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INVESTIGATIONS ON THE INFLUENCE OF FERTILIZATION AND OF *ONOBRYCHIS VICIIFOLIA* SCOP. AND *BROMUS INERMIS* LEISS. MIXTURE ON SOIL MICROFLORA

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ABSTRACT - This paper presents the results of an experiment conducted on two temporary meadows, situated in the Southern area of the Moldavian Plain, set up by sowing a mixture made up of *Onobrychis viciifolia* Scop. (sainfoin) and *Bromus inermis* Leiss. (smooth bromegrass) at different rates. We have analysed the soil microbiological activity of the two temporary meadows, as influenced by the fertilization with an unconventional product (Vinasse Rompak - 3% N, 0.5% P, 7% K and pH 7-8) and with different rates of organic and chemical fertilizers. The objective of this investigation was to isolate and quantify the microbial population found in soil (Gram-positive bacteria, Gram-negative bacteria, micromycetes and nitrogen-fixing bacteria), thus establishing their percent, main fungus genera, which activate in soil and their activity level for each variant. The results have shown the influence of the fertilization on the total number of microorganisms, on the relationships between the main groups (bacteria and fungi) and on the various micromycete species, determined in each variant of our experiment.

Keywords: fertilization, microbial activity, soil, temporary meadow

REZUMAT - Cercetări privind influenţa fertilizării şi a amestecului de *Onobrychis viciifolia* Scop. şi *Bromus inermis* Leiss. asupra microflorei solului. Lucrarea prezintă rezultatele obţinute în cadrul unor experienţe, amplasate în două pajişti temporare din zona sudică a Câmpiei Moldovei, înfiinţate prin însămânţarea unui amestec de *Onobrychis viciifolia* Scop. (sparcetă) şi *Bromus inermis* Leiss. (obsigă nearistată), în proporţii diferite. S-a urmărit activitatea microbiologică a solului din cele două pajişti temporare, sub influenţa fertilizării cu un produs neconvenţional (Vinassa Rompak - 3% N, 0.5% P, 7% K, pH 7-8), cu îngrăşăminte organice şi chimice. S-au determinat grupele principale de microorganisme prezente în sol (bacterii Gram pozitive, bacterii Gram negative, micromicete şi fixatori de azot), stabilindu-se procentul de participare al acestora, principalele genuri de ciuperci care activează şi nivelul activităţii acestora pentru fiecare variantă. Rezultatele au

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evidențiat o activitate microbiană diferită şi un spectru divers de micromicete între toate variantele avute sub observație, ca urmare a fertilizării diferențiate şi a raportului diferit între cele două specii.

Cuvinte cheie: activitate microbiană, fertilizare, sol, pajişte temporară

INTRODUCTION

Maintaining or improving soil organic matter is an important objective for each sustainable agriculture system. The organic matter loss has a negative effect on soil chemical, physical and biological characteristics. The diminution in soil microbial activity is alarming, because of the active role of microorganisms from soil: decay of biological residues, mineralization of the organic matter, formation and stabilization of soil aggregates, etc. (Cardon et al., 2007; Papacostea, 1976).

Under natural ecosystems, the losses of main mineral elements from soil, necessary to plant nutrition, are compensated by fixing and solubilisation processes, while in the systems used in agriculture, these losses exceed the rate of replacement. This leads to a lack of balance between the needs of nutritive elements and the opportunity of covering these needs (Saratchandra et al., 1988; Varma et al., 2004).

This problem may be solved by using conventional (organic and mineral fertilizers) or unconventional products (Vinasse), on the condition of their application in well-determined rates and combinations. This is necessary for soil balance as concerns the natural microflora from soils, which maintains soil health and, implicitly, a constant productive potential.

The goal of this study was to analyse and compare the microbiological activity from soil, occupied with two types of temporary meadows, as influenced by the fertilization with an unconventional product (Vinasse Rompak) and with organic and chemical fertilizers.

