Pestic. Phytomed. (Belgrade), 29(3), 2014, 213–219
DOI: 10.2298/PIF1403213S

UDC 632.954:631.427.2
Original scientific paper

Assessment of microbial activity and biomass in different soils exposed to nicosulfuron

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Received: June 17, 2014
Accepted: September 18, 2014

SUMMARY

The effects of the herbicide nicosulfuron on the abundance of cellulolytic and proteolytic microorganisms, activity of β-glucosidase and protease enzymes, and microbial phosphorus biomass were examined. A laboratory bioassay was set up on two types of agricultural soils differing in physicochemical properties. The following concentrations were tested: 0.3, 0.6, 3.0 and 30.0 mg a.i./kg of soil. Samples were collected 3, 7, 14, 30 and 45 days after treatment with nicosulfuron.

The results showed that nicosulfuron significantly reduced the abundance of cellulolytic microorganisms in both soils, as well as microbial biomass phosphorus in sandy loam soil. The herbicide was found to stimulate β-glucosidase and protease activity in both types of soil and microbial biomass phosphorus in loamy soil. Proteolytic microorganisms remained unaffected by nicosulfuron.

Keywords: Soil; Phosphorus; Nicosulfuron; Microorganisms; Biomass

INTRODUCTION

Modern agricultural production relies on herbicides as a necessity in efforts to suppress weed species. After incorporation in soil, such chemicals may significantly interfere with microbial processes in soil. Microorganisms play an important role in the circulation of nutrients within soil ecosystems, affecting carbon, nitrogen and phosphorus transformation, and thereby securing and modifying soil fertility (Rezende et al., 2004; Blagodatskaya & Kuzyakov, 2013). Soil properties such as microbial species diversity, enzymatic activity, microbial biomass and soil respiration can be used as parameters in monitoring changes that occur in agricultural practice (Allen & Schlesinger, 2004; García-Ruiz et al., 2008). Primary populations of microorganisms normally break down soil-incorporated herbicides within days. Secondary populations produce enzymes and degrade the remaining compounds after a period of adaptation (Milošević, 2008). The relationship between soil microorganisms and herbicides is therefore very important as it affects the fertility and quality of soils (Dinelli et al., 1998; Crouzet et al., 2010).

Sulfonylureas are a group of herbicides with good selectivity and a broad spectrum of weed control in many cereal crops. Those herbicides typically degrade by a combination of bridge hydrolisis through chemical

route and microbial degradation, and they are effective at very low application rates, and have various effects on microbial activity in soil (Sofo et al., 2012).

Other studies have shown that the effects of sulfonylurea herbicides on microbial activity in soil may be of varying degrees. Bensulfuron-methyl, nicosulfuron and rimsulfuron treatments have been found to decrease significantly the abundance of bacteria in top soil (Saeki & Toyota, 2004; Djuric & Jarak, 2006). Also, metsulfuron-methyl and rimsulfuron have been observed to cause significant change in the content of microbial biomass and enzymatic activity in soil (Vischetti et al., 2000; Zabaloy et al., 2008).

The present study was based on an assumption that nicosulfuron, being a toxicant, would affect the microbial populations and their activity in tested soils. The objective was to examine, in the laboratory, the herbicide's effects on microbial abundance, enzymatic activities and microbial biomass.

MATERIAL AND METHODS

The herbicide nicosulfuron 2-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-*N,N*-dimethylpyridine (product Motivell, BASF) was applied at the following rates: 0.3, 0.6, 3.0 and 30.0 mg a.i./kg of soil. The lowest tested concentration (0.3 mg) equalled the recommended field rate, while the other three were double, ten-fold and hundred-fold higher. The bioassay was performed on a loamy soil from Zemun Polje and sandy loam soil from Tavankut, and the physicochemical properties of both are shown in Table 1.

