ACETOCHLOR INFLUENCE ON SOIL MICROBIAL COMMUNITIES

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Abstract

The chloroacetanilide herbicide acetochlor [2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)-acetamide] was used to control the weeds on a maize (*Zea mays* L.) field experiment. Acetochlor was applied to soil at three different concentrations between 2.2 and 4.0 l/ha after sowing the crop. Before sowing a control soil sample was collected. At days 7, 14 and 21 after application soil sample were collected and analyzed to determine the herbicide effect on the microbial communities (Gram positive bacteria, Gram negative bacteria and micromycetes).
The objective of this research was to determine the influence of acetochlor on the total number of microorganisms, on the relationship between the main groups (bacteria and fungi), and on the micromycetes spectrum determined in each variant of our experiment.

Key words: herbicide acetochlor, *Zea mays* L., soil microbiota

Acetochlor is a selective systemic herbicide which is applied to the soil as a preemergence treatment to control most annual grasses and certain broadleaf weeds in maize, soybean and the duration of its effect is 8 to 15 weeks. It is compatible with most other pesticides and liquid fertilizers when used at recommended rates. Usually 10-15 mm of rainfall within 7-10 days of application is required to leach acetochlor into the root zone of the weeds (Rao, 2000; Hiller et al., 2009).

Soil, plant and climatic factors are known to affect the efficacy and activity of herbicides (). Soil factors that affect herbicide activity include temperature, moisture content, microorganisms, pH and organic matter (Franze and Zollinger, 1997 Rao, 2000; Schwab et al., 2006). High soil moisture content can enhance herbicide activity, while soil organic matter reduces activity by adsorbing the herbicide (Vasilakoglou, 2001; Steckel et al., 2003). Soil microorganisms are one of the factors that affect herbicide degradation (Zimdahl and Clark, 1982; Rao, 2000).

The purpose of our investigation was to determine the effect of acetochlor on soil microflora and soil biological activity. Also, we analyzed the acetochlor influence on the total number of microorganisms, on the relationship between the main groups (bacteria and fungi), and on the micromycetes spectrum determined in each variant of our experiment.

MATERIAL AND METHOD

The trial was conducted with 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 P_2O_5, 314-336 ppm K_2O 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, herbicide Phoenix- active ingredient acetochlor - was applied as a preemergence treatment in three different concentrations: 2.2 l/ha (V1), 3.1 l/ha (V2) and 4.0 l/ha (V3). For microbiological analyses soil was collected at four dates, every seven days for a month. First time soil sample were collected before applying the herbicide. 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 DISCUSSIONS

The analysis of the total number of microorganisms in the sampling soils, before (the control soils) and after herbicide application, shown significant increases of soil biological activity in all variants were acetochlor was applied.

The greatest number of microorganisms/g soil was determined in case of sample taken from the application area with 40% acetochlor increased rates (V2). For other two variants, recommended (V1) and plus 80% (V3) herbicide rates, the biological activity was almost equal, and some lower in compare to the first variant. In case of variant with 40% acetochlor increased rates (V2) the number of microorganism ranged during one month from 21.2 x 10^4 to 72.8 x 10^4 cells per one gram dry weight of soil (*figure 1*).

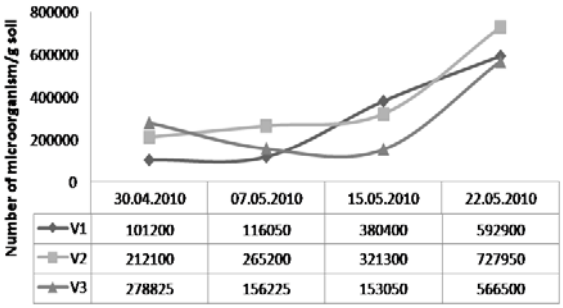

Figure 1 **Number of microorganism/g soils for each herbicide variant and date of application**

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

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

The best represented microorganism group for all variants and sampling period is that of Gram-negative bacteria (G-).

In case of recommended dose (V1), G- bacteria represent between 62.1 and 84.6% from total number of microorganism. One week after herbicide application the number of G+ bacteria was very low and starts to increase again after 21 days. This can be explained through the accommodation period needed for some microbial population to the new condition. The numbers of micromycetes ranged from 2.0 to 12.1% (*figure 2*).