MATERIALS AND METHODS

The trial was conducted on a 2-3% slope field from the Ezăreni Farm, which belongs to the University of Agricultural Sciences and Veterinary Medicine, Iaşi. Soil is a clayey loam cambic chernozem, weakly degraded, with pH comprised between 6.7 and 6.8, humus content 2.73-2.93%, 51-55 ppm P_2O_5, 314-336 ppm K_2O and 184-187 ppm CaO. The area is characterized by mean annual temperatures of 9.6^o C, annual rainfall of 517.8 mm and air relative humidity of 69%.

From the physical-geographical viewpoint, this territory is found in the Southern area of the Moldavian Plain, which is named the Lower Jijia Plain and the Bahlui Plain, being situated in the South-Western extremity of this natural zone.

The studied factors were:

Factor A = mixture of perennial legumes and grasses: A_1 = *Onobrychis viciifolia* 20% + *Bromus inermis* 80%; A_2 = *Onobrychis viciifolia* 70% + *Bromus inermis* 30%.

Factor B = fertilization: B_1 = unfertilized control; B_2 = $N_{200}P_{100}$ kg/ha;

B_3 = 30 t manure /ha and B_4 = 5 t/ha Vinasse.

For determining the number of microorganisms per 1 g soil, we have used the culture method in Petri dishes. Soil samples were gathered in paper bags, by means of a metallic spatula and the used material was previously sterilized. Soil was sampled at 10 cm depth and then samples were processed by grinding and homogenization in a sterile mortar. Soil dilutions were prepared according to the method of successive dilutions and sowing was done in Petri dishes, by the incorporation in medium.

For an easy identification of colonies, we have used different culture mediums, specific to systematic groups. Thus, for determining the total number of microorganisms, we have used the simple PDA (potato-dextrose-agar) medium, for determining the number of Gram-positive bacteria, we have used the PDA with streptomycin (35 ppm) medium and for determining the number of micromycetes, we have used the PDA with Bengal Pink (33 ppm) medium. Nitrogen fixing-bacteria of *Azotobacter* and *Clostridium* genera were emphasized by using the Ashby medium (Constantinescu, 1974).

Sowing was done by introducing a ml of dilution in each Petri dish with melted and cooled medium at 45°C. The sown dishes were incubated in a thermostat at 28°C. The number of bacterial colonies was determined at 24 hours and the fungus colonies at 5 days; counting was done by naked eye, using a marker. At high densities, the Wolfhügel plate was used (Larpent et al., 1990).

RESULTS AND DISCUSSION

The analysis of the obtained data on the two types of temporary meadows has shown a significant increase of soil biological activity in all the fertilized variants, compared with the unfertilized control, except the variant sown with a mixture made up of *Onobrychis viciifolia* 20% + *Bromus inermis* 80%, fertilized with Vinasse, where the number of microorganisms per soil gram was equal to their number from the control (*Table 1*).

Table 1 – Biological activity of soil occupied with temporary meadows

Mixture	Fertilization variant	Total micro-organisms	Bacteria		Fungi		N_2 Fixing bacteria
			thousands/ g soil	%	thousands/ g soil	%	
A_1	Unfertilized control	277	157	56.7	120	43.3	x
A_2		444	250	56.3	194	43.7	x
A_1	$N_{200}P_{100}$ kg/ha	1103	1033	93.7	70	6.3	x
A_2		940	810	86.2	130	13.8	x
A_1	30 t/ha manure	414	314	75.8	100	24.2	x
A_2		885	785	88.7	100	11.3	x
A_1	5 t/ha Vinasse	270	210	77.8	60	22.2	x
A_2		599	549	91.7	50	8.3	x

A_1 = *Onobrychis viciifolia* 20% + *Bromus inermis* 80%; A_2 = *Onobrychis viciifolia* 70% + *Bromus inermis* 30%; x - The presence of nitrogen-fixing bacteria.

In all the studied variants, we isolated nitrogen-fixing species belonging to *Azotobacter* and *Clostridium* genera.

In both mixtures used for sowing the two types of temporary meadows, the highest biological activity was recorded in the variants fertilized with nitrogen and phosphorus-based fertilizers. In the mixture made up of *Onobrychis viciifolia* 20% + *Bromus inermis* 80% (A1), the highest activity was recorded in B2 Variant ($N_{200}P_{100}$ kg/ha), followed by B3 Variant (30 t/ha manure). In the mixture made up of *Onobrychis viciifolia* 70% + *Bromus inermis* 30% (A2), the same fertilization variant has signalled the highest number of microorganisms/g soil, followed by B3 (30 t/ha manure N150) and B4 (5 t/ha Vinasse) (*Figure 1*).

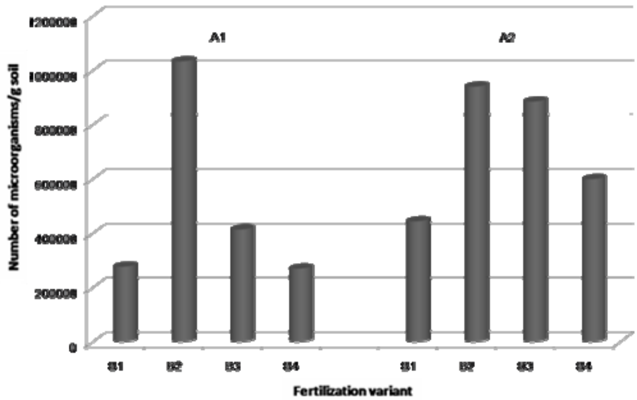

Fig. 1 – Differentiation of the microbiological activity per fertilization variants and type of temporary meadow

Analysing the ratio between the main groups of microorganisms found in the soil occupied by the two types of temporary meadows, we found significant differences among all the fertilization variants, except the unfertilized control, where the ratio between bacteria and micromycetes was identical for the two types of meadows. The best represented microorganism group for all the fertilization variants and for each type of meadow is that of bacteria that are between 56.7% (B1) and 93.7% (B2) of the total microorganisms, in case of A1 mixture, and between 56.3% (B1) and 91.7% (B3), in case of A2 mixture (*Figure 2*). The change of the ratio between the main systematic groups, in order to favour the numerical increase in bacteria or fungus species to the prejudice of the other microorganism group may be explained by the application of fertilizers or other unconventional products (Vinasse Rompak). The high rate of soil bacteria in all the fertilized variants and for both types of meadows may be explained by their competition against micromycetes and plants as concerns some nutrients, and by increasing the soil concentration in nutrients, due to fertilizers (especially, nitrogen and phosphorus fertilizers), which determined an exponential increase in the bacterial populations (Ulea et al., 1999; Wood, 1989).

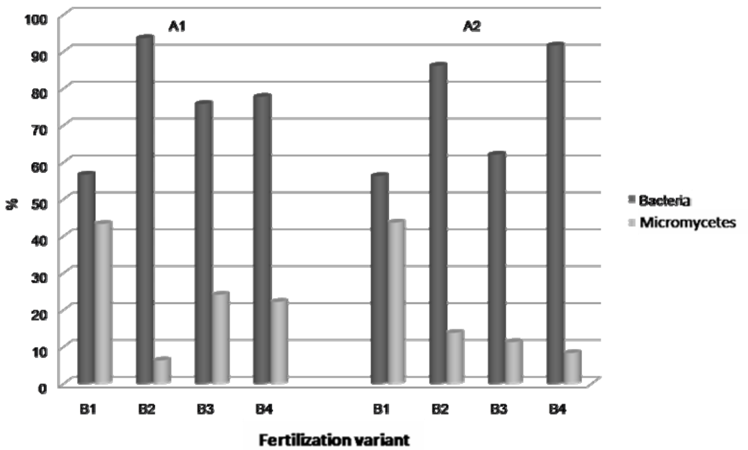

Fig. 2 – Main groups of microorganisms for each fertilization variant and type of temporary meadow

The investigations conducted on the frequency of micromycete genera have shown a diminution in the number of genera, in the fertilized variants, compared to the unfertilized control, at both used mixtures (Bontea, 1986; Gilman, 1959) (*Table 2*).

Table 2 – Isolated micromycete genera found in the soil occupied by temporary meadows

Mixture	Fertilization variant	Isolated micromycete genera
A_1	Unfertilized control	*Penicillium*, *Aspergillus*, *Fusarium*, *Alternaria*, *Rhizopus*, *Mycogone*
A_2		*Penicillium*, *Aspergillus*, *Fusarium*, *Alternaria*, *Robillarda*, *Cladosporium*, *Trichoderma*, *Mucor, Rhizopus*
A_1	$N_{200}P_{100}$ kg/ha	*Penicillium*, *Aspergillus*, *Alternaria*, *Rhizopus*
A_2		*Penicillium*, *Fusarium*, *Alternaria*, *Robillarda*, *Cladosporium*, *Trichoderma*, *Rhizopus*
A_1	30 t/ha manure	*Penicillium*, *Fusarium*, *Aspergillus*, *Rhizopus*, *Mycogone*
A_2		*Penicillium*, *Aspergillus*, *Fusarium*, *Melanospora*, *Cladosporium*, *Trichoderma*, *Mucor, Rhizopus*
A_1	5 t/ha Vinasse	*Penicillium*, *Trichoderma*, *Rhizopus*
A_2		*Penicillium*, *Aspergillus*, *Fusarium*, *Trichoderma*, *Rhizopus*

A_1 = *Onobrychis viciifolia* 20% + *Bromus inermis* 80%;
A_2 = *Onobrychis viciifolia* 70% + *Bromus inermis* 30%;

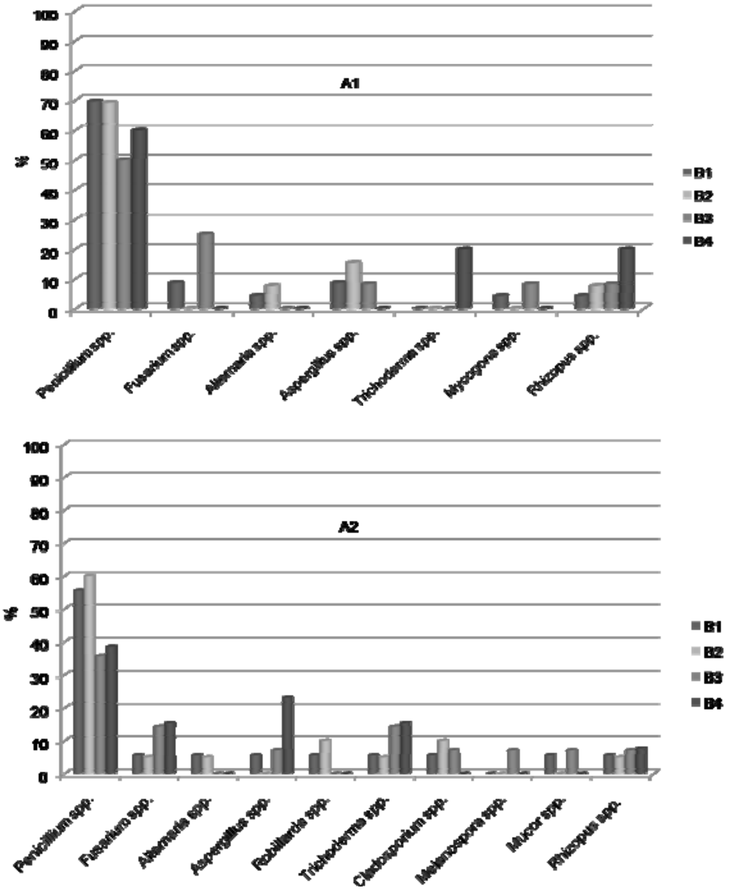

Fig. 3 – Micromycete species found in each fertilization variant (B1, B2, B3 and B4) and type of temporary meadow (A1, A2)

We have also noticed that the number of isolated fungus genera, in the meadow sown with A1 mixture was more reduced than in case of A2 mixture. In the mixture made up of *Onobrychis viciifolia* 20% + *Bromus inermis* 80% (A1), we have isolated species belonging to six micromycete genera (*Penicillium*, *Aspergillus*, *Fusarium*, *Alternaria*, *Rhizopus*, *Mycogone* and *Trichoderma*), while in the mixture made up of *Onobrychis viciifolia* 70% + *Bromus inermis* 30% (A2), we identified species belonging to 10 genera (*Penicillium*, *Aspergillus*, *Fusarium*, *Alternaria*, *Robillarda*, *Cladosporium*, *Trichoderma*, *Melanospora*, *Mucor* and *Rhizopus*). This difference may be explained by the selection of some specific fungus genera, according to the biochemical features of root secretions in the two sown mixtures (Yevdokimov et al., 2008; Zarnea, 1994). Thus, the species belonging to *Robillarda*, *Cladosporium*, *Melanospora* and *Mucor* genera were isolated only from the temporary meadow, sown with the mixture made up of *Onobrychis viciifolia* 70% + *Bromus inermis* 30%, while *Mycogone* genus was identified only in the mixture made up of *Onobrychis viciifolia* 20% + *Bromus inermis* 80% (*Figure 3*).

Among the determined micromycetes in all the studied variants, we pointed out *Penicillium* genus, which was isolated at a rate comprised between 50 and 69.9% of the total identified genera for the A1 mixture and respectively, between 35.7 and 60%, for the A2 mixture.

The micromycete species from the A1 mixture were completed by *Fusarium*, *Aspergillus* and *Rhizopus* genera, and from the A2 mixture, by *Trichoderma*, *Fusarium*, *Cladosporium* and *Rhizopus* genera (*Figure 3*)

The analysis on the number of micromycete colonies/Petri dish has shown that among all the fertilization variants, the lowest number of colonies was recorded in the variant fertilized with Vinasse 5 t/ha (B4), and the highest number, in the unfertilized control (B1) (*Figure 4*)

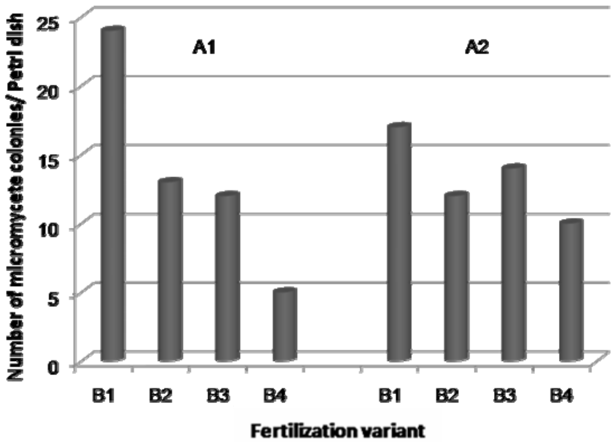

Fig. 4 – Determination of the number of micromycete colonies/ Petri dish, for each fertilization variant and type of temporary meadow

CONCLUSIONS

Our observations have shown a very good activity of aerobe and anaerobe nitrogen-fixing bacteria that live free in soil and complete the activity of bacteria found in the nodosities of legumes.

Between the two mixtures used for setting up the meadows, the highest microbial activity in B1 (unfertilized), B3 (30 t/ha manure) and B4 (Vinasse 5 t/ha) was recorded at the mixture made up of *Onobrychis viciifolia* 70% + *Bromus inermis* 30% (A2), and in B2 ($N_{200}P_{100}$ kg/ha) at the mixture made up of *Onobrychis viciifolia* 20% + *Bromus inermis* 80% (A1).

The microbial activity has shown a significant increase at both mixtures used for sowing, in all the fertilized variants, compared to the unfertilized control.

At both mixtures made up of *Onobrychis viciifolia* 20% + *Bromus inermis* 80% (A1) and *Onobrychis viciifolia* 70% + *Bromus inermis* 30% (A2), the highest number of microorganisms was recorded in the variant fertilized with $N_{200}P_{100}$ kg/ha.

In both trials, we found a diminution in the number of identified fungus genera at the fertilized variants, compared with the unfertilized control.

In all the studied variants, from all the isolated micromycete species, *Penicillium* genus had the highest frequency; it was followed by *Fusarium*, *Aspergillus*, *Trichoderma* and *Rhizopus* genera.

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