Table 1. Physicochemical properties of agricultural soil samples

Soil	Loamy soil (Zemun Polje)	Sandy loam soil (Tavankut)
Sand (%)	49.80	91.44
Silt (%)	33.40	1.32
Clay	16.80	7.24
Total C (%)	2.30	0.53
Total N (%)	0.25	0.06
P_2O_5 (mg/100g)	46.00	24.50
K_2O(mg/100g)	65.00	22.00
Organic matter (%)	3.96	0.91
pH (H_2O)	7.64	8.04

Soil was sampled from 0-10 cm depth in locations that had never been treated with herbicides, then cleaned from aboveground and underground plant parts, fully air dried and sieved using a 5 mm sieve. Each herbicide concentration was applied to 1 kg of soil by a thin layer chromatography sprayer and the samples were homogenized on a rotational mixer for 30 minutes. After homogenization, the soil samples were poured into vegetation pots. Control samples remained untreated. The bioassay had four replications. The vegetation pots were kept under semicontrolled conditions at 20 ± 2°C. Soil moisture was set to 50% field capacity. Sampling was carried out 3, 7, 14, 30 and 45 days after herbicide application.

A plate count method was used to determine microorganism abundance. Diluted soil suspensions were spread upon a Waksman-Cery nutrient medium for enumeration of cellulolytic microorganisms and upon agar medium supplemented with gelatin for estimation of proteolytic microorganisms. The counts are presented as the number of microorganisms per 1 gram of dry soil (Jarak & Đurić, 2006).

β-glucosidase activity was estimated by a method proposed by Tabatabai (1982) based on spectrophotometric determination of p-nitrophenol (p-NP) formed from p-nitrophenyl-b-D-glucoside as a product of enzymatic activity. Measurements were made at 410 nm wavelength and enzyme activity expressed as μg p-NP/gram of soil/second.

Protease activity was determined using Romeiko's (1969) method. It is based on titrimetric determination of protease activity in gelatin medium. The proteolytic activity of soil was calculated from an applied 4% $FeCl_3$. Protease activity is expressed as the number of gelatin units per 1 g of air dry soil. Ten gelatin units required 0.2 ml of $FeCl_3$.

A method developed by Brookes et al.(1982) was used to determine microbial biomass phosphorus. The samples were incubated in limestone [calcium carbonate ($CaCO_3$)] and caustic soda [sodium hydroxide (NaOH)] under high moisture, and then fumigated with alcohol-free chloroform ($CHCl_3$). After incubation, phosphorus was extracted with a 0.5 M solution of sodium hydrogen carbonate ($NaHCO_3$). The acquired data are presented as μg P/g soil.

Data were statistically processed in the Statistica 8.0 software. A three-way analysis of variance was used to compare means of the examined microbial parameters: the abundance of microbial groups, and amounts of microbial enzymes and biomass. The LSD test was used to compare treatments and assessments of each parameter when differences in F values were statistically sigificant ($p<0.05$).

RESULTS AND DISCUSSION

The results showed that the effects of nicosulfuron on soil microbial activity depended on its application rate, duration of activity and type of soil, and it was either stimulating or inhibiting.

Cellulose becomes metabolized in soil through the presence of abundant aerobic and anaerobic cellulolytic microorganisms (Schellenberger et al., 2012). Over the initial three days of our bioassay, nicosulfuron caused no change in the abundance of cellulolytic microorganisms in loamy soil (Table 2). However, 0.6, 3.0 and 30.0 mg concentrations significantly reduced the abundance of that group of microorganisms ($p<0.05$) from the 7th until 30th day. At the end of the bioassay (45th day), nicosulfuron was found to have a stimulating effect as cellulolytic microorganisms significantly increased in numbers, i.e. by 34.7% against the untreated control. In contrast to loamy soil, the herbicide caused a decrease in the abundance of cellulolytic microorganisms in sandy loam soil as early as three days after treatment with the two top concentrations (3.0 and 30.0 mg), and the inhibitory effect extended into the 14th day (48.5%). Fourty-five days after treatment, 0.6 and 3.0 mg concentrations of nicosulfuron significantly increased, i.e. by 44.1%, the counts of that group of microorganisms. Differences between all other variants were statistically nonsignificant.

