EFFECT OF HERBICIDE S-METOLACHLOR ON SOIL MICROORGANISMS

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Abstract

The chloroacetanilide herbicide S-metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(1S)-2-methoxy-1-methylethyl]-acetamide] is an important selective product used for the control of several annual grass weeds and certain broad-leafed weeds in soybean (*Glycine max* Merr.).
This paper studied the effects of different concentrations S-metolachlor (1.5, 2.1 and 2.7 kg/ha), an enantiomer of metolachlor, on soil microorganism activity (Gram positive bacteria, Gram negative bacteria and micromycetes).Before sowing a control soil sample was collected. At days 7, 14 and 21 after herbicide application, soil sample were collected and analyzed to determine the herbicide effect on the structure of microbial populations. The influence of S-metolachlor on the total number of microorganisms, relationships between the main groups (bacteria and fungi) and the micromycetes spectrum from our experiment were established.

Key words: herbicide S-metolachlor, *Glycine max* Merr., soil microbiota

The acetanilide herbicide S-metolachlor (Dual Gold 960 EC) is registered for control of most annual grasses and certain broadleaf weeds in soybean (*Glycine max* Merr.).

Soil fertility often depends on the balance of the diferent genera of soil fungi and other soil inhabiting microorganisms whose activities determine the efficiencies of the various metabolic cycles (nitrogen, carbon, minerals). It is clear that the addition to the soil of any potentially toxic herbicide constitutes a threat to this equilibrium and hence to the future fertility of the soil (Edwards, 1989; Moorman, 1989). The equilibrium can be altered by direct toxic action on microorganisms in the soil, by selective toxicity for certain groups of microorganisms thus indirectly altering the population equilibrium, or by promoting the growth of one or more types of soil organism. These depressive or stimulating effects depend upon the kind of chemical and its concentration, possibly moderated by environmental conditions (Moorman, 1989).

The purpose of our investigation was to determine the effect of S-metolachlor on soil microflora and soil biological activity. Also, we analyzed the S-metolachlor 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 soybean (*Glycine max* Merr.) 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^oC, 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 Dual Gold 960 EC - active ingredient S-metolachlor - was applied as a preemergence treatment in three different concentrations: 1.5 kg/ha (V1), 2.1 kg/ha (V2) and 2.7 kg/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

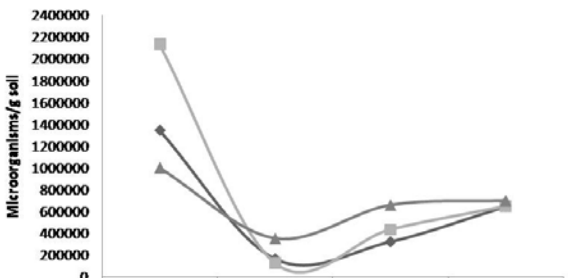
(G+), we have used the PDA with streptomycin (35 ppm) medium and for determining the number of micromycetes, we have used the PDA with rose bengal (33 ppm) medium (Constantinescu, 1974).

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 populations of soil fungi and bacteria were affected by treatment S-metolachlor. The analysis of the total number of microorganisms in the sampling soils, before (the control soils) and after herbicide application, shown significant decreases of soil biological activity in all variants were herbicide was applied. An increase of metolachlor with 40 (V2) and 80% (V3) showed no additional reduction of microbial population.

Initially, the greatest number of microorganisms/g soil was noted under recommended S-metolachlor dose and lower, but almost equal for both increased doses. At the end of experimental period microbial population in all three variants were almost equal. In case of recommended dose the number of microorganism ranged during one month from 134.45 x 10^4 to 64.42 x 10^4 cells per one gram dry weight of soil (*fig. 1*).