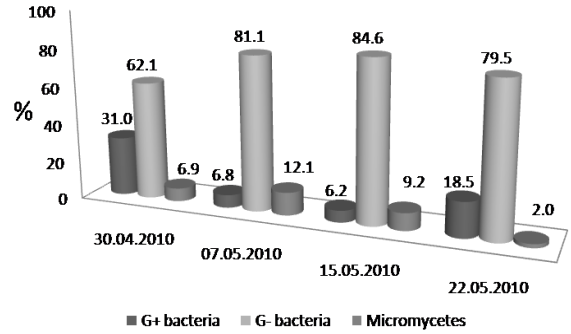

Figure 2 **Main groups of microorganisms measured during a month for recommended acetochlor dose (V1)**

In the soil where acetochlor was applied in increased rates (plus 40%; V2) the number of G- bacteria decreased during the first week from 92.5 to 53.3% and increase until the end of the observed period to 84.1%. The number of G+ bacteria increased during the first seven days from 3.7 to 41.4%, because of their ability to metabolize the herbicide and his major metabolites.

Micromycetes were present in range from 3.8 to 7.2% (Figure 3).

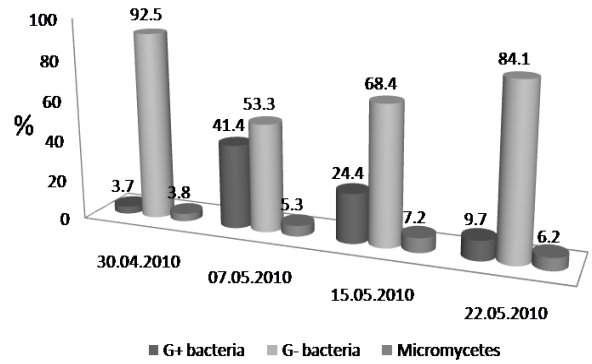

Figure 3 **Main groups of microorganisms measured during a month for +40% increased acetochlor dose (V2)**

In case of recommended acetochlor dose plus 80% (V3), the percent of G- bacteria ranged

from 61.6 to 94.3%. The content in mycromycetes is variable with value between 2.9 and 33.3% (*figure 4*).

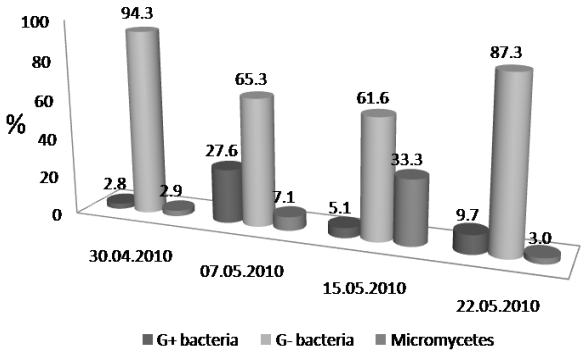

Figure 3 **Main groups of microorganisms measured during a month for +80% increased acetochlor dose (V3)**

Soil-applied herbicides can be lost through microbial degradation whereby the herbicide is broken down by microorganisms present in the soil. This occurs when microorganisms such as fungi and bacteria use the herbicide molecule as a food source. Conditions that favour microbial growth will result in faster degradation of the herbicide, leading to reduced persistence. Factors favouring microbial growth include warm temperatures, favorable pH levels, adequate soil moisture, oxygen and fertile soils (Rao, 2000).

Vasilakoglou and Eleftherohorinos (2003) reported that microbial degradation is the most important factor affecting the activity and dissipation of the acetochlor in soil.

The investigations conducted on the frequency of micromycetes genera have shown a diminution their number with increasing of acetochlor rates (Bontea, 1986; Gilman, 1959).

We noticed that the number of isolated fungus genera in the all three observed variants were identical. The isolated species belonging to six micromycetes genera (*Penicillium*, *Trichoderma*, *Rhizopus*, *Fusarium* and *Aspergillus*, *Nigrospora*).

Among the determined micromycetes in all the studied variants, we pointed out *Penicillium* genus, which was isolated at a rate comprised between 22.2 and 91.9% of the total identified genera for V1, between 21.4 and 94.3% for V2 and also, ranged from 22.2 to 90.0% for V3, respectively (*figure 4*).

