisms is 83.07% at Haplic Vertisols and 87.53% at Chromic Cambisols at the rhizosphere of sunflower plants infected by *O. cumana* Wallr. There a significant decrease also at the oligotrophic microorganisms numbers - 68.04% at the first soil type, and 66.91% at the second type. The specific differences compared with controls at both trophic groups of microorganisms statistical at the highest level ($p \leq 0.001$) were justified. Statistically evaluation the effect of the factor "broomrape" on the numbers at autochthonous microorganisms was 24.71% and was 17.66% at oligotrophic microorganisms.

The force of impact of the factor "soil type" was respectively 43.79% at autochthonous and 60.61% at oligotrophic microorganisms. The impact of both factors was proven with significant level ($p \leq 0.01$).

The next researched groups microorganisms – microscopic fungi and actinomycetes are soil-born, too. The features of their populations are broadly applied for evaluation of the soil biological properties. These microorganisms possess powerful and varied physiological means for organic compounds conversion, and are key sections in microbial communities. Beside their role as important determinants of rhizosphere microbial community structure and functional biodiversity, they are also substantial source of biologically active substances and have major part in mineralization processes in soil (Werner, 2001). In the rhizosphere of sunflower plants infected by broomrapes, the numbers of microscopic fungi populations, in both soil types, were lowered by more than 40%. The differences between infected and control hosts plants were statistically very significance ($p \leq 0.01$). The effect of the factor "broomrape" was found 52% of total variance with very significant ($p \leq 0.01$). Only at this group of microorganisms impact of factor "soil type" has was weak (3.86%) and statistically an undistinguishable. The observed decline in quantities in the other hyphal group microorganisms - actinomycetes in the presence of broomrape, was by 74.62% at Haplic Vertisols, and by 67.83% at Chromic Cambisols. The differences were statistically significant ($p \leq 0.05$). The force of impact of the both factors was shown to be respectively 21.75 % for "broomrape", and 40.74% for "soil type" with confidence level ($p \leq 0.01$).

The next trophic groups of microorganisms which were analyzed are associated with some of the key transformations of nitrogen in the soil. These are: Aerobic nitrogen-fixing bacteria of the genus *Azotobacter*, Ammonifying and Mineral nitrogen assimilating microorganisms (table 2).

The possibility for nitrogen-income through fixation could be depicted using the quantities of the free living aerobic bacteria of the genus *Azotobacter*. The decrease of their numbers in the rhizosphere of infected by *O. cumana* Wallr. plants is clearly pronounced – 77.46% at Haplic Vertisols, and 74.89% at Chromic Cambisols. Statistically very significant at confidence level ($p \leq 0.01$) differences compared to plants-controls were found. The force of impact of "broomrape" factor was 22.61% and of factor "soil type" - 23.75%. The effects of the factors were statistically significant at also confidence level ($p \leq 0.01$).

The role of the ammonifying microorganisms is to complete the organic compounds mineralization and to produce available for the plants ammonium nitrogen. Their quantity in infected plants rhizosphere was also altered in the direction to reduction. The percentage of decrease compared to control hosts was over 40% in both soil types more pronounced at Haplic Vertisols. Specific differences between infected and uninfected sunflower plants were significant ($p \leq 0.05$). The impact of the factor "broomrape" is slightly - 8.27% of the influence of all factors, but a statistically reliable at level ($p \leq 0.01$). The power of influence of the factor "soil type" on the density of this group of microorganisms is very high - 71.12% and statistically significant at level ($p \leq 0.01$).

The microbes, assimilating the mineral forms of nitrogen immobilizing it in their own biomass, and thus provide dynamic reserve of fixed nitrogen, but the other hand they to be at plants competitors for it.

The numbers of these microbes were reduced by 42.15% at Haplic Vertisols and by 35.38% at Chromic Cambisols in the presence of

broomrape. Quantitative differences with uninfected plants did not statistically proven ($p \geq 0.05$). The effects of the factor "broomrape" on nitrogen immobilizing microorganisms were detected 4.11% (statistically not proven), and of the factor "soil type" - 53.31% statistically significant at level ($p \leq 0.01$).