Reports from many other studies (Ghinea et al., 1998; Radivojević et al. 2007) have shown significant decreases in that group of microorganisms after nicosulfuron treatments. Pampulha and Oliveira (2006) observed a significant inhibition of cellulolytic bacteria (by 38-68%) after application of a combination of bromoxynil and prosulfuron herbicides. Also, the presence of brominal (a herbicide) and profenofos (an insecticide) have been found to significantly reduce the abundance of cellulolytic fungi (Omar & Abdel-Sater, 2001).

Proteolytic microorganisms belong to aminoheterotrophs and they require proteins as nitrogen sources. Data in Table 3 show that nicosulfuron caused no statistically significant changes ($p<0.05$) in the abundance of proteolytic microorganisms in loamy soil. Their abundance was minimal seven days after treatment with 30.0 mg ($7.59 \times 10^4 g^{-1}$ soil), and 4.2% lower than the control value. However, neither that one nor any other difference in that type of soil was statistically significant. Similarly, proteolytic microorganisms reacted without a significant change to the application of nicosulfuron in sandy loam soil. Seven and 14 days after treatment with the highest concentration (30.0 mg), their abundance increased around 15%, but the differences were not statistically significant.

Data from other studies have shown different herbicide effects on this group of microorganisms. A study by Przybulewska and Taborska (2008) showed that the use of triazine herbicides over many successive years had increased the abundance of proteolytic microorganisms resistant to that group of herbicides. On the other hand, Latha and Gopal (2010) observed in their laboratory incubation studies that pyrazosulfuron ethyl acted inhibitory on proteolytic bacteria (7.85% reduction against control).

Table 2. Effects of nicosulfuron on the abundance of cellulolytic microorganisms ($10^4 g^{-1}$dry soil)

Nicosulfuron mg kg^{-1}	Loamy soil					Sandy loam soil				
	Days after treatment					Days after treatment				
	3	7	14	30	45	3	7	14	30	45
0.0	104.02 cdef*	111.89 bcd	97.44 def	109.40 dce	107.6 cde	53.22 b	52.68 b	54.25 b	55.95 b	59.23 b
0.3	94.85 ef	90.81 fg	99.37 def	97.47 def	142.91 a	51.61 b	55.37 b	53.76 b	54.68 b	64.51 b
0.6	100.40 def	69.72 hij	76.48 hi	98.77 def	130.04 ab	54.34 b	51.90 b	53.19 b	53.09 b	85.48 a
3.0	92.82 fg	46.38 k	47.87 k	81.14 gh	145.03 a	35.48 c	29.63 cd	28.49 cd	53.09 b	84.95 a
30.0	97.88 def	33.88 l	61.33 j	67.36 ij	119.72 bcd	32.79 cd	27.12 d	28.72 cd	50.51 b	64.36 b

(*LSD test at $p<0.05$ significance)

Table 3. Effects of nicosulfuron on the abundance of proteolytic microorganisms ($10^4 g^{-1}$ dry soil)

Nicosulfuron mg kg^{-1}	Loamy soil					Sandy loam soil				
	Days after treatment					Days after treatment				
	3	7	14	30	45	3	7	14	30	45
0.0	8.05 a*	7.92 a	8.07 a	8.11 a	8.20 a	4.35 a	4.52 a	4.25 a	3.97 a	4.13 a
0.3	8.14 a	8.02 a	8.30 a	8.11 a	8.18 a	4.43 a	4.34 a	4.42 a	4.08 a	3.91 a
0.6	8.09 a	8.10 a	8.29 a	7.89 a	8.20 a	4.35 a	4.47 a	4.58 a	4.14 a	4.21 a
3.0	7.85 a	7.63 a	7.93 a	8.21 a	8.08 a	4.25 a	4.63 a	4.63 a	4.18 a	4.01 a
30.0	7.73 a	7.59 a	7.95 a	7.96 a	8.15 a	4.12 a	5.23 a	5.15 a	3.94 a	4.13 a

