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QUANTITATIVE REPRESENTATIVITY OF DIFFERENT ECOLOGIC GROUPS OF MICROORGANISMS, IN SOIL OF SECALE CEREALE PLANTS, ARTIFICIAL INFECTED WITH DIFFERENT STRAINS OF *CLAVICEPS PURPUREA*

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INTRODUCTION

The distribution of the different ecologic micro organism groups in the soil, for the cultivated terrains, depends on the different types of fertilisers (mineral or organic) but, in great measure, also on the species of cultivated plants.

In the measure that the composition of the micro flora in the soil has a considerable influence on growth and development of the plants (contributing at fertilizing the soil) in the same measure the plants also constitute an especially important factor in the life of micro organisms, influencing their quantitative and qualitative composition. Their role manifests visible in the course of their life (through the radicular system), as well as after harvesting the plants.

Taking account of the major influence that the composition of the radicular excreta could have on the micro organism distribution in the soil, the current research studied the spectre modification of some ecophysiologycal micro organism groups involved in the nitrogen and carbon circulation in nature (ammonifying, denitrifying, nitrifying micro organism, free fixators, aerobes and type N anaerobes and cellulosolytic aerobes) in the soil of a *Secale cereale* culture infected with strains that belong to another type of alkaloids different from *Claviceps purpurea*.

The proposed research is justified from the point of view of the anterior results that demonstrated the metabolism altering of the infected plants, considering this aspect as having influence on the radicular exudate composition and implicitly on the micro organism distribution in the soil belonging to different ecophysiologycal groups.

MATERIAL AND METHOD

The determination of the micro flora represented by ecophysiologycal micro organism groups, implicated in the development of the different biochemical reactions that take place in the cultivated soil, has been performed on special nutritious environments (selective culture environments). The dilution technique was applied and the cultivation on liquid environments, the micro organism number belonging to each physiological group, being measured either through emphasing a chemical reaction, or visual, through observing the development of the culture.

Taking the soil samples and performing the dilutions The soil sampling was performed with a special probe; there were 10 different partial harvests for each sample, the depth was 15 cm, right next to the plant roots. After grinding the samples were transferred (10 g of soil for each sample) in flask (300ml capacity) with 100ml sterile water, performing the 1/10 or 10^{-1} dilution. The decimal dilutions (10^{-1}- 10^{-23}) were performed, the suspensions obtained in this way being immediately seeded on the specific environments for each ecophysiologycal group studied (3 test - tubes for each dilution).

1. For the determination of the ammonifying micro organisms, it was tested, with the help of the Nessler reactive (confirmed by the yellow-orange colouring), if there is any presence of NH_3 in the culture environment. The reading of the results was done, at 3, 5, 7, 10 and 15 days of incubation at 28^0C.

2. Determination of the free atmospheric nitrogen fixating micro flora: a) aerobe micro organisms – the presence of fixating bacteria atmospheric N (especially *Azotobacter*) was studied after 7 – 10 days from seeding through the presence, of a grey-white coloured, then a dark brown coloured, film at the surface. of the liquid; b) aerobe micro organisms - the presence of fixating bacteria atmospheric N (especially *Clostridium pasteurianum*) was made evident through the presence of the gas bubbles that develop in the Durham tube, as a consequence to the butyric fermentation.

3. Determination of the nitrifying micro flora – the evidencing, of the presence of the bacteria groups corresponding to the two nitrifying processes (nitration, performed by nitrous bacteria, nitration, performed by nitrifying bacteria) was performed with the help of the sulphuric diphenylamine. For evidencing the nitrites the hemolysistubes are almost completely destroyed, 10 drops of sulfuricacid and 10 drops of sulphuric diphenylamine are added. The

positive reaction results from the blue colouring. For evidencing the nitrites in the culture environment 50mg urea (for eliminating the nitrites in the sulphuric environment), 10 drops of sulfuricacid and 10 drops of sulphuric diphenylamine are added. The presence of nitrites results from the same blue colouring.

4. Determination of the denitrifying micro flora – for evidencing this micro organism group from the soil it is tested if there any nitrites are present in the culture environment. The reading of the samples was made after 7 –15 days of incubation at 28^0C, the positive tubes being those that had no colour (indicate the lack of nitrites).

5. Determination of the cellulosolytic aerobe micro flora – the presence of the cellulosolytic bacteria was determined after 15 days of incubation, due to the appearance of pigmented marks on the earlier introduced Whatman filter paper

The alkaloids were assayed in the sclerotia, at different stages of growth by Rumpel method (1954), while the ratio of different types of ergot alkaloids was determined by thin layer chromatography (Brevet 1977).

RESULTS AND DISCUSSIONS

The soil samples were harvested after 30 days from the artificial rye head infection made with different *Claviceps purpurea* strains (T1, T2, T3, T4 and T5). The sclerotia, at this age, have an intense activity, they are almost mature. The soil samples were harvested right next to the infected plant roots.

The results obtained from the microbiological and biochemical analysis are having a graphical representation. The tested strains are characterized by different capacity of producing sclerotia. The biosynthetic level of the strains is similar concerning the total quantity of alkaloids. The values are between 0.5-0.7g%. The strains differences, considered by us to be important, refer to the proportion between the biosynthesised peptide alkaloids (figure 1). For the T2 and T2 strains we mention a predominant presence of ergotamine in the alkaloid complex. At the other strains, 40-50% from the total quantity, it is represented by ergotoxinic alkaloids (ergocristine and less, ergocryptine).

Our researches concerning the presence of bacteria in the infested and not infested rye cultivated soil show the presence of the principal ecofiziologice groups. We mention that, for the different groups of micro organisms, there are quantitative differences, even if they are relatively reduced. They are correlated with the different pathogen systems variants present in the culture. Our investigations, concerning the free nitrogen fixating bacteria, present in the infected plant and not infected plant soil, have showed in most of the cases, the anaerobe numeric nitrogen fixators are predominant. There are 2 exceptions: the T1 and T5 samples where the anaerobe nitrogen fixators have a more reduced number than the control. At the T2 and T3 the lack of aerobe N fixatores is remarked. The number of aerobe nitrogen fixators is best represented at sample T5. The parasite sclerotia belonging to the eperimentated T2 and T3 variants are different from the sclerotia belonging to the other strains because they predominantly produce ergotamine.

Through the correlation of the obtained results, we consider that this numeric growth of the anaerobe nitrogen fixators isn't of great importance for boosting the fertility of the soil. The reasons are: the proliferation of this bacteria group in the cultivated soil could be due to the supplementary quantity of available nitrogen, as a result of the intensification of the organic substance mineralization, through ammonification. In this case, as we know, the capacity to fixate the atmospheric nitrogen is falling. Increasing the NH_3 amount in the soil (as a result of ammonification) acts as a strong inhibiting factor regarding the nitrogenase synthesis (the enzymatic system that characterises the atmospheric nitrogen reaction).

In consequence, the intensification of the ammonification process in the soil, expressed by the numeric growth of the process participating micro organisms, is a favourable phenomenon for the *Claviceps purpurea* infected rye plants because, in these conditions, the quantity of ammoniacal nitrogen is growing, and indirectly, the nitrate amount that plants can absorb directly.

Knowing the fact that the ammonification and nitrification processes are in direct relationship, and the nitrification intensity depends on the ammonium amount in the soil and its provenience, we can explain the numeric growth of the nitrous micro organisms (that participate at the second stage of the nitrification: nitration), identified in the samples harvested from the plot with *Claviceps purpurea* (T2, T4 and T5) cultures infected strains. Concerning the number of nitrous bacteria, the controls are the samples harvested from the *Claviceps purputrea* T1 and T3 infected strains plot. In the well refined soils, with corresponding humidification (the case of our harvested samples), the nitrogen in form of nitrites is rarely evidenciated, it rapidly oxidised by the nitrates. On the whole, activity intensification of the nitrous bacteria is favourable; especially that it corresponds the maximum absorption period of the plants, for its own metabolic assurance, but especially for those of the parasite

The participating micro organisms, at the denitrification process, were not yet identified in the studied samples. This process is considered negative for the soils, because nitrogen contained by the nitrites and nitrates is lost in form of ammonium, and the assimilable amount of nitrogen is reduced. The obtained results are positive, from this point of view, because the nitrogen that is still in the soil contributes at boosting its fertilisation. Between the microrganisms participating at the carbon circuit in nature, the ones that were researched were the cellulosic aerobe micro organisms. In the analysed samples, the activity of this micro organisms varies very little in comparison to the control, higher values being found just in the case of the soil samples that

belong to the *Claviceps p.* T3 and T5 infected strains plot. The sclerotia belonging to these strains are remarkable because they produce the highest amount of total alkaloids.