When analyzing the microbial communities as a whole, it was found that the average density of the microbial population is reduced by over 65% in the presence of broomrape at both types of soils (67.14% in Haplic Vertisols and 68.41% in Chromic Cambisols). The influence of the factor "broomrape" - 7.12% and of the factor" soil type" - 23.45% from the general variation was found. The impacts were statistically proven at significance level ($p \leq 0.01$).

Interesting changes are observed in the distribution of different trophic groups, calculated as a proportion of the overall population density in microbial communities. Into control plants, the partitions of autochthonous and oligotrophic microorganisms are approximately equal, with a slight preponderance of autochthonous. A similar ratio there was between ammonifying and mineral nitrogen assimilating microorganisms.

In microbial communities formed at presence of broomrape, regardless of the lower density were predominant partitions of oligotrophic microorganisms and mineral nitrogen assimilating microorganisms, i.e. habitually biological equilibrium is disrupted. The proportion of microscopic fungi in them was increased and the share of actinomycetes was practically unchanged (fig.1).

This distribution is an indicator of changes in the structure of microbial communities, while maintaining their integrity as well as an indicator the depletion of nutrients in the rhizosphere of the infected plants-hosts and intensive immobilization processes of assimilable nitrogen.

All included in present study trophic groups of microorganisms essentially are organotrophic and soil indigenous. According to Ishida and Kodota (1981) depending on their specific needs from energy resources, regulating factor for the growth and development of them is the amount of incoming nutrients. In many nutrient-rich surroundings ammonifying microorganisms are predominant. Cultivating on a soil-agar authochthonous microorganisms are mesotrophic. At greatly reduced nutrient flow - below 0.1 mg/l organic carbon per day oligotrophic microorganisms increase the quantity and activity. Nutrient impoverishment in the rhizosphere environment of patho-system sunflower / *O. cumana* Wallr could be the cause of the render an account numerical preponderance of oligotrophic microorganisms and of mineral nitrogen assimilating bacteria. Because of the presence general trophic units microorganisms of different physiological groups are closely interlinked. Depending on the conditions for development they complement each other or interchange. A microbial community naturally is replaced with another, thereby regulates the structure and supports dynamic equilibrium in micro-biocenosis (Berendsen *et al*., 2012). Probably, the observed structural changes in the percentage distribution between the trophic groups of microorganisms at infection by broomrape are the result of a compensatory mechanism to counteract of reduction of microbial numbers and natural striving of microbial communities towards preserve the stability and properly functioning.

Quantitative changes in the density of the investigated trophic groups soil microorganisms are well expressed and have occurred regardless of the type of soil. Similar

trends at reducing population density and oligotrophic characters of microbial communities were outlined in previous studies the rhizosphere microflora under various plant systems hosts and several species broomrape (Hristeva at al., 2013). These researches, as and the present were conducted at the phase "flowering" of the parasitic weed. What happens in the microbial communities during the early phases of the development of the broomrape? Organotrophic microorganisms are associated with the plant roots in one degree or another. Depending on the conditions and the phases of plants the microbial rhizosphere communities are constructed. The conditions and the composition of the microenvironment should be different in the early stages from development of parasite. Will there be changes in population density of rhizosphere microorganisms and will they be similar to those observed in phase "flowering" of the parasite or not? Surveys are needed under controlled conditions, as type "microcosm", committed to dynamics covering as much as possible a more detailed phases of development of *O. cumana* Wallr: since the germination of seeds, attachment to host and its appearance above the surface. Besides the phases of development of the parasite would be useful to make a comparative study of the rhizosphere microbiota at tolerant and intolerant sunflower varieties to different races *O. cumana* Wallr.

