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THE MICROBIOLOICAL ACTIVITY IN THE RHIZOSPHERIC SOIL UNDER THE SOYBEAN CROP AFTER THE APPLICATION OF HERBICIDES

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Microorganisms use herbicide molecules as sources of biogenic elements and energy for their physiological processes. This leads to an increase in microbial biomass, which indicates a stimulating effect of herbicides on part of a microbial population. However, microorganism populations without an enzymatic system of herbicide degradation are inhibited in their development, which leads to their reduced number and enzymatic activity.

We aimed to investigate the effect of herbicides on the dynamics of microorganism abundance in the rhizosphere. The following herbicides were applied: H_1- quizalafop-p-tefuril (Pantera) 48 g ha^{-1}; H_2 - oxasulfuron (Dynam) 60 g ha^{-1}; H_3 - imazethapyr (Pivot) 0.8 l ha^{-1}; H_4 - clomazone (Command) 0.75 l ha^{-1}; and H_5 - control (no herbicides).

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The herbicides applied on the average reduced the abundance of investigated parameters, mostly up to the 90th day, with the exception of actinomycetes, whose abundance grew as early as on the 14th day, while the abundance of fungi was raised after 30 days.

Key words: microorganism, rhizospheric soil, soybean, herbicides

INTRODUCTION

The contemporary maize growing practices imply a compulsory application of herbicides. Although there are many positive effects of this measure, there are some drawbacks. Weed flora has been changed and some species resistant to herbicides have spread (JANJIĆ, 1985). Rising amounts of herbicides per area unit have been used with little or no control of their effects on micro life in the soil. Soybean growing is not possible without the application of highly selective herbicides for efficient control of the most susceptible broad-leaf weeds (*Abutilon theophrasti, Amaranthus retroflexus, Ambrosia spp. Chenopodium album, Solanum nigrum, Datura stramonium* etc.), as well as, the most susceptible grass weeds, such as *Digitaria sanquinalis, Sorghum halepense* (from the seeds), *Setaria* spp.

Many herbicides that could be found on our market are not sufficiently tested, hence there is a great risk of disturbing microbiological processes in the soil (ANDERSON, 1978). The applied herbicides reach the soil and rhizosphere of cultivated plants and in a different way affect microbiological and microchemical processes in the soil via microorganisms that participate with 60-90% in the total metabolic soil activity (LEE, 1994). Considering their great activity and biodiversity, microorganisms are a very good bioindicator of all changes occurring in the soil, as their role in degradation of herbicides is irreplaceable. However, if microorganisms are not capable to decompose the whole amount of pesticides and their metabolites, then there is a danger of their accumulation in the soil, which could result in their getting into plants or underground waters. Decomposition in the soil lasts for a long time and without microorganisms it would be almost impossible. As microbes are very dynamic and sensitive to all changes, it is possible to recognise different effects of herbicides on microorganisms on the basis of microbial vigorous activities (GOVEDARICA *et al.*, 1995b, 1996; CERVELLI *et al.*, 1976). Effects of herbicides on soil microbes depends, first of all, on chemical and physical properties of applied compounds, their amounts/rates and the method of application, as well as, on climatic and soil factors.

MATERIAL AND METHODS

A five-replicate trial was set up in a randomised block design on slightly calcareous chernozem in the experimental field of the Maize Research Institute at Zemun Polje. All cropping practices were performed in due time and were of high quality. Prior to sowing, the soybean seeds were inoculated with

Nitragin microbiological fertiliser that includes highly efficient strains of *Bradyrhizobium japonicum.*

Four herbicides were applied within the crop control system in the following recommended rates:

H1- quizalafop-p-tefuril (Pantera) 48 g ha^{-1}

H2 - oxasulfuron (Dynam) 60 g ha^{-1}

H3 - imazethapyr (Pivot) 0.8 l ha^{-1}

H4 - clomazone (Command) 0.75 l ha^{-1}

H5 - control (without herbicides)

The herbicide Command was applied immediately after sowing, but prior to emergence, while the other herbicides were applied when the first soybean trifoliate leaf was developed.

All applied herbicides are selective wide spectrum herbicides that provide lasting protection to legumes against all more important broad-leaf and grass weeds.

Soil sampling for microbiological analyses was done from the soybean root rhizosphere. Samples were taken on the 3rd, 14th and 90th day upon the herbicides application. The dynamics of the abundance of microorganisms was determined on the basis of the following parameters: the total number of bacteria (UBB), distribution of *Azotobacter* (Az), aminoheterotrophs (AMN), abundance of actinomycetes (ACT), fungi (F) and dehydrogenase activity (DHA).