(*LSD test at $p<0.05$ significance)

Several studies have shown that enzymes and microbial biomass are more sensitive parameters than microbial abundance for detecting changes in microbial population. Microbial phosphorus makes 0.5-11% of total phosphorus in soil and a significant contribution to total microbial biomass (Jeannotte et al., 2004). Data in Figure 1 show that nicosulfuron applied to loamy soil had a stimulating effect on microbial biomass phosphorus. A statistically significant increase in that parameter ($p<0.05$) was detected on the 3rd day of bioassay after treatment with the highest nicosulfuron concentration (30.0 mg) and the trend continued into the 7th, 14th and 30th day, when a significant increase was also found in pots treated with ten-fold higher herbicide concentrations (6.9-22.8%). The maximal microbial biomass phosphorus of 62.48 µg P/g soil was recorded 14 days after treatment with 30.0 mg of nicosulfuron. There were no significant changes in the other treatment variants. In sandy loam soil, nicosulfuron demonstrated negative effects seven and 14 days after treatment with the two highest concentrations. Microbial biomass phosphorus was found to have significantly decreased, by 13.3-30.1%, and its lowest value was 18.58 µg P/g soil ($p<0.05$). However, 30 days after the application of double and ten-fold higher concentrations of nicosulfuron the values of that parameter significantly increased around 15%, reaching a maximum of 31.99 µg P/g soil. No significant changes against the untreated control were detected in the other bioassay variants.

The content and amount of microbial biomass phosphorus may have an impact on the potential of soils to make phosphorus available to plants through microbial mineralization (Jeannotte et al., 2004; Makoi & Ndakidemi, 2008; Li et al., 2009). The studies of Das and Dey (2013) showed that paraquat increased microbial biomass phosphorus in soil by 15.2% compared to untreated soil.

ß-glucosidase belongs to a group of enzymes that play an important role in degradation of organic carbon components in soil. Data in Figure 2 show that nicosulfuron had a stimulating effect on the activity of ß-glucosidase in loamy soil. The increased enzyme activity was statistically significant ($p<0.05$) and recorded from the 7th through the 30th day (3.3-14.4%), and a maximum of 209.66 µg-pNP/g soil/s was observed after treatment with 30.0 mg of nicosulfuron. The treatments and control showed no significant difference on the 3rd and 45th day of bioassay. In loamy soil, both stimulating and inhibitory effects of nicosulfuron were detected regarding the activity of ß-glucosidase enzyme. A statistically significant increase in its activity of 5.6-29.4% was recorded seven and 14 days after treatment with the two highest concentrations (3.0 and 30.0 mg). The maximal enzyme activity over the period was 176.55 µg-pNP/g soil/s. On the 45th day all nicosulfuron concentrations reduced the activity of ß-glucosidase. The changes were statistically significant and the minimal enzyme value of 123.62 µg-pNP/g soil/s was found in sandy loam soil. After three and 30 days of bioassay, statistically significant changes were not found in any variant.

Similarly, Sofo et al. (2012) had noted in their research that the effect of four sulphonylureas (cinosulfuron, prosulfuron, thifensulfuron methyl and triasulfuron) on enzymatic activity depended on the concentration applied.