The results raise the question - Directly or indirectly consequence of the presence of the parasite are registered quantitative changes? Nutrient violations to the host and the reduction of nutrients in the rhizosphere zone might be a logical explanation for the established trends towards reduction of microbial numbers. The poor surroundings undoubtedly have an impact on the quantitative development of the microorganisms, but the processes in the rhizosphere are too complex and interconnected to be the only reason.

Pretty data of the crucial importance of the root microbiota as a whole in many aspects of plant development, including the occurrence of immunity have accumulated (Berendsen *et al*., 2012). The plants form root microbiota by adjusting the root secretion in the form of carbon-rich exudates and it influence of microbial diversity, density and activity (Dennis *et al*., 2010). There has been considerable progress in understanding the signaling pathways and molecules emitted by plants in rhizosphere zone which involved in the recruitment of certain groups of microbes A number of experimental data show specificity of the interaction between plant species and associated rhizobacterial communities (Badri *et al*., 2009; Sugiyama *et al*., 2013). In this respect, the specificity of rhizosphere at the patho-system with broomrapes is much more complicated. As biological components - host, broomrape and microbial communities are connected.

The host intervenes and by phytohormones inducing seed germination of broomrapes. New and more in-depth knowledge of them suggest that they should also influence the quantity, composition and structure of microbial communities. It is considered that most plants produce and secrete lactones in the rhizosphere and they have a profound effect on the communications there (Liu *et al*., 2013; Pandey *et al*., 2016). This class of compounds characterized by enormous structural diversity (Tarkowski at al., 2015), which further strengthens the specifics of the various patho-systems. Besides clearly proven role in the formation of symbiosis between plants and arbuscular mycorrhizal fungi there are reports of an inhibitory effect on the growth of some phytopathogenic fungi from genus *Botrytis* and genus *Cryphonectria* (Ćavar *et al*., 2015), but on the growth of

Fusarium oxisporum not found such (Foo *et al*., 2016), i.e. there is a divergence of action.

The results of this study suggest necessity from a detailed study of species diversity inside within the groups of microscopic fungi and actinomycetes and the influence of sunflowers root secretions on them. The numbers in these groups decreased, but the share of their participation in microbial communities remains unchanged or increased slightly. These microorganisms possess potent enzyme system, which determines their ability to transformed complex organic substances that resist the action of the majority of bacteria. They are producers of various biologically active substances - antibiotics, toxins, amino acids (Vurro *et al*., 2009; Rodriquez-Conception and Boronat, 2012). Not accidentally, many of the microorganisms offered as bio-agents against broomrapes belong to these groups.

Concerning to the broomrapes, there is evidence that, after attachment deviated hormones and photo-assimilates from host, mainly auxins and sucrose (Abbes *et al*., 2009), by expression of genes involved in metabolism of carbohydrates. (Péron *et al*., 2012). According to Veronesi *et al*. (2005) one the reasons for the virulence of the different races of *O. cumana* Wallr maybe is the synthesis, the intensity and the secretion of pectinolytic enzymes exuded from broomrape during the attachment to the host. That means that the broomrapes also secreted metabolites and altered chemical composition in the rhizosphere. Probability broomrapes to have a direct impact on the population density of rhizosphere microorganisms should not be ignored.

For the participation of soil microorganisms in processes at plant- systems with broomrape have been guessed years ago. According Petzoldt (1979), the penetration of *O. crenata* Forsk. in the root of *Vicia faba* is stimulated by infection with *Rhizobium leguminosarum.* Influence of various metabolites (toxins, amino acids) by fungal and bacterial origin have been tested (Vurro *et al*., 2009; Sun, 2011). Exudate of *Streptomyces enissocaesilis* inhibit seed germination of *O. cumana*, but increase polyphenoloxidase activity of the roots of sunflower, which according to the authors is beneficial for microflora of the rhizosphere (Chen *et al*., 2016).