The abundance of the stated groups was determined by the thinning method on the selective nutritive media: UBB on the medium of the tyrpton soybean agar TCA (TCA=trichloroacetic acid), (POCHON and TURDIUX, 1962), AMN on mesopetptone agar, abundance of Az on the Fiodor agar by the method of "fertile caps", ACT on the Waksman-Carey agar, and F on the Chapek-Dox agar.

Oxidoreduction process, i.e. dehydrogenase activity DHA, was determined on the 90th day by the method of Thalman 1968, and based on the measurements of extinction intensity of triphenylformazane TPF that was produced by the reduction of triphenyl-phenol-tetrazolium chloride TTC (TTC=2,3,5-Triphenyl-2*H*-tetrazoilium chloride).

RESULTS AND DISCUSSION

Microorganisms, due to their abundance and enzymatic activity affect and response to all changes occurring in their environment, i.e. the soil. Therefore, it often might be mistakenly spoken of herbicide effects on microorganisms, as persistence of herbicides considerably depends on the vital activities of microorganisms, hence it can be spoken of the effects of microorganisms on herbicide persistence in the soil. Herbicide persistence depends not only on herbicides adsorption by the organic part of the soil and colloid clay, but also on the activity of microorganisms that have a very important role in herbicides metabolism.

Persistence of chemicals incorporated into soil depends on the environmental pH, soil type and structure, and also on the composition and

activities of microorganisms. Many scientists have studied this problem (MILOĆŠEVIĆ *et al.*, 1995, 1995b, 1996, 2000; HANCE, 1979; RADIVOJEVIĆ and STANKOVIĆ-KALEZIĆ, 2000), but due to certain difficulties a general conclusion cannot be drawn. The greater microbiological activities are the greater herbicide degradation is.

Providing that the total area cultivated with soybean amounts to 105,000 ha and that the annual use of herbicides amounts to 2.6 l ha^{-1} (STEPIĆ and DAKIĆ, 2000), and with a view to protecting the fundamental agricultural resources, a special attention has to be paid to the type of herbicides, i.e. to their a.i., as well as to the application rates.

Growth of microorganisms can be either inhibited or stimulated in the contact with herbicides (CERVELLI *et al.*, 1978.) The effects of applied herbicides on microbiological activities in the trial depended, first of all, on the type of herbicides and microorganisms, as well as on the period of the herbicides activity.

The total number of bacteria and their composition, as one of the parameters of total soil biogeny, affect the decomposition rate of herbicides. The results obtained (Table 1) point out to the downward trend of UBB up to the 90th day, with an increase in their number of only 1% at the end of the investigation period. The herbicide Command (H-4) alone reduced, on the average for all 90 days, the UBB distribution (16%), while other herbicides reduced the total number of bacteria by 1-9%.

Table 1. - Total number of bacteria 10^6 ha^{-1}

Herbicides Herbicidi	Herbicide activity period (in days) Vreme delovanja herbicida u danima								$\bar{X}$	
	3	I.N.	14	I.N.	30	I.N.	90	I.N.	No	I.N.
H-1	42.8	79	49.6	83	44.3	51	259.9	123	99.2	92
H-2	39.4	73	75.2	125	93.2	107	218.5	104	106.6	99
H-3	32.5	61	83.9	139	85.6	98	187.9	89	97.5	91
H-4	36.5	68	40.4	67	104.9	120	179.4	85	90.3	84
$\bar{X}$	37.8	71	62.2	104	82.0	94	211.4	101	-	-
Kontrola Control	53.5	100	60.0	100	87.4	100	209.9	100	107.7	100

I.N. - index level/indeksni nivo

The applied herbicides reduced the bacteria within the nitrogen cycle. First of all, the abundance of aminoheterotrophs, the group of bacteria that decompose protein molecules to peptones, peptides and amino acids by proteolytic enzymes, was reduced. Their abundance was, on the average, reduced up to the 14th day for all applied herbicides (23-30%), but their growth was stimulated by herbicides at the end of the investigation period (Table 2). The increase of their abundance on the 90th day amounted to 6%, which is however relatively small and slow. The herbicide Command (H-4) showed the greatest inhibitory effect, which agrees with the results obtained by MILOŠEVIĆ *et al.* (2000).