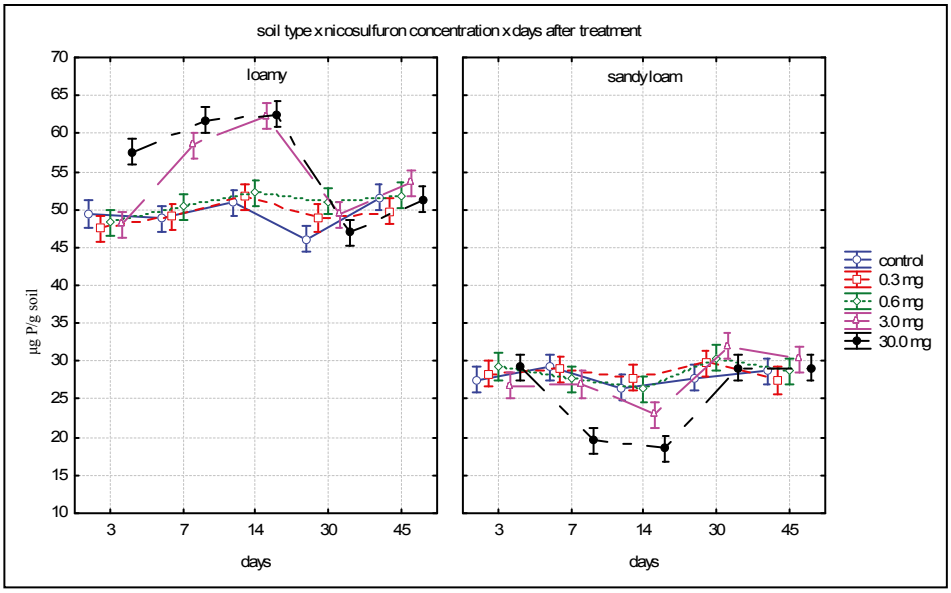

Figure 1. Effects of nicosulfuron on microbial biomass phosphorus (µg P g^{-1} soil)

Thus, ß-glucosidase data showed an increase in samples containing triasulfuron at ten-fold the field rate, whereas data for thifensulfuron methyl at normal field rate and ten-fold the field rate were not statistically different from the untreated control. In a study involving metribuzin, which is a triazine herbicide, Šantrić et al. (2008) had observed its inhibitory effect on acid phosphatase and dehydrogenase and stimulating effect on urease, while no change was observed in ß-glucosidase values.

The enzyme protease is crucial for nitrogen mineralization and its activity in soil largely depends on various biotic and abiotic factors. The data presented in Figure 3 show that nicosulfuron had a stimulating effect on the activity of protease in loamy soil. A statistically significant increase in the activity of that enzyme was recorded from the 7th until the 30th day of bioassay ($p<0.05$). On the 7th and 30th day, its activity was found to have increased after treatments with 3.0 and 30.0 mg nicosulfuron rates, and the 0.6 mg rate also had a stimulating effect on the 14th day (10.6-43.6%). The maximal protease activity was 33.75 g.u./g soil in that period. At the beginning and the end of the experiment (3rd and 45th day), changes were statistically nonsignificant.

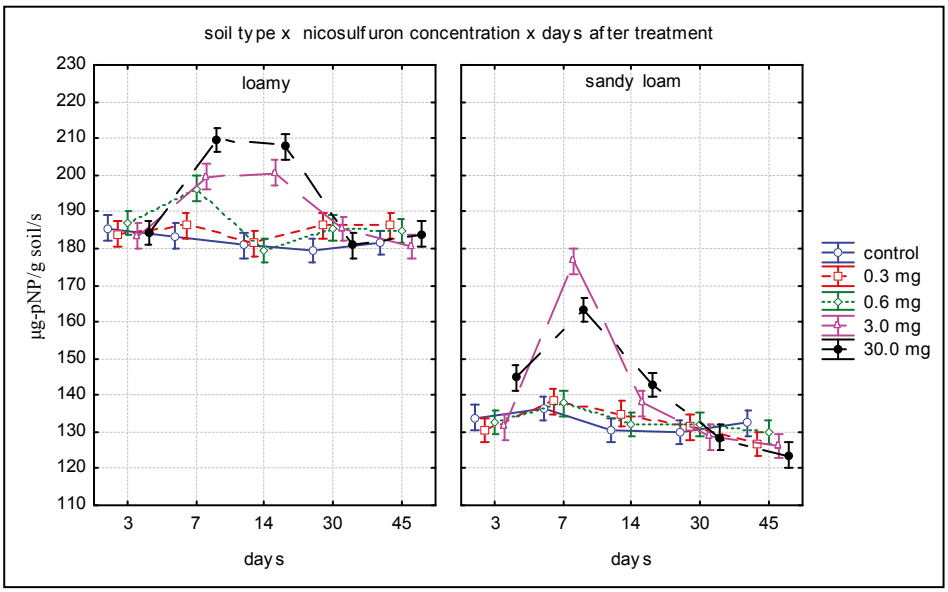

Figure 2. Effects of nicosulfuron on β-glucosidase (µg-pNP g^{-1} soil)

