

EFFECTS OF CHLORSULFURON ON SOIL MICROBIAL POPULATION

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Abstract

The sulfonylurea herbicide chlorsulfuron [2-chloro-N-[[[4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-benzenesulfonamide] is effectively used in wheat (*Triticum aestivum* L.) to control a wide spectrum of weeds and has low toxicity to humans and animals. Chlorsulfuron controls weeds by inhibiting acetolactate synthase (ALS), an enzyme required for leucine, valine and isoleucine biosynthesis in plants and microorganisms. Tolerance of wheat to chlorsulfuron is based on its ability to metabolize this substance to herbicidally inactive conjugate. Different concentrations of chlorsulfuron ranged from 31.5 to 56.7 mg/l were used and their effect on the soil microflora was investigated. The influence of chlorsulfuron on the existing microbial population in soil (Gram positive bacteria, Gram negative bacteria and micromycetes) was measured every seven days for a month.

The objective of this research was to determine the influence of chlorsulfuron 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 chlorsulfuron, wheat, soil microbiota.

Chlorsulfuron is a sulfonylurea herbicide that controls select broadleaf weeds and undesirable grasses in wheat fields and contributes to increased yield and improved quality. Frequently, herbicides not only affect plant growth but also have a detrimental effect on soil microorganisms, growth and metabolism.

Some studies have evaluated the rate of chlorsulfuron degradation. Chlorsulfuron degraded rapidly in a silt loam soil (pH 6.4) at 25°C under aerobic laboratory conditions, with estimated DT50 and DT90 values of 20 and 50 days, respectively (Rapisarda et al., 1982). Microbial processes appeared to be most important in the period immediately following application, but then became less important to the dissipation mechanism, especially under field conditions (Joshi et al., 1985). The persistence of chlorsulfuron increased with increasing soil depth, which can be attributed to the decline in the microbial population down the profile (Sarmah et al., 1998).

The objective of this research was to determine the chlorsulfuron influence on the total number of microorganisms and on the relationship between the different soil microbial populations (Gram negative and positive bacteria, micromycetes). Also, the micromycetes spectrum for each variant of our experiment was determined.

MATERIAL AND METHOD

The trial was conducted with wheat (*Triticum aestivum* L.) grown on a 2-3% slope field from the Ezăreni Farm, which belongs to the University of Agricultural Sciences and Veterinary Medicine, Iași. 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, herbicide Glean - active ingredient chlorsulfuron - was applied after emergence in three different concentrations: 31.5 mg/l (V1), 44.1 mg/l (V2) and 56.7 mg/l (V3). For microbiological analyses soil was collected at four dates, every seven days for a month. First time soil samples 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 (Larpen 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 chlorsulfuron was applied.

The greatest number of microorganisms was noted under recommended chlorsulfuron dose and lower, but almost equal for both increased doses. In case of recommended dose the number of microorganism ranged during one month from 19.54×10^4 to 82.74×10^4 cells per one gram dry weight of soil (figure 1).

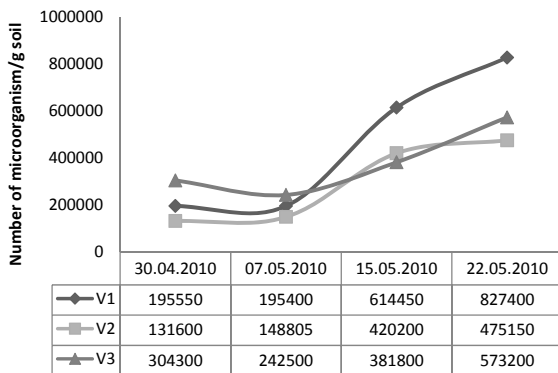


Figure 1 Number of microorganism/g soils for each herbicide variant and date of application

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 48.2 and 96.8% from total number of microorganism. Interesting was the observation that one week after herbicide application the number of G- bacteria was very low and equal with number of G+ bacteria. This can be explained through accommodation for a little period to the new condition. The numbers of micromycetes go lower during observation period and ranged from initially 7.7 to 1.3% (figure 2).

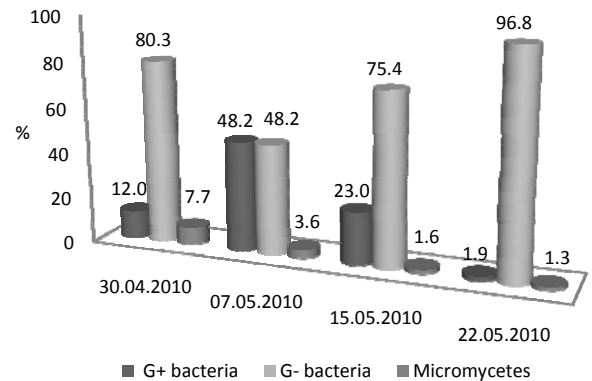


Figure 2 Main groups of microorganisms measured during a month for recommended chlorsulfuron dose (V1)

In the soil where chlorsulfuron was applied with +40% increased dose (V2) the number of G-bacteria decreased continuously from 89.5 to 59.5%. The number of G+ bacteria increased from 6.0 to 38.0%, because of their resistance and ability to metabolize the herbicide and his degradation compounds. Micromycetes were present in range from 2.5 to 8.7% (figure 3).