Table 2. - Abundance of aminoheterotrophs 10^6 ha^{-1}

Herbicides Herbicidi	Herbicide activity period (in days) Vreme delovanja herbicida u danima								$\bar{X}$	
	3	I.N.	14	I.N.	30	I.N.	90	I.N.	No	I.N.
H-1	18.7	54	47.2	79	97.3	106	249.2	109	103.1	100
H-2	7.83	23	46.9	78	92.8	101	245.1	107	100.1	97
H-3	30.3	89	42.5	71	96.5	105	234.6	103	100.9	97
H-4	12.8	38	31.2	68	82.7	90	242.5	106	92.3	89
$\bar{X}$	26.2	77	41.9	70	92.3	101	242.8	106	-	-
Control Kontola	34.1	100	59.9	100	91.8	100	228.1	100	103.5	100

I.N. - index level/indeksni nivo

Azotobacter is any of several soil bacteria important as nitrogen fixers that convert atmospheric nitrogen into forms available to plants and is a very important parameter for determination of soil biogeny. This group of bacteria is very sensitive to all changes occurring in the soil and respond to them by its abundance and vigorous activities. Hence, its abundance, as a responce to herbicide presence in soil, was reduced by 11-14% up to the 30th day after the application (Table 3). An increase of their abundance was registered no sooner than on the 90th day and only by 8%. The obtained results indicate that the application of clomazone and quizalafop-p-tefuril within the 90-day period affected the abundance reduction of this bacterial group by a great percentage of 21-19%. However, the herbicides oxasulfuron and imazethapyr stimulated the growth of *Azotobacter* from 15% to 17% at the end of the 90th day.

Table 3. Abundance of Azotobacter 10^4 ha^{-1}

Herbicides Herbicidi	Vreme delovanja herbicida u danima Herbicide activity period (in days)								$\bar{X}$	
	3	I.N.	14	I.N.	30	I.N.	90	I.N.	No	I.N.
H-1	20.8	86	12.3	84	62.4	80	76.4	76	43.5	79
H-2	30.1	114	13.1	89	70.3	91	139.4	138	63.2	115
H-3	12.1	45	15.1	102	92.1	119	125.9	124	64.1	117
H-4	26.5	100	11.8	79	42.6	54	97.2	96	44.3	81
$\bar{X}$	22.8	86	13.7	89	66.6	86	109.5	108	-	-
Control Kontrola	26.5	100	14.7	100	77.4	100	100.9	100	54.9	100

I.N. - index level/indeksni nivo

The applied herbicides lowered the number of fungi up to the 14th day by 10-14% on the average. After this period, they had a stimulatory effect (Table 4). The clomazone application inhibited the fungal growth up to the 30th day on the average. The highest inhibitory effect was recorded on the 14th day, hence the application of this herbicide resulted in the reduction of fungi abundance at the end of the investigation period.

Table 4. Abundance of fungi 10^4 ha^{-1}

Herbicides Herbicidi	Herbicide activity period (in days) Vreme delovanja herbicida u danima								$\bar{X}$	
	3	I.N.	14	I.N.	30	I.N.	90	I.N.	No	I.N.
H-1	6.17	87	8.64	122	6.04	137	7.35	138	7.05	110
H-2	4.82	68	4.82	68	7.36	167	5.83	108	5.71	89
H-3	9.64	136	8.43	119	3.86	87	7.25	135	7.29	114
H-4	4.70	66	2.35	33	3.67	83	5.72	106	5.61	87
$\bar{X}$	6.33	90	6.06	86	4.73	107	6.53	121	-	-
Control Kontrola	7.06	100	7.05	100	4.41	100	5.38	100	6.41	100

I.N. - index level/indeksni nivo

Actinomycetes similarly to the previously described group of microorganisms, showed an upward trend after the 14th day of the herbicide application. On the average for the whole period of investigation, the application of clomazone and imazethapyr inhibited the growth of actionmycetes by a great percentage (29% on the 3rd and 14th day after application). The number of actinomycets reached maximum on the 30th day (Table 5).

Based on the presented results it can be observed that certain groups of microorganisms degrade herbicides a few days after their applications (14 days in our study). Only the very susceptible microbial population was inhibited after an adaptation period that differed depending on the toxicity of herbicides.

Table 5. Abundance of Actinomycetes 10^4 ha^{-1}

Herbicides Herbicidi	Herbicide activity period (in days) Vreme delovanja herbicida u danima								$\bar{X}$	
	3	I.N.	14	I.N.	30	I.N.	90	I.N.	No	I.N.
H-1	48.1	61	95.6	112	38.1	123	77.9	123	64.9	117
H-2	31.3	40	73.5	149	50.0	162	84.5	133	59.8	107
H-3	65.1	82	25.6	52	30.4	98	70.6	111	47.9	86
H-4	56.5	71	35.4	71	34.7	113	75.9	119	50.6	91
$\bar{X}$	50.2	64	57.5	116	38.3	124	74.2	117	-	-
Control Kontrola	79.0	100	49.4	100	30.8	100	63.5	100	55.7	100

I.N. - index level/indeksni nivo

Besides the total number of bacteria and abundance of *Azotobacter*, dehydrogenase activity is also a very important indicator of soil biogeny. The applied herbicides affected the DHA reduction at the end of the 90-day investigation period, which correlates to results on the abundance of UBB and Az (Table 5). The greatest reduction of dehydrogenase activity was registered after the application of clomazone (H-4) (37.85%) and imazethapyr (H-3) (25.17%).