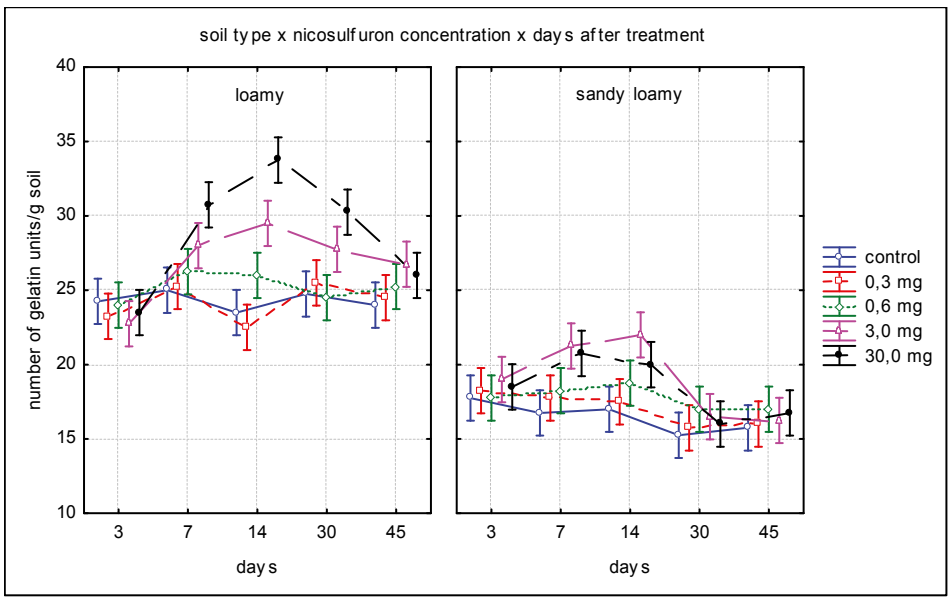

Figure 3. Effects of nicosulfuron on protease (g.u. g^{-1} soil)

In sandy loam soil, nicosulfuron also increased the activity of protease. Stimulating effect of the two top concentration rates was observed on the 7th and 14th days (17.6-29.4%) and all differences were statistically significant ($p<0.05$). Protease activity ranged from a minimum of 15.25 g.u./g soil up to 22.00 g.u./g soil. In all other bioassay variants (3rd, 30th and 45th day) no statistically sigificant differences were found regarding the activity of that enzyme.

Other studies have shown rimsulfuron effects that depended on parameters monitored in each. So herbicide treatments in laboratory bioassays at 10- and 100-fold higher rates than the recommended have been found to reduce significantly the microbial biomass carbon and increase the activity of protease and ß-glucosidase (Martins et al., 2001; Radivojević et al., 2011).

The results confirmed our original assumption on which we had based our experimental concept that nicosulfuron, being a toxicant, would change the abundance of microorganisms and enzymatic activity in soil samples. The effects caused by the herbicide ranged from inhibitory to stimulating, depending on application rate, duration of activity, soil type and parameter tested.

ACKNOWLEDGEMENT

The study was part of the project TR 31043 of the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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Određivanje mikrobiološke aktivnosti i biomase u različitim zemljištima nakon primene nikosulfurona

REZIME

Ispitivan je uticaj herbicida nikosulfurona na brojnost celulolitskih i proteolitskih mikroorganizama, aktivnost enzima β-glukