EFFECT OF LIGNOHUMATE (HUMIC FERTILIZER) ON SOIL MICROORGANISMS

Florin Daniel LIPŞA[1], Eugen ULEA[1], Evelina Cristina MORARI[1], Daniel GALEŞ[1], Iulian Constantin ARSENE[1]
e-mail: flipsa@uaiasi.ro

Abstract

Lignohumate is a humic fertilizer obtained through oxidation/hydrolytic destruction of lignin-containing raw materials as brown coal and peat. This compound contains both macro- and microelements, and can be applied in combination with other fertilizers or plant protection products as growth stimulant agent. The objectives of these trials were to evaluate the influence of Lignohumate on soil microorganism activity (Gram positive bacteria, Gram negative bacteria and micromycetes) and structure of microbial populations in case of two species: maize (*Zea mays* L.) and soybean (*Glycine max* Merr.). In addition to the basic fertilization on maize, Lignohumate was applied in two steps: as treatment of seed (100 g t^{-1}) and in combination with herbicide (60 g ha^{-1}) at 3-4 leaf stage. In case of soybean, seeds were treated with 100 g t^{-1} Lignohumate and second treatment was applied before flowering time (60 g ha^{-1}). The obtained results show that Lignohumate concentration stimulates growth and development of microfungi and bacteria in case of maize (*Zea mays* L.) with 54.8% and 39.0%, respectively. In case of soybean (*Glycine max* Merr.) the procentual growth was 146.0% for microfungi and 25.4% for bacteria.

Key words: Lignohumate, structure of microbial populations, soybean, maize

Lignohumate is a humic fertilizer obtained through oxidation/hydrolytic destruction of lignin-containing raw materials as brown coal and peat. This compound contains both macro- and microelements (sodium, potassium, calcium, sulphur, silicon, magnesium, iron, copper, manganese), and can be applied in combination with other fertilizers or plant protection products as growth stimulant agent.

Lignohumate applied at the foliar level leads to improved foliar nutrition of the plants, increases production, quality, capacity and energy for seed germination. In addition, it prevents stress generated by the treatment with pesticides, frost or drought effect on plants, improving growth, plant development and reducing the vegetation period.

Tugarinov et al. demonstrated that Lignohumate promotes growth and development of numerous groups of bacteria, such as *Pseudomonas*, *Agrobacterium*, *Flavobacterium*, *Bacillus* and *Arthrobacter*. In minor concentrations stimulates, particularly nitrogen-fixing bacteria. Highly-concentrated Lignohumate may inhibit growth of some bacteria species and act as a preserving agent.

The objectives of these trials were to evaluate the influence of Lignohumate on soil microorganism activity (Gram positive bacteria, Gram negative bacteria and micromycetes) and structure of microbial populations in case of two species: maize (*Zea mays* L.) and soybean (*Glycine max* Merr.).

MATERIAL AND METHOD

The trial was conducted with soybean (*Glycine max* Merr.) and maize (*Zea mays* L.) grown 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°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.

To assess the effect on soil microflora in case of maize (*Zea mays* L.) and soybean (*Glycine max* Merr.), Lignohumate was applied in addition to the basic fertilization. In case of maize, was applied in two steps: as treatment of seed (100 g t^{-1}) and in combination with herbicide (60 g ha^{-1}) at 3-4 leaf stage (V2P). In case of soybean (V2S), seeds were treated with 100 g t^{-1} Lignohumate and

[1] University of Agricultural Sciences and Veterinary Medicine Iaşi

second treatment was applied before flowering time (60 g ha^{-1}). Non-treated plots were used as a blank (V1P, V1S).

Soil sample were collected for each cultivated species four weeks after second treatment. 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 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 (G+), we have used the PDA with streptomycin (35 ppm) medium and for determining the number of micromycetes, we have used the PDA with rose bengal (33 ppm) medium (Constantinescu, 1974).

Sowing was done by introducing an 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 soil microflora in collected soil samples shown significant increases of soil biological activity in both variants were Lignohumate was applied.

A close examination of the biological activity from rhizosphere of maize (*Zea mays* L.) and soybean (*Glycine max* Merr.) show a great variability on soil microorganism activity and structure of microbial populations.

It should be noticed that the structure of microbial populations was different; thus, fungi from soybean treated with Lignohumate (V2S) represent 5.9% and overcome the untreated area (3.1%). Among bacteria, the G^- species overcome the G^+ species with 76.4% to 17.7% in case of V2S and with 75.0% to 21.9% in case of V1S.

In maize (*Zea mays* L.) cultivated soils with Lignohumate (V2P), the number of G^- bacteria increased from 64.9 to 75.7%, because of their ability to metabolize the herbicide and his major metabolites. The number of G^+ bacteria decreased from 31.8 to 20.6%. Micromycetes were present in range from 3.4 to 3.7% (Figure 1).

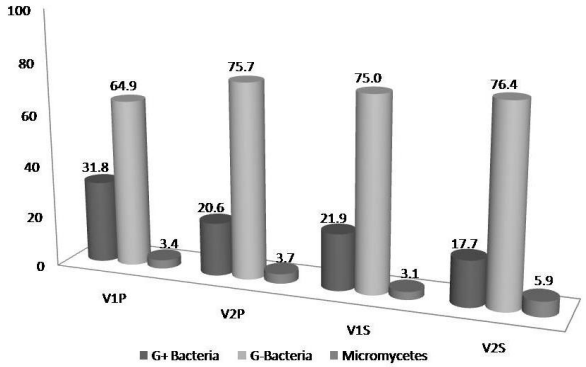

Figure 1 **Main groups of microorganisms/g soils for each experimented variant (%)**

The results interpretation about the biological activity on soybean (*Glycine max* Merr.) rhizosphere soils show that the microbial activity is more intense than of maize (*Zea mays* L.), and even when compared to the blanck, represented by non-treated soil specimen.

In case of soybean, the greatest number of microorganisms/g soil was determined in case of sample taken from the application area with Lignohumate (V2S) in compare to the blank variant (V1S). In case of variant with Lignohumate the number of microorganisms were 52.5 x 10^4 cells per one gram dry weight of soil in compares to 40.7 x 10^4 determined on blank soil sample. The same aspect appears in the case of fungi where the biological activity was three times bigger than the control sample.

The total number of microorganisms determined in maize rhizosphere ranged from 31.3 x 10^4 (blank soil sample - V1P) to 43.6 x 10^4 cells per one gram dry weight of soil (area treated with Lignohumate - V2P).

As noted in our experiment, and many other studies (Atlas et al., Lewis et al. 1978, Ulea et al. 2002), soil microorganisms generally react to xenobiotic substances by increasing their biomass and activity, although inhibitory effects have also been noted (Sawicka et al. 1996, Schuster et al. 1990).

We noticed that the number of isolated fungus genera in the all observed variants were not identical. The isolated species belonging to ten micromycetes genera: *Aspergillus*, *Fusarium Penicillium*, *Trichoderma*, *Rhizopus*, *Humicola Cladosporium*, *Alternaria*, *Acremonium*, and *Nigrospora*. Among the determined micromycetes in all the studied variants, we pointed out *Aspergillus* genus, which was isolated at a rate comprised between 22.0 and 61.8% of the total identified genera.

The identification of fungi genera which activates in the rhizosphere area of soybean shows

a relative small number of genera before Lignohumate application: *Aspergillus*, *Fusarium Penicillium*, *Trichoderma*, *Rhizopus* and *Alternaria*. After application of the humic fertilizer the number o genera increased with two: *Cladosporium* and *Nigrospora*.

However, the ratio between these groups is very different, with *Aspergillus*, *Fusarium* and *Penicillium* as dominant genera in both cases (V1S and V2S) with approximatively 84.0% from all fungi (Figure 2).

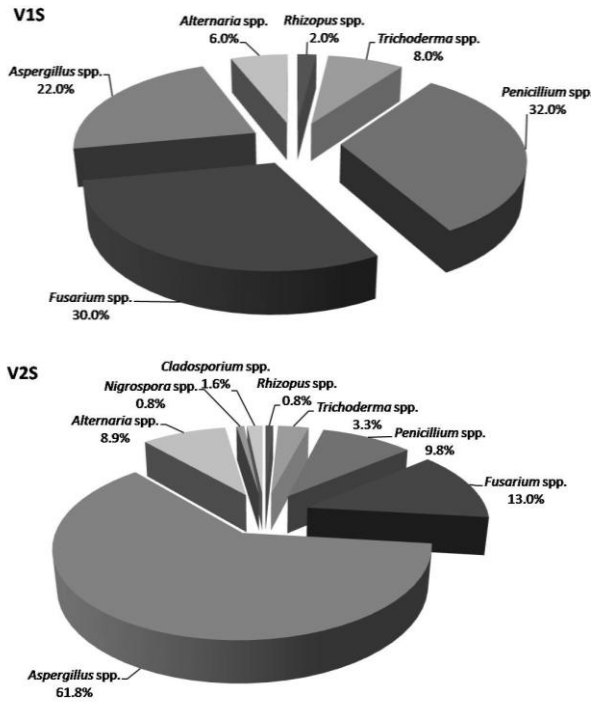

Figure 2 **Micromycetes genera isolated from soybean field for each variant (V1S and V2S).**

At maize treated with Lignohumate (*Zea mays* L.) the synthesis of results shows a fungal activity less intense than that of soybean, but greater than that of the control soil sample. Thus, the total number of fungi determined in the rhizosphere area was 1.6 x 10^4 cells per one gram dry weight of soil compared with 1.0 x 10^4 cells per one gram dry weight of soil founded in case of control sample.

The greatest number of fungal genera (eight) was determined in case of sample taken from the application area without Lignohumate (V1P). For the other variant (V2P) the number of fungi genera was almost equal (seven), but some lower in compare to the first variant (Figure 3).

The ratio between the variants with or without humic fertilizer is more equilibrated compared to soybean, with an advantage for *Aspergillus* spp. (47.7 vs. 38.1%) followed by the genera *Fusarium*, *Penicillium*, *Trichoderma*, *Rhizopus* and Alternaria (Figure 3).

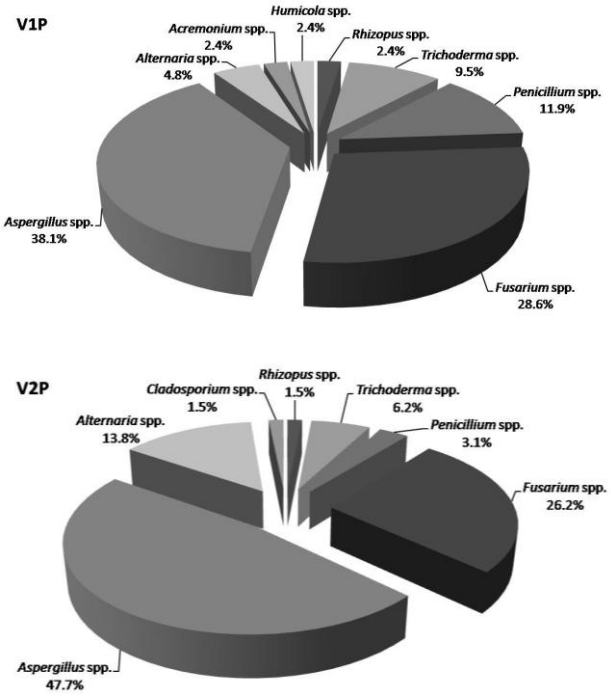

Figure 3 **Micromycetes genera isolated from maize field for each variant (V1P and V2P).**

The identification of fungi genera which activates in the rhizosphere area of maize without Lignohmate treatment shows a different spectrum of genera compares with the treated variant. In very small ratios were present the following micromycetes genera: *Acremonium* and *Humicola* (both 0.5%).

In generally, the investigations conducted on the frequency of micromycetes genera have shown an increasing number in the presence of Lignohumate.

CONCLUSIONS

Our observation on the total number of microorganisms/g in the sampling soils shown significant increases of soil biological activity in all variants were Lignohumate was applied.

Between the analyzed variants the highest microbial activity was recorded in the sampling soils collected from soybean (*Glycine max* Merr.) variant were the humic fertilizer was applied (V2S).

The biological soil activity in other three trials (V1P, V2P and V1S) was lower compared to the V2S variant.

In all the studied variants, from all the isolated micromycetes genera, *Aspergillus* spp. has the highest frequency; it was followed by,

Fusarium, *Penicillium*, *Trichoderma*, *Alternaria*, *Rhizopus* and *Nigrospora*.

In very small ratios and only in the rhizosphere area of maize without Lignohmate treatments (V1P) were isolated the following micromycetes genera: *Acremonium* and *Humicola*.

REFERENCES

Atlas R. M., Pramer D., Bartha R., 1978 - *Assessment of pesticide effects on non-target soil microorganisms*, Soil Biol. Biochem. 10, p. 231-239.

Lewis J. A., Papavizas G.C, Hora T.S., 1978 - *Effect of some herbicides on soil microflora*. Soil Biol. Biochem. 10, 137-143.

Constantinescu Ovidiu, 1974 - *Metode şi tehnici în micologie (Methods and techniques in mycology)*, Edit. Ceres, Bucureşti.

Eliade G., Ghinea L., Ştefanic G., 1975 - *Microbiologia solului (Soil microbiology)*, Edit. Ceres, Bucureşti. *microbiological characteristics of high-producing pastoral soil*, Biology and Fertility of Soils 6, p. 328-335.

Larpent J.P., Larpent-Gourgand M., 1990 - *Mémento technique de Microbiologie*, Lavoisier, Paris.

Schuster E., Schroder D., 1990 - *Side-effects of sequentially-applied pesticides on non-target soil microorganisms: field experiments*. Soil Biol. Biochem. 22, p. 367-371.

Tugarinov L. V. et al., 2009 - available from:www.amagro.com/content/file/Doklad_tug_cz. pdf.

Sawicka A., Skrzypczak G., Blecharczyk A., 1996 – *Influence of imazethapyr and linuron on soil microorganisms under legume crops*. Proceedings of the Second International Weed Control Congress. Copenhagen, vol. 1: 361-365

Ulea E., Ilisescu Isabela, Zaharia M., 2002 – *Influenţa unor erbicide asupra echilibrului microbiologic din sol*, Lucr. şt., seria Agronomie, vol. 45, U.Ş.A.M.V. Iaşi, p. 90-96.