Table 6. Dehydrogenase activity

Herbicides Herbicidi	μg10 TPF g^{-1} soil/zemljišta	DHA Index level - Indeksni nivo
H-1	513	89.06
H-2	420	72.92
H-3	431	74.83
H-4	358	62.15
$\overline{X}$	455	78.99
Control - Kontrola	576	100

CONCLUSION

The microbiological activity in the rhizosphere of the soil under soybean crop after herbicide application depended on the type of herbicide, activity period, as well as on the group of microorganisms.

The applied herbicides reduced on the average the abundance of observed parameters mostly up to the 90th day, with the exception of aminomycetes and fungi, whose abundance increased after the 14th and 30th day, respectively. The abundance increase of the other observed groups was detected no sooner than on the 90th day in the amount of 1-8%, which is relatively low. Furthermore, dehydrogenase activity did not reach the level of the control variant, not even on the 90th day.

The results acquired strongly suggest that herbicides should be applied according to recommended rates. Additionally, attention should be paid to proper choice of herbicides, since the annual production of herbicides over the period 1995-1999 reached 4,046 t, or 43% of the overall production of pesticides (STEPIĆ and DAKIĆ, 2000). Uncontrolled and inadequate application of herbicides could adversely affect the potential and productive capacity of soil.

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MIKROBIOLOŠKA AKTIVNOST U RIZOSFERNOM ZEMLJIŠTU POD USEVOM SOJE NAKON PRIMENE HERBICIDA

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Izvod

Mikroorganizmi koriste molekule herbicida kao izvore biogenih elemenata i energije za svoje fiziološke potrebe. Ovo uslovljava povećanje mikrobijalne biomase, pa se može govoriti o stimulativnom delovanju herbicidan na deo mikrobne populacije. Međutim, populacije mikroorganizama koji nemaju razvijen enzimatski sistem za razgradnju herbicida bivaju inhibirani u svom razvoju, što uslovljava smanjenu brojnost i enzimatsku aktivnost.

Cilj rada je bio da se ispita uticaj herbicida na dinamiku brojnosti mikroorganizama u rizosferi soje nakon njihove primene. Primenjeni su sledeći herbicidi: H_1- kvizalofop-p-tefuril (Pantera) 48 g.a.m./ha; H_2-oksasulfuron (Dynam) 60g.a.m./ha; H_3-imezetapir (Pivot) 80 g.a.m./ha i H_4- klomazon (Command) 75 g.a.m./ha i K-kontrola bez primene herbicida.

Biološka aktivnost zemljišta određivana je standardnim mikrobiološkim metodama (POCHON and TARDIEUX 1968; KRASILJNIKOV 1962) 3, 14, 30 i 90-og dana nakon primene herbicida na osnovu sledećih parametara: ukupnog broja bakterija, zastupljenosti azotobaktera, aminoheterotrofa, gljiva i aktinomiceta, kao i dehidrogenazne aktivnosti. Uticaj herbicida na biogenost zemljišta zavisio je od herbicida, parametara istraživanja i vremena uzorkovanja.

Primenjeni herbicidi su u proseku smanjili brojnost ispitivanih parametara uglavnom do 90-og dana, osim aktinomiceta kod kojih je zabeleženo povećanje njihove brojnost već posle 14-og dana, a kod gljiva posle 30-dana. Kod ostalih ispitivanih grupa bakterija zabeleženo je povećanje njihove brojnosti tek 90-og dana od primene herbicida u vrednosti od 1-8%, što je relativno malo. Međutim dehidrogenazna aktivnost ni 90-og dana nije dostigla nivo kontrolne varijante. Rast gljiva i aktinomiceta bio je stimulisan u većem intezitetu nakokon tretiranja zemljišta herbicidima što ukazuje da ova grupa mikroorganizama učestvuje u njihovoj razgradnji.

S obzirom da su mikrobi važna karika u sistemu zemljište-biljka ovo pokazuje potrebu da ispitivanja mikrobiološke aktivnosti u zemljištu posle primene herbicida.

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