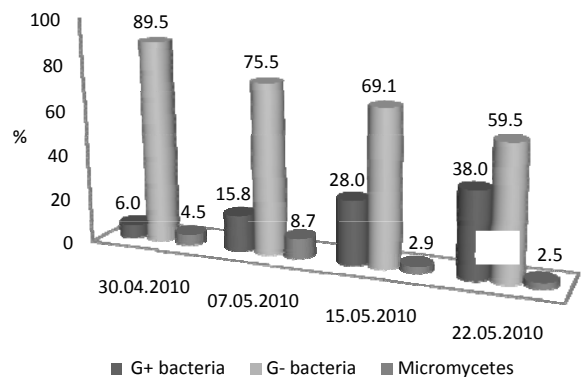


Figure 3 Main groups of microorganisms measured during a month for +40% increased chlorsulfuron dose (V2)

In case of recommended chlorsulfuron dose plus 80% (V3), the percent of G- bacteria ranged from 67.1 to 96.6%. The content in micromycetes was very low, with value between 1.3 and 2.9%. The very low content could be explained through the toxicity of herbicide. In this variant the number of present micromycetes genera was also the lowest in compare to another two variant (fig.4).

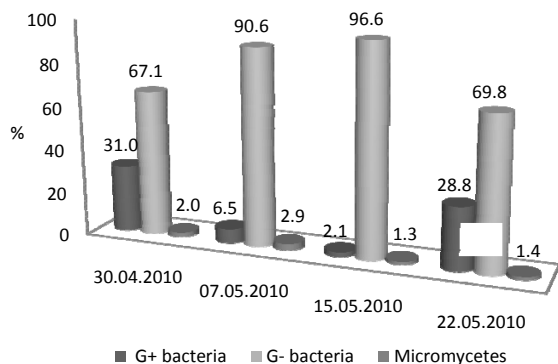


Figure 3 Main groups of microorganisms measured during a month for +80% increased chlorsulfuron dose (V3)

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 chlorsulfuron in different doses.

The high rate of soil bacteria in all variants may be explained by their competition against microfungi as concerns some nutrients, and by increasing the soil concentration in degradation compounds of chlorsulfuron, which determined an exponential increase in the bacterial populations (Ulea et al., 2002; Wood, 1989).

The investigations conducted on the frequency of micromycetes genera have shown a diminution their number with increasing of chlorsulfuron doses (Bontea, 1986; Gilman, 1959). We noticed that the number of isolated fungus genera in the variant with recommended chlorsulfuron dose (V1) was higher than in case of variants with increased doses (V2 and V3). In V1 we have isolated species belonging to seven micromycetes genera (*Penicillium*, *Trichoderma*, *Rhizopus*, *Fusarium*, *Aspergillus*, *Nigrospora* and *Verticillium*), while in V2 and V3 their number were reduced to six (no species of *Verticillium* spp.).

Among the determined micromycetes in all the studied variants, we pointed out *Penicillium* genus, which was isolated at a rate comprised between 10 and 52.9% of the total identified genera for V1, between 7.7 and 57.1% for V2 and

also, ranged from 20 to 44.4% for V3, respectively (Figure 4).

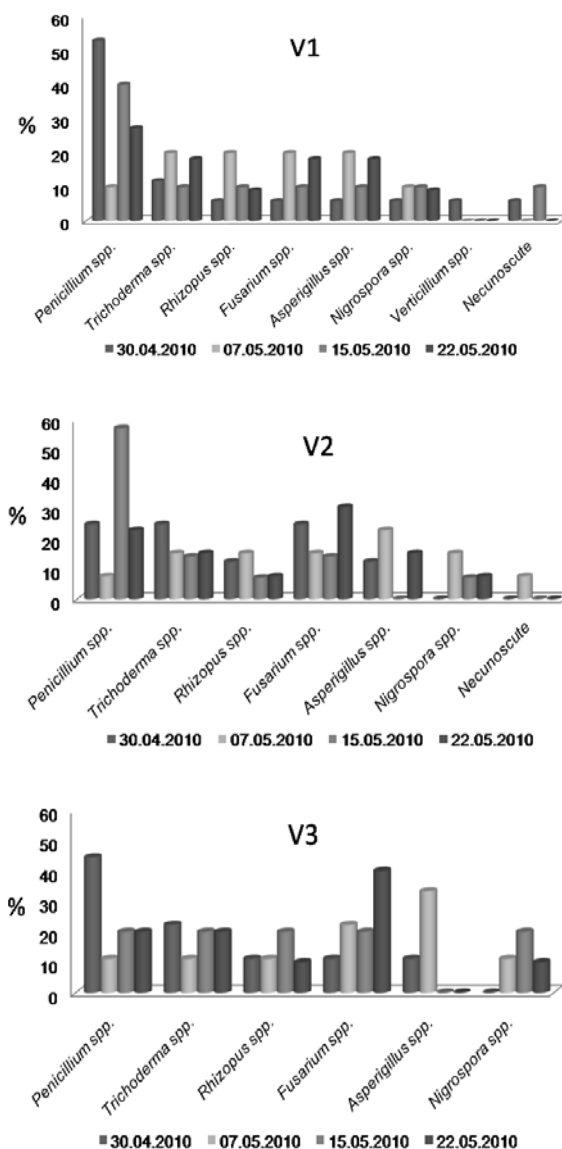


Figure 4 Micromycetes genera isolated during the observation period for each variant (V1, V2 and V3)

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 chlorsulfuron was applied.

Between the analyzed variants the highest microbial activity was recorded in the sampling soils from the variant were the herbicide chlorsulfuron was applied in the recommended dose (V1).

The microbial activity in both trials with higher doses of chlorsulfuron (V2 and V3) was lower compared to the first 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.

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