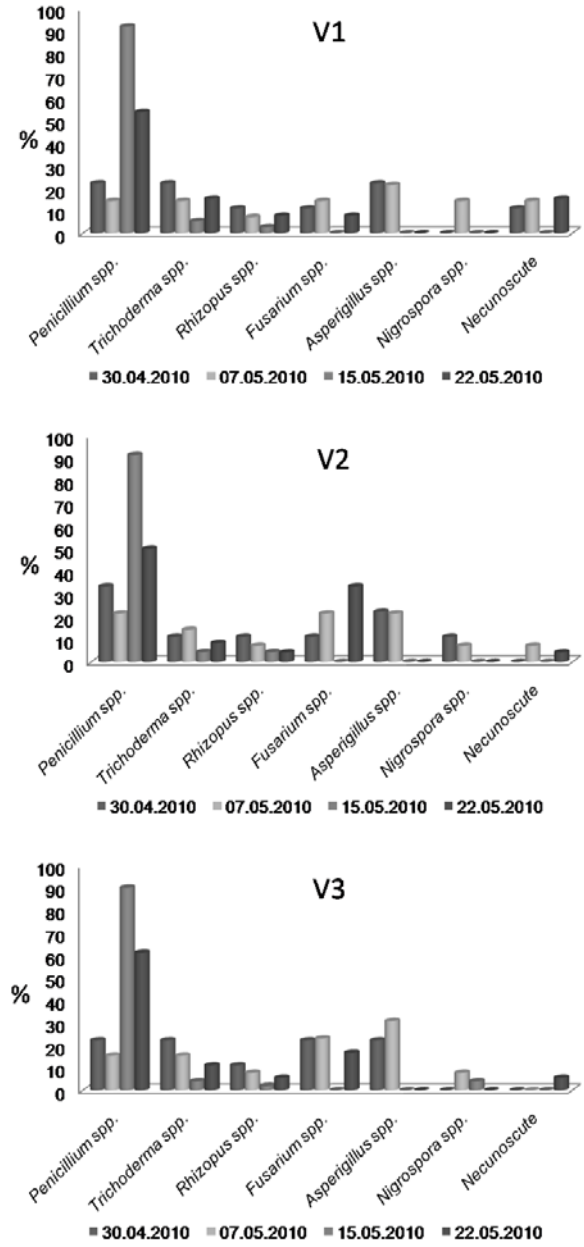

Figure 4 **Micromycetes genera isolated during the observation period for each variant (V1, V2 and V3)**

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 acetochlor was applied.

Between the analyzed variants the highest microbial activity was recorded in the sampling soils from the variant were the herbicide acetochlor was applied in increased rates (plus 40%; V2).

The biological soil activity in other two trials (V1 and V3) was lower compared to the second variant.

In all the studied variants, from all the isolated micromycetes genera, *Penicillium* spp. had

the highest frequency; it was followed by, *Trichoderma*, *Rhizopus*, *Fusarium*, *Aspergillus* and *Nigrospora* genera.

BIBLIOGRAPHY

Bontea, Vera, 1986 - *Ciuperci parazite şi saprofite din România (Parasite and saprophyte fungi from Romania)*.

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.

Gilman, J., 1959 – *A Manual of Soil Fungi*, The Iowa University Press, Iowa, USA.

Hiller, E., Krascesenit, S.Z., Čňeranský, S., Milička J., 2009 - *Effect of soil and sediment composition on acetochlor sorption and desorption*, Environ. Sci. Pollut. Res. 16, p. 546 - 554.

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

Papacostea, P., 1976 - *Biologia solului (Soil biology)*, Edit. Ştiinţifică şi Enciclopedică, Bucureşti.

Rao, V.S., 2000 - *Principles of weed science***.** 2nd edn, Science publisher.Inc, USA.

Varma, S., Abbott, L., Werner, D., Hampp, R., 2004 - *Plant surface microbiology*, Springer Verlag Berlin, Germany.

Vasilakoglou, I.B., Eleftherohorinos, G.I., 2003 - *Persistance, efficacy and selectivity of amide herbicides in corn***,** Weed Technol. 17, p. 381-388.

Wood, M., 1989 - *Soil Biology*, Blackie and Son et al, London a Saint-Amand.C

Zarnea, G., 1994 - *Tratat de Microbiologie generală (Treatise of general microbiology)*, Edit. Academiei Române, Bucureşti, vol. V.

Zimdahl, R.L., Clark, S.K., 1982 - *Degradation of three acetanilide herbicides in soil***.** Weed Sci. 30, p. 545 - 548.