	30.04.2010	07.05.2010	15.05.2010	22.05.2010
V1	1344500	164150	320300	644150
V2	2132500	124750	431900	647000
V3	1000025	352550	658700	698950

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

As noted in our experiment, and other studies (Sawicka et al., 1996, Schuster et al., 1990), herbicide could have inhibitory effects on soil microorganisms, but generally the microbial populations react by increasing their biomass and activity (Atlas et al. 1978, Lewis et al., 1978, Ulea et al., 2002).

Analyzing the ratio between the main groups of microorganisms found in the soil occupied by soybean 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 69.8 and 92.6% from total number of microorganism. After herbicide application the numbers of G+ bacteria start to increase until the end of our experiment. The numbers of micromycetes ranged from 0.7 to 9.1% (*fig. 2*).

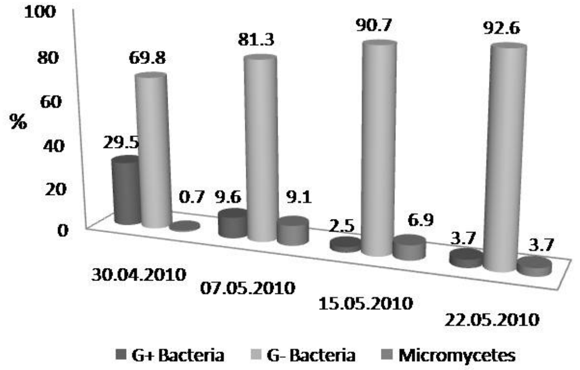


Figure 2 **Main groups of microorganisms measured during a month for recommended S-metolachlor dose (V1)**

In the soil where S-metolachlor was applied in increased rates (+40%; V2) the number of G- bacteria decreased during the first week from 94.8 to 81.8% and increase until the end of the observed period again to 94.6%. This can be explained through the accommodation period needed for some microbial population to the new condition. The number of G+ bacteria increased during the first seven days from 4.6 to 12.6%, because of their ability to metabolize the herbicide and his major metabolites. Micromycetes were present in range from 0.6 to 5.6% (*fig. 3*).

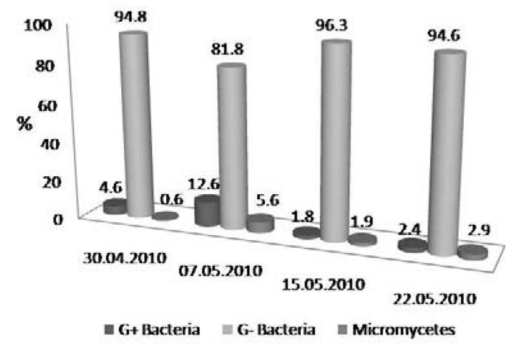


Figure 3 **Main groups of microorganisms measured during a month for +40% increased S-metolachlor dose (V2)**

In case of recommended S-metolachlor dose plus 80% (V3), the percent of G- bacteria ranged from 85.7 to 96.6%. The content in mycromycetes is variable with value between 0.7 and 4.3%. (*fig.4*).

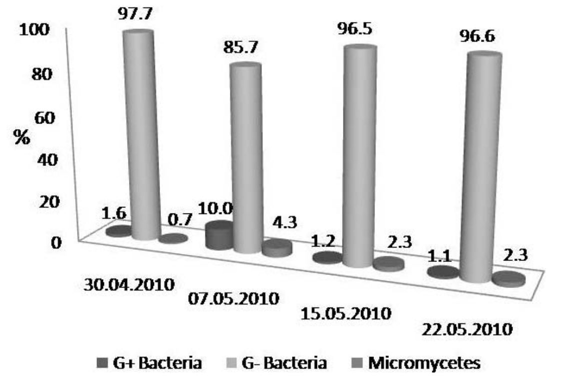
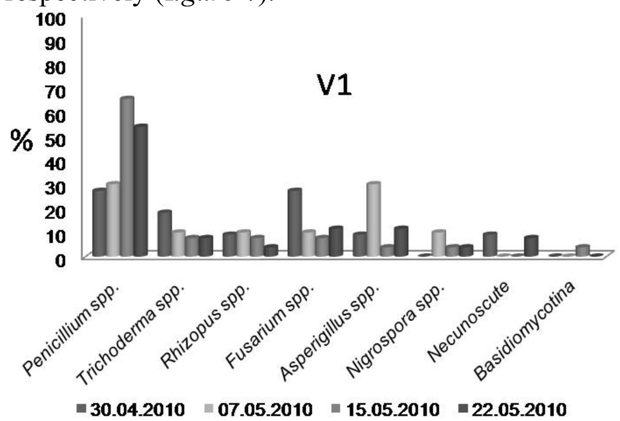


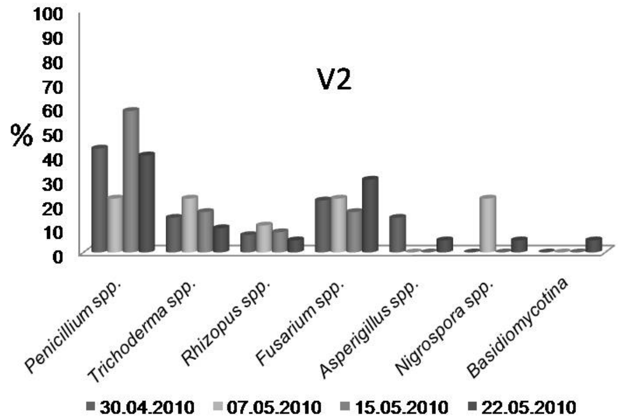
Figure 3 **Main groups of microorganisms measured during a month for +80% increased S-metolachlor dose (V3)**

Organic matter decomposition through microbial populations is one of the most important life processes occurring in the soil. Generally metolachlor gave greater reduction in fungal numbers in soil. The investigations conducted on the frequency of micromycetes genera have shown a diminution their number with increasing of S-metolachlor rates (Bontea, 1986; Gilman, 1959). Fungal population seemed to fluctuate but generally decreased following a pattern similar to that in the control. The results also show that metolachlor was more toxic to G+ bacteria than to G- bacteria.

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 Aspergillus* and *Nigrospora*). Interesting was the fact that with increasing herbicide doses the genus *Penicillium* reduced in favor of *Fusarium* spp.

Among the determined micromycetes in all the studied variants, we pointed out *Penicillium* genus, which was isolated at a rate comprised between 27.3 and 65.4% of the total identified genera for V1, between 22.2 and 58.3% for V2 and also, ranged from 20.0 to 50.0% for V3, respectively (*figure 4*).





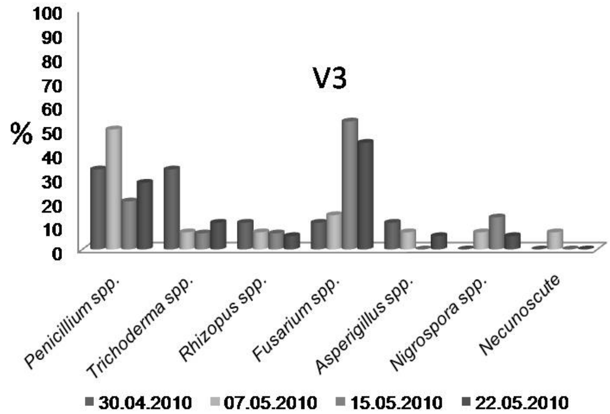


Figure 4 **Micromycetes genera isolated during the observation period for each variant (V1, V2 and V3)**

CONCLUSIONS

Our observation on the total number of microorganisms/g in the sampling soils shown dicreases of soil biological activity in all variants were S-metolachlor was applied. Between the analyzed variants, at the end of experimental period, the microbial activity was equal recorded in the sampling soils from the all variant were the herbicide S-metolachlor was applied.

Generally metolachlor gave greater reduction in fungal numbers in soil. Interesting also, was the fact that with increasing herbicide doses the genus *Penicillium* present reduced percentage from total fungi in favor of *Fusarium* spp.

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