INVESTIGATIONS ON THE INFLUENCE OF FERTILIZATION AND MAIZE (*ZEA MAYS* L.) ROOT EXUDATES ON SOIL MICROFLORA

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Abstract

Researches were carried out on maize (*Zea mays* L.) field trials located in the south region of Moldavian plain (Ezareni Farm), studying the effects of mineral fertilization (N - 150 kg/ha, P and K - 75 kg/ha) and different compost (organic fertilizer) rates (10 t/ha, 20 t/ha and 30 t/ha).
The objectives of this investigation were to isolate and quantify the existing microbial population in soil (Gram positive bacteria, Gram negative bacteria, micromycetes) establishing their participation ratio, the main fungus genres which activate in soil and their activity level for each variant.
Mineral fertilizer has caused a increasing in the number of microorganisms. The inhibitory effect of the organic fertilizer used was pronounced in case of compost application in dosage of 30 t/ha. This dosage caused a reduction in the number of microorganisms and microbial activity in soil.
The results illustrate the influence of the fertilization on the dynamic of microorganisms population, on the relationship between the main groups (bacteria and fungi), and on the micromycetes spectrum determined in each variant of our experiment.

Key words: microbial activity, *Zea mays* L., fertilization

Modern agriculture is not possible without fertilizer application, which not only maintains, but even gradually increases field crop yield. Modern fertilization systems are not just an isolated element of plant production, but are indispensable link in the practice. Organic and mineral fertilization provides plants with nutrients at appropriate proportions and quantities which enable maximum yield increase of crops with high biological and technological quality.

Biomass of all microorganisms living in soil play an important role in the functioning of entire soil ecosystems because their enormous biochemical activity. Soil microflora is involved in biochemical transformations of fertilizers, synthesis of biologically active substances and nitrogen fixation from the air. Because of active role of soil micro-population, the diminution in microbial activity is always alarming (Barabasz, W., Albińska, D., Jaśkowska, M., Lipiec, J., 2002)

The purpose of present investigation was to analyse the demography of soil microflora (bacteria, micromycetes) and their relationship under different fertilization systems.

MATERIAL AND METHOD

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 P2O5, 314-336 ppm K2O 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 experiment was conducted in five experimental variants: V1 – unfertilized (control); V2 – mineral fertilization $N_{150}P_{75}K_{75}$; V3 – organic fertilization by application of 10 t/ha compost; V4 – organic fertilization by application of 20 t/ha compost; V5 – organic fertilization by application of 30 t/ha compost.

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, using a metallic spatula. All material and instruments were 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 inoculation was made through incorporation of 1 ml dilution in medium.

For an easy identification of colonies, we have used different culture mediums, specific to

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each systematic group. 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 medium with streptomycin (35 ppm) and for determining the number of micromycetes, we have used the PDA medium with rose bengal (33 ppm) (Constantinescu, O., 1974).

Inoculation was done by introducing 1 ml 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 by counting using a marker. At high densities, the Wolfhügel plate was used (Larpent, J.P., Larpent-Gourgand, M., 1990).

RESULTS AND DISCUSSIONS

The analysis of the obtained results regarding the evolution of the biological activity from the soils cultivated with corn (*Zea mays* L.) under fertilization underlined an important diversity, in what concerns the ratio between the microorganism groups as well as the spectrum of the reported micromycetes.

Concerning the total number of the reported microorganisms, a considerable difference is noticed between the unfertilized control, where the total number exceeds 1.17 million microorganisms/g soil compared to the variants fertilized with mineral elements and compost (10 t/ha and 20 t/ha) where the average registered values were between 1.2 and 1.7 millions microorganism/g soil. Interesting is the fact that the lowest number of microorganisms is registered at the variants fertilized with 30 t/ha compost, fact which demonstrated that compost applied in higher amount induces a temporary decreasing of soil microbial activity (*tab. 1*).

