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THE EFFECT OF SULPHONYLUREA HERBICIDES ON THE MICROBIAL ACTIVITY IN SOIL UNDER MAIZE

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ABSTRACT

Depeneding on the quality and quantity of herbicides and their mode of action and soil type, the number of different groups of microorganisms in soil increased or decreased.

Sulphonylurea herbicides belong to a group of pesticides which in doses of only a few grams per hectar are sufficient to control weeds. The mode of action of these herbicides is based on blocking the enzyme acetolactate synthase (ALS, EC 4.1.3.18) which is involved in the biosynthesis of amino acids valin, leucine and isoleicine, thus making them non-toxic for animals but very toxic for sensitive plants and microorganisms.

The aim of this investigation was to investigate the effect of Nicosulfuron and Rimsulfuron, sulphonylurea based herbicides, on the total number of bacteria, number of Azotobacter sp. and dehydrogenase activity in surrounding soil and rhizosphere under the maize crop.

The total number of bacteria and the number of Azotobacter showed a stronger reaction to herbicide application than did dehydrogenase activity. The negative effect was the most conspicuous seven days after herbicide application because microbiological degradation of herbicides was at its lowest at that time. The changes in microbial activity were greater in the rhizosphere than in the surrounding soil, as the number of micro-organisms and dehydrogenase activity were higher in it.

KEY WORDS:

sulphonylure herbicides, total number of bacteria, azotobacter, dehydrogenase activity, soil.

1. INTRODUCTION

Soil, with its physical and chemical properties, enables the growth and the development of different systematic and physiologic groups of microorganisms. Microbiological activity is one of the important factors in soil fertility. The number and enzymatic activity of micro-organisms could be a reliable indicator of potential and effective soil fertility during the vegetation period of different vegetables and crops [2].

Contemporary agricultural production uses different kinds of herbicides. Introduction of large quantities of herbicides in soil leads to perturbation in the normal flow of microbiological processes in soil, and consequently in the flow of nutrients and energy. Depeneding on the quality and quantity of herbicides and their mode of action and soil type, the number of different groups of microorganisms in soil increased or decreased [1].

Some microbial groups use herbicides as a source of energy and nutrients, whereas to other microorganisms herbicides may be toxic [5]. Micro-organisms in adverse ecological conditions and in soil with low quality decompose herbicides weakly, hence, this could have a toxic effect on sensitive plants.

Sulphonylurea herbicides belong to a group of herbicides which in doses of only a few grams per hectar are sufficient to control weeds. The mode of action of these herbicides is based on blocking the enzyme acetolactate synthase (ALS, EC 4.1.3.18) which is involved in the biosynthesis of amino acids valin, leucine and isoleicine, thus making them non- toxic for animals but very toxic for sensitive plants and micro-organisms.

Nicosulfuron and Rimsulfuron, sulphonilurea derivatives, are the active ingredients of herbicides Motivell and Tarot 25 WG which are used in maize crop to control annual graminea and broadleaf weeds, and some perenial broadleaf weeds.

The aim of this investigation was to investigate the effect of Nicosulfuron and Rimsulfuron on the total number of bacteria, number of *Azotobacter sp*. and dehydrogenase activity in the surrounding soil and rhizosphere under the maize crop.

2. MATERIAL AND METHODS

The experiment was conducted on the location of Rimski Šančevi, in the experimental fields of the Scientific Institute of Field and Vegetable Crops, near the city of Novi Sad (province of Vojvodina). The trial was conducted by random block system in four turns, in the calcerous chernozem soil type with the field size of 25 m^2 (5 x 5 m) in each turn. The field was planted with maize hybrid NS 640 on 23April, 2004. Herbicide treatments were performed when the maize was in 6-7 leaves growth stage and weeds in stage of 3-4 leaves, with shoulder sprayer and 250 – 300 l/ha of water (2,3 – 3,0 l/100 m^2). Two herbicides were used in the experiment in recommended doses for weed control in maize crop (table 1).