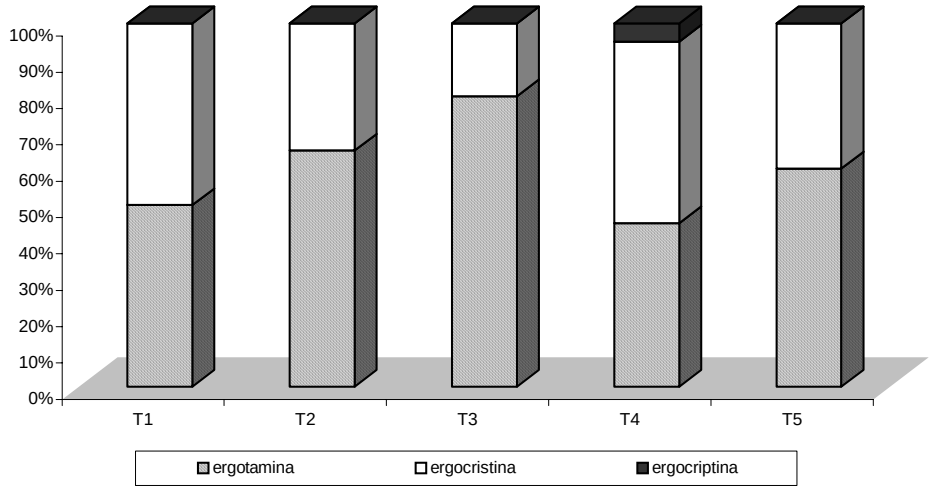

Fig. 1. Alkaloid spectrum of *Claviceps purpurea* sclerotia

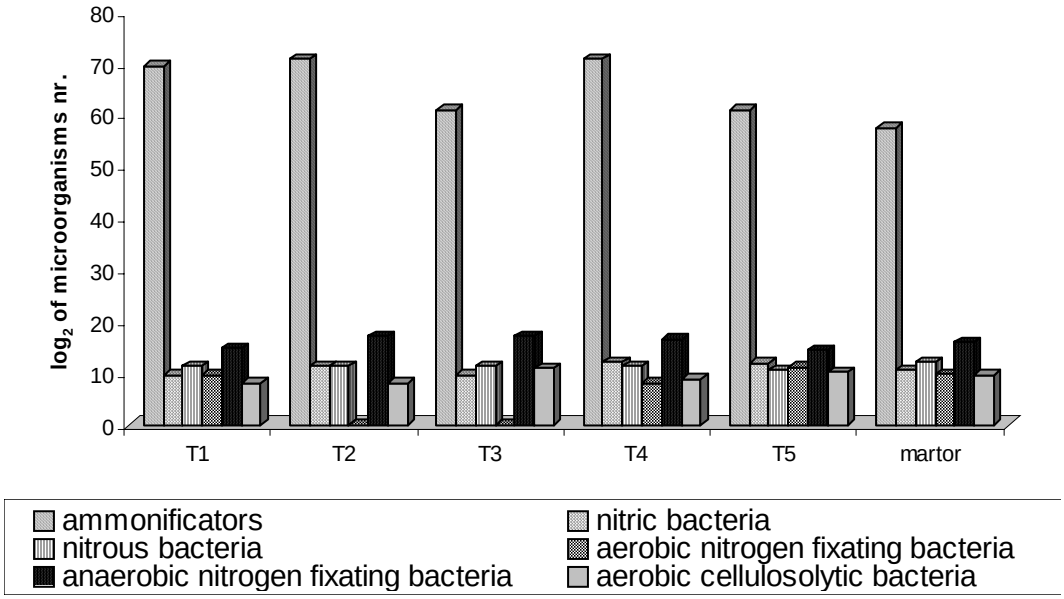

Fig. 2. Quantitative representatives of different ecologic groups of micro organisms, in soil of *Secale cereale* plants infected with *Claviceps purpurea*

CONCLUSIONS

1. In conclusion, we think that that the representativity of the ecologic micro organisms groups implicated in the biological circuits of the biogene C and N elements is different in the soil samples, because it is influenced by the action of the pathogen system (plant- parasite).
2. The parasite presence with a strong intensification of the ammonification process, with the proliferation of the nitric bacteria and the quantitative nitrous bacteria reduction in the soil samples that come from the infected plants plots, in comparison to the control plot.
3. We can concluded that the parasite, by total quantity and quality of alkaloids produced, influence some ecophysiologycal groups of micro organisms in soil of *Secale cereale* plants infected by strains of *Claviceps purpurea* producing a large quantity of alkaloids (increase the number of cellulosolytic bacteria, absence of aerobic nitrogen fixating bacteria, semnificative increase of anaerobic nitrogen fixating bacteria).
4. In analysed soil samples was not noticed the presence of denitrification bacteria.

REZUMAT

Cercetările privind prezența bacteriilor libere fixatoare de azot, în solul provenit din loturile plantelor infectate cu ciuperca *Claviceps purpurea* şi, respectiv, neinfectate, a scos în evidență faptul că, în majoritatea cazurilor, fixatorii anaerobi de azot predomină numeric, atât față de fixatorii aerobi, cât şi față de martor, se intensifică puternic procesul de amonificare şi creşte numărul microorganismelor nitrice. Microorganismele participante la procesul de denitrificare nu au fost identificate în nici una dintre probele cercetate. De asemenea, s-a constatat că, dintre microorganismele participante la circuitul carbonului în natură, evoluția microorganismelor celulozolitice aerobe în solurile plantelor infectate, este nesemnificativă în comparație cu probele martor.

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