Table 1

Soil microbial activity

	Fertilization variant	Total microorg./g soil	Gram negative bacteria		Gram positive bacteria		Fungi	
			microorg./g soil	%	microorg./g soil	%	microorg./g soil	%
1	Unfertilized (control)	1,172,000	588,750	50.2	353,250	30.1	230,000	19.7
2	$N_{150}P_{75}K_{75}$	1,691,500	824,250	48.7	667,250	39.4	200,000	11.9
3	Compost 10 t/ha	1,486,000	392,500	26.4	863,500	58.1	230,000	15.5
4	Compost 20 t/ha	1,220,500	392,500	32.2	628,000	51.5	200,000	16.3
5	Compost 30 t/ha	1,023,500	157,000	15.3	706,500	69.1	160,000	15.6

Analyzing the ratio between the main groups of microorganisms found in the soil occupied by maize during the observation time, we found significant differences among all variants after fertilizer application.

The best represented microorganism group in case of variants unfertilized (control) and with mineral fertilizer is that of Gram-negative bacteria (G-), which represent 50.2 and 48.7%, respectively. Gram-positive bacteria (G+) represent 30.1% in case of control variant and 39.4% when a mineral fertilizer was used. Fungi represent 19.7% and 11.9% from total number of microorganism, respectively.

We noticed that in the case of all variants fertilized with compost (10, 20 and 30 t/ha), compared to the other variants, a significant increasing of G+ bacteria took place, fact which can be explained by the appearance of hard accessible substances for G- bacteria. Also, this situation can be explained through the accommodation period needed for some microbial population to the new condition.

The analysis on the number of micromycetes colonies/Petri dish has shown that among all the analysed variants, the lowest number of colonies was recorded in the variant fertilized with mineral elements (NPK), and the highest number in the unfertilized control (*tab. 1*).

Obtained results are important for the ecological agriculture because they put in evidence the possibility of compost usage in quantity of 20 t/ha as organic fertilizer in ecological farms.

As noted in our experiment and other studies, fertilizer could have inhibitory effects on microbial populations, but generally the microbial populations react by increasing their biomass and activity (Lipşa, F. D., Ulea, E., Chiriac, Irina Paraschiva, Coroi Irina Gabriela, 2010;. Pesakovic, M., Mandic, L., Djukic, D., 2003).

The investigations conducted on the frequency and spectrum of micromycetes genera shown different values depending of fertilization.

The greatest number of isolated fungus genera was in case of unfertilized variant (10) followed by the variant fertilized organic with 10t/ha compost (9) and mineral elements (7).

Among the determined micromycetes in all analysed variants, we pointed out the fungus genera *Penicillium* (20 - 52%), *Trichoderma* (4 - 40%), *Aspergillus, Fusarium* and *Rhizopus* (*fig. 1*). These genera are well-known for their wide spreading presence on almost all kind of soils from our area. We notice also, the appearance of the

micromycetes genera with cellulolytic and pectinolytic activity (*Trichoderma*). Important places in all variants have micromycetes known as antagonists, which might be a cause for the decreasing of microbial activity in some cases.

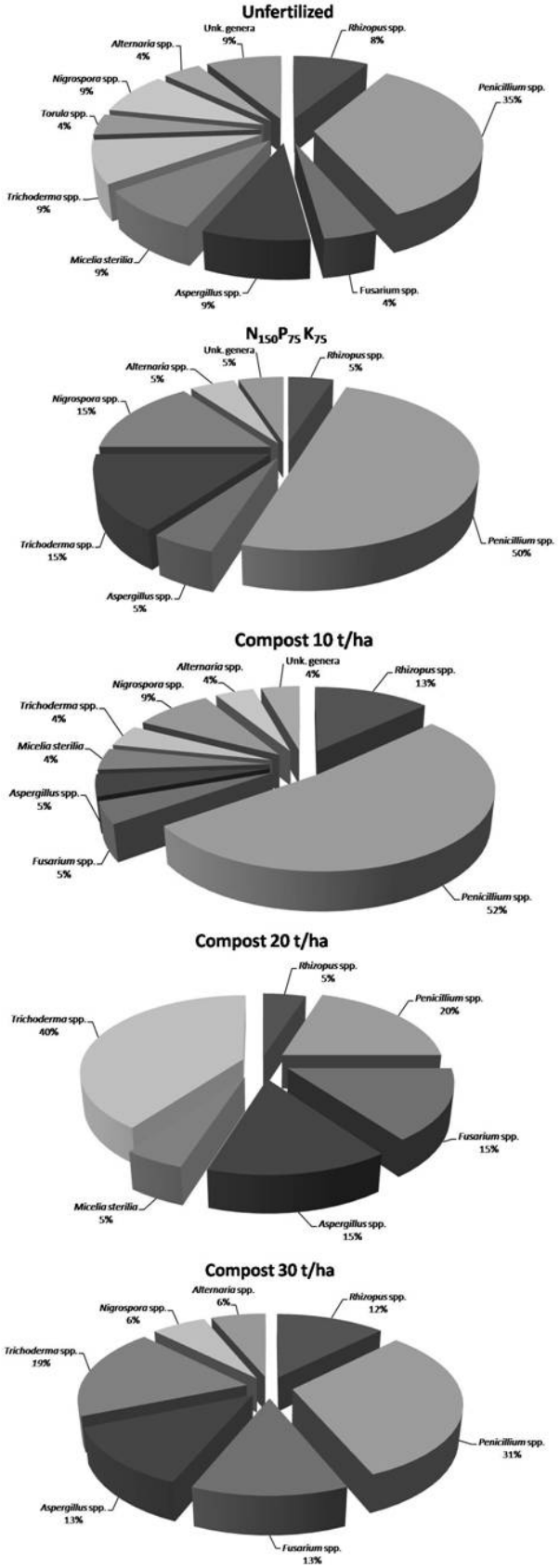

Figure 1 **Micromycetes genera isolated during this study with or without fertilization**

The reducing number of soil fungi genera in case of all the fertilized variants compare to control variant, may be explained by their intern competition or against bacteria and plants for some nutrients. Increasing the soil concentration in nutrients, due to fertilizers (especially, nitrogen and phosphorus fertilizers), will be favourable to some fungi genera and will determine their population exponential increasing (Ulea, E., Samuil, C., 1999,. Wood, M., 1989)

The presented results show that the compost applied on maize fields has in general, a positive influence on soil microflora structure and biological activity, which can be compared with the mineral elements influence. Exception: quantity of 30 t/ha compost caused a reduction in the number of microorganisms and soil microbial activity.

In case of control variant were isolated species from one unknown plus nine identified soil fungi genera: *Penicillium*, *Aspergillus*, *Fusarium*, *Alternaria*, *Rhizopus*, *Micelia sterillia, Torula, Nigrospora* and *Trichoderma*. The lowest number of genera was observed in the sample analyzed from the field fertilized with 20 t/ha compost. The identified genera were *Trichoderma*, *Penicillium*, *Aspergillus*, *Fusarium*, *Micelia sterillia* and *Rhizopus*. The difference may be also explained by the selection of some specific fungus genera, according to the biochemical features of root secretions (Yevdokimov, I., Gattinger, A., Buegger F., Munch, J.C., Schloter, M., 2008 9. Zarnea, G., 1994).

CONCLUSIONS

The current paper presents several experimental results achieved by using either mineral ($N_{150}P_{75}K_{75}$) or organic products (10, 20 and 30 t/ha compost) for fertilization of maize from Ezareni Farm in the period 2009-2010.

The aim of this work consisted in isolating and quantifying the soil microbial population (Gram-positive bacteria, Gram-negative bacteria and mycromicetes) by determining the percentage of participation, the main genera and the level of their activity in each variant.

The microbial activity, compared to the unfertilized variant, has shown a significant increase in all the fertilized variants, with one exception. Quantity of 30 t/ha compost caused a reduction in the number of microorganisms and microbial activity in soil.

In the unfertilized and mineral fertilized variants, the G- bacteria represented the majority of the isolated microorganisms. Contrary, in the organic fertilized variants G+ bacteria was better

represented with a procentage between 51.5 and 69.1%.

The highest number of microorganisms/g soil was recorded in the variant fertilized with $N_{150}P_{75}K_{75}$.

In this trial, a diminution in the number of identified fungus genera at the fertilized variants, compared with the unfertilized control, was found.

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

The appearance of the antagonistic micromycetes like *Trichoderma* spp. was signaled in all fertilized variants.

These results evidence the possibility of compost usage in quantity of 20 t/ha as organic fertilizer in ecological farms for maize.

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