Surrounding soil samples for laboratory analyses were taken aseptic between maize rows at 0 – 10 cm depth and rhizospheric soil samples at 0,5 cm from maize root . The dynamics of sampling were 7, 14 and 28 days after herbicide application.

Table 1. Used herbicides, active ingredient (a.i.) and quantity

VARIANTS	HERBICIDE	A. I.	QUANTITY l or kg/ha	QUANTITY per 100m^2
1	CONTROL - WITHOUT HERBICIDE TREATMENT			
2	Motivell	Nicosulfuron	1,25	12,5 cm^3
3	Tarot 25 WG + Trend	Rimsulfuron	0,06 + 0,1 %	0,6g + 0,1 %

The number of microorganisms was estimated by standard microbiological methods, by planting an appropriate soil dilution in selective nutrient mediums [14]. The total number of bacteria was determined in soil extract agar from 10^{-7} soil dilution. The number of *Azotobacter sp*. was estimated on Fjodor selective agar from 10^{-2} soil dilution by fertile drops method. Dehydrogenase activity was measured spectrophotometrically [9, 13] and represented in □g of TPF per gram of air dry soil. The number of colony forming units (CFU) was counted and calculated per gram of absolute dry soil and the log of number were showen.

The results obtained in the experinemt were statistically analysed by two-way LSD test to identify significant differences between the treatments. The significance levels of 95% ($P < 0.05$) and 99% ($P < 0.01$) were selected and interaction between factors were calculated.

3. RESULTS AND DISCUSSION

The investigated herbicides had different effects on the fertility of soil under the maize crop. They had different effect on the number of microorganisms an on their enzymatic activity.

The total number of bacteria were represented by large-cell bacteria from soil wich could form colonies on agar media and had great ecological significance despite their low number [11].

Both applied herbicides significantly decreased the total number of bacteria in maize rhizosphere, while in the surrounding soil, no significant changes were observed seven days after the application (table 2).

TABLE 2. THE EFFECT OF HERBICIDES ON THE TOTAL NUMBER OF BACTERIA (LOG OF NUMBER g^{-1} SOIL)

VARIANTS	TIME OF SAMPLING AFTER HERBICIDE APPLICATION					
	7 davs		14 davs		28 davs	
HERBICIDES FACTOR A	FACTOR B		FACTOR B		FACTOR B	
	Soil	Rhizosphere	Soil	Rhizosphere	Soil	Rhizosphere
Control	9,43	10,07	9,50	9,87	9,83	9,97
Motivell	9,37	9,53	9,40	9,80	9,67	10,20
Tarot 25 WG	9,57	9,67	9,77	9,67	9,70	10,00
LSD	1%	5%	1%	5%	1%	5%
factor A	0,27	0,19	0,21	0,15	0,47	0,33
factor B	0,22	0,15	0,17	0,12	0,38	0,27
interaction AxB	0,38	0,27	0,30	0,21	0,66	0,47

With the variants where Nicosulfuron was applied, 14 days after the treatment, no negative effect in the rhizosphere was observed. Contrary to this, Rimsulfuron decreased the total number of bacteria in the rhizosphere, while in the surrounding soil, a significant increase of the number of this group of microorganisms was detected 14 days after herbicide application. Investigating the effects of a large number of herbicides on the number of microorganisms in wheat rhizosphere, Govedarica et al. (2000) got the similar results with variants where Triasulfuron + Dicamba was applied - the total number of microorganisms decreased 14 days after herbicide application.

The total number of bacteria did not change significantly regardless of the applied herbicide 28 days after the treatment.

Azotobacter sp. are the major group of soil aerobic N-fixing free-living bacteria that keep N cycling in the biosphere and have often been used for testing the effects of various environmental toxicants on the growth and nitrogen fixation [10]. The number of *Azotobacter sp.* significantlly decreased in the surrounding soil and rhizosphere seven days after the application of the both investigated herbicides (table3).