Some authors suggest that the root secretions from the host maybe are transformed by enzymes produced by microorganisms and after that can to induce germination of the seeds of broomrape (Perez de Luque *et al*., 2001; Bouwmeester *et al*., 2007; Cardoso *et al*., 2011). Some of these studies concern to the AMF, in others were discovered mechanisms of strigolactones degradation from the soil microscopic fungi - *Fusarium* and *Thrichoderma spp*. (Boari at al., 2016).

Actually, all microorganisms synthesize and produce in the environment a huge variety of extracellular enzymes, which is related to their living strategy. Inducers for commencement or cessation of enzyme synthesis, secretion and speed are not only local substrate concentrations, but population densities of microbes.

Microorganisms respond to signals that serve to inform them about their immediate surroundings, so-called "quorum - sensing". Considered, that this phenomenon is process that controls all sorts of interactions at the rhizosphere (Burns *et al*. 2013).

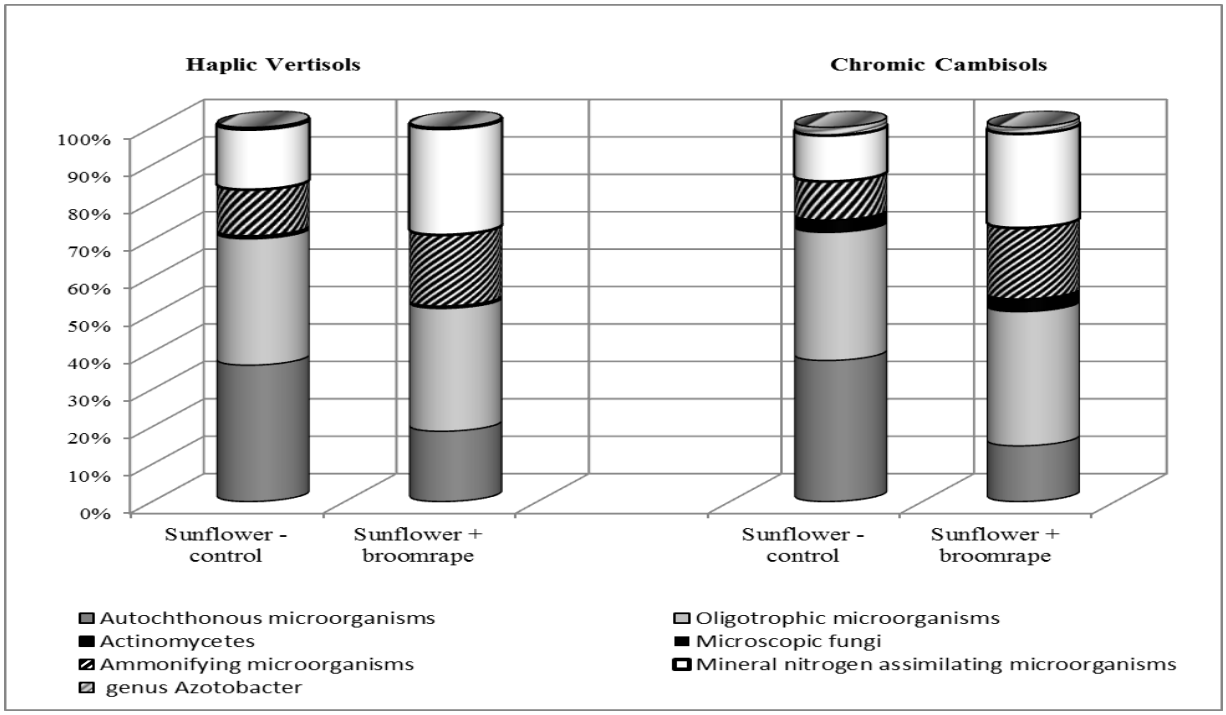
Table.1 Quantities (CFU/g a.d.s.) of trophic groups of microorganisms related to transformation of carbon compounds and percentage reduction in rhizosphere of sunflower plants infected by *O. cumana* Wallr., in relation to the quantities in rhizosphere of the uninfected plants

Variants / Trophic groups micro-organisms	Average number of trophic groups microorganisms / soil type				Effects of the factors (ηx^2) and statistical significance according to the Fisher's criterion: $F_{tab.95\%} = 3.49$; $F_{tab.99\%} = 5.85$			
	Haplic Vertisols		Chromic Cambisols		Factor "soil type"		Factor "broomrape"	
	$\bar{x} \pm \sigma_x$	Reduction %	$\bar{x} \pm \sigma_x$	Reduction %	$F_{exp.}$	ηx^2	$F_{exp.}$	ηx^2
Autochtonous microorganisms								
Sunflower – control	$1.85x10^9 \pm 21514$	100.00	$52.32x10^6 \pm 3582$	100.00	55.18	0.438^{***}	31.14	0.247^{***}
Sunflower + broomrape	$0.31x10^9 \pm 8852$	- 83.07	$6.40x10^6 \pm 1265$	- 87.53				
Statistical significance of the differences between the variants - "broomrape" and control: $D = 0.791x10^{9\,(***)}$; $S_d = 0.174x10^9$; $t_{exp.}$ 4.556 > $t_{tab.\ 0.1\%}$ 4.073; $GD_{5\%} = 0.370x10^9$; $GD_{1\%} = 0.512x10^9$; $GD_{0.1\%} = 0.708x10^9$								
Oligotrophic microorganisms								
Sunflower – control	$1.714x10^9 \pm 20699$	100.00	$46.614x10^6 \pm 3414$	100.00	137.2	0.606^{***}	40.64	0.180^{***}
Sunflower + broomrape	$0.548x10^9 \pm 11702$	- 68.04	$15.425x10^6 \pm 1964$	- 66.91				
Statistical significance of the differences between the variants - "broomrape " and control: $D = 0.599x10^{9\,(***)}$; $S_d = 0.115\ x10^9$; $t_{exp.}$ 5.205 > $t_{tab.\ 0.1\%}$ 4.073; $GD_{5\%} = 0.245\ x10^9$; $GD_{1\%} = 0.339\ x10^9$; $GD_{0.1\%} = 0.468x10^9$								
Microscopic fungi								
Sunflower – control	$63.335x10^3 \pm 126$	100.00	$74.182x10^3 \pm 136$	100.00	1.06	0.039^{ns}	14.29	0.520^{***}
Sunflower + broomrape	$36.388x10^3 \pm 95$	- 74.62	$41.730x10^3 \pm 102$	- 67.83				
Statistical significance of the differences between the variants - "broomrape " and control: $D = 29699^{(**)}$; $S_d = 9625$; $t_{exp.}$ 3.086 > $t_{tab.\ 1\%}$ 2.947; $GD_{5\%} = 20512$; $GD_{1\%} = 28366$; $GD_{0.1\%} = 39204$								
Actinomycetes								
Sunflower – control	$42.652x10^6 \pm 3265$	100.00	$4.409x10^6 \pm 1645$	100.00	21.64	0.407^{***}	11.55	0.217^{***}
Sunflower + broomrape	$10.826x10^6 \pm 1050$	- 42.55	$1.418x10^6 \pm 595$	- 43.75				
Statistical significance of the differences between the variants - "broomrape " and control: $D = 17.408\ x10^{6\,(*)}$; $S_d = 6.273x10^6$; $t_{exp.}$ 2.775 > $t_{tab.\ 5\%}$ 2.131; $GD_{5\%} = 13.367x10^6$; $GD_{1\%} = 18.486x10^6$; $GD_{0.1\%} = 25.549x10^6$								

Table.2 Quantities (CFU/g a.d.s.) of trophic groups of microorganisms related to transformation of nitrogen compounds and percentage reduction in rhizosphere of sunflower plants infected by *O. cumana* Wallr. in relation to the quantities in rhizosphere of the uninfected plants.

Variants / Trophic groups micro-organisms	Average number of trophic groups microorganisms				Effects of the factors (ηx^2) and statistical significance according to the Fisher's criterion: $F_{tab.95\%}$ = 3.49; $F_{tab.99\%}$ = 5.85			
	Haplic Vertisols		Chromic Cambisols		Factor "soil type"		Factor "broomrape"	
	$\bar{x} \pm \sigma_x$	Reduction %	$\bar{x} \pm \sigma_x$	Reduction %	$F_{exp.}$	ηx^2	$F_{exp.}$	ηx^2
Aerobic nitrogen-fixing bacteria of the genus *Azotobacter*								
Sunflower – control	$0.286x10^9 \pm 8463$	100.00	$2.957x10^6 \pm 860$	100.00	8.98	0.237^{***}	8.55	0.226^{***}
Sunflower + broomrape	$6.457x10^6 \pm 1271$	- 77.46	$0.742x10^6 \pm 431$	- 74.89				
Statistical significance of the differences between the variants - "broomrape " and control: $D = 0.141\ x10^{9\ (*)}$; S_d =59.108x10^6; $t_{exp.}$ 2.388 > $t_{tab.\ 5\%}$ 2.131; $GD_{5\%}$ =0.126x10^6; $GD_{1\%}$ =0.174 x10^9; $GD_{0.1\%}$ =0.241x10^9								
Ammonifying microorganisms								
Sunflower – control	$0.624x10^9 \pm 12491$	100.00	$14.046x10^6 \pm 1874$	100.00	66.48	0.712^{***}	7.72	0.083^{***}
Sunflower + broomrape	$0.317x10^9 \pm 8902$	- 49.21	$8.162x10^6 \pm 1428$	- 41.89				
Statistical significance of the differences between the variants - "broomrape " and control: $D = 0.157x10^{9\ (*)}$; S_d =69.008x10^6; $t_{exp.}$ 2.268 > $t_{tab.\ 5\%}$ 2.131; $GD_{5\%}$ = 0.147x10^9; $GD_{1\%}$ = 0.203 x10^9; $GD_{0.1\%}$ =0.281x10^9								
Mineral nitrogen assimilating microorganisms								
Sunflower – control	$0.818x10^9 \pm 14300$	100.00	$16.753x10^6 \pm 2047$	100.00	16.51	0.533^{***}	1.27	0.041^{ns}
Sunflower + broomrape	$0.473x10^9 \pm 10876$	- 42.15	$10.826x10^6 \pm 1645$	- 35.38				
Statistical significance of the differences between the variants - "broomrape " and control: $D = 0.175\ x10^{9\ (ns)}$; S_d = 0.190x10^9; $t_{exp.}$ 0.921 < $t_{tab.\ 5\%}$ 2.131; $GD_{5\%}$ = 0.406 x10^9; $GD_{1\%}$ = 0.561x10^9; $GD_{0.1\%}$ = 0.776x10^9								

Fig.1 Distribution of the different trophic groups microorganisms, calculated as a proportion of the overall population density in microbial communities at the patho-system with *O. cumana* Wallr and at the control plants



Most microbiological studies concerning of broomrapes target to using specific types of microorganisms such as bio-agents of control. Understand and clarify the processes in patho-systems with the presence of *Orobanchaceae*, profound complex investigations in different aspects, including biochemical and molecular genetic on rhizosphere communities in their entirety, unity and simultaneously specifics are need

In conclusion, reduction of population density of basic trophic groups microorganisms and distinct structural changes in microbial communities formed in the rhizosphere of sunflower plants infected by broomrape /*O.cumana* Wallr./ regardless of the soil type have been established.

The results of the present study does not answer the issues discussed, but they clearly support the opinion to role of rhizosphere microorganisms such as biotic factor in the ongoing processes in systems host-parasite.

The results allow to develop hypothesis for two-way impact: by side of microorganisms towards broomrape and by side of broomrapes towards rhizosphere microbiota.

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Abbreviations

AMF - arbuscular mycorrhizal fungi
CFU/g a. d. s. - colony forming units per g absolutely dry soil.

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