TABLE 3. THE EFFECT OF HERBICIDES ON THE NUMBER OF *AZOTOBACTER SP*. (LOG OF NUMBER g^{-1} SOIL)

VARIANTS HERBICIDES FACTOR A	TIME OF SAMPLING AFTER HERBICIDE APPLICATION					
	7 davs		14 davs		28 davs	
	FACTOR B		FACTOR B		FACTOR B	
	Soil	Rhizosphere	Soil	Rhizosphere	Soil	Rhizosphere
Control	4,27	4,17	4,07	4,03	3,97	3,57
Motivell	3,80	3,43	4,00	4,10	3,63	3,97
Tarot 25 WG	3,93	3,87	4,07	4,10	3,73	3,57
LSD	1%	5%	1%	5%	1%	5%
faktor A	0,30	0,21	0,26	0,18	0,27	0,19
faktor B	0,24	0,17	0,21	0,15	0,22	0,16
interaction AxB	0,42	0,30	0,37	0,26	0,39	0,27

In the field where Nicosulfuron was applied, the effect was powerful in rhizosphere, but not in the case where Rimsulfuron was applied. Regardless of the applied herbicide and the place of sampling (surrounding soil, rhizosphere), the number of *Azotobacter sp*. did not significantly change 14 days after the application. The negative effect of herbicide application on the number of this group of microorganisms disappeared 28 days after the treatment. At the same time, Nicosulfuron induced an increase of the number of *Azotobacter sp*. in maize rhizosphere.

Dehydrogenases are a group of enzymes that catalize the transfer of H^+ from donors to acceptors, deppending on O_2 availabilty. Their activity has been proposed as an indicator of soil fertility and soil biology [4]. Dehydrogenase activity in the surrounding soil in the field where Rimsulfuron was applied significantlly decreased in comparison with the

control and the rhizosphere seven days after herbicide application (table 4). With other variants there were no significant changes.

There was no difference in dehydrogenase activity 14 days after Nicosulfuron application.

TABLE 4. THE EFFECT OF HERBICIDES ON DEHYDRIGENASE ACTIVITY
(µg TPF/ g^{-1} soil)

VARIANTS HERBICIDES FACTOR A	TIME OF SAMPLING AFTER HERBICIDE APPLICATION					
	7 days		14 days		28 days	
	FACTOR B		FACTOR B		FACTOR B	
	Soil	Rhizosph.	Soil	Rhizosph.	Soil	Rhizosph.
Control	593,00	632,00	381,33	438,00	648,67	523,00
Motivell	600,67	588,67	430,00	414,33	653,33	438,33
Tarot 25 WG	712,67	581,67	460,33	377,67	577,33	573,33
LSD	1%	5%	1%	5%	1%	5%
factor A	93,08	65,44	73,74	51,84	119,72	84,17
factor B	76,00	53,43	60,21	42,33	97,75	68,73
interaction AxB	131,64	92,55	104,28	73,32	169,32	119,04

The application of Rimsulfuron caused a significant decrease of dehydrogenase activity in rhizosphere and a significant increase in the surrounding soil at the same time of sampling. Regardless of the applied herbicide, there was no change in dehydrogenase activity 28 days after the treatment. The activity of dehydrogenase was greater in rhizosphere where Nicosulfuron was applied, however, it was not observed in the field where Rimsulfuron was applied. Perucci et al. (1999) found that decreases in the dehydrogenase activity and increase in the global hydrolytic capacity, in comparation with the untreated soil control, were found at the higher Rimsulfiron dosages (10-fold and 100-fold higher concentration than recommende field dose).

4. CONCLUSION

The negative effect of the investigated sulphonylurea herbicides was the most conspicuous seven days after the aplication. Both preparations induced a statistically significant decrease of the total number of bacteria in the rhizosphere and the number of *Azotobacter sp*. in the surrounding soil and rhizosfere under the maize crop.

Dehydrogenase activity significantlly increased in the surrounding soil 7 and 14 days after the application of Rimsulfuron, while the effect of the same herbicide in rhizosphere was opposite 14 days after the treatment.

The total number of bacteria and the number of *Azotobacter sp*. were more sensitive to herbicide application than was dehydrogenase activity.

The changes in microbial activity were greater in the rhizosphere than in the surrounding soil under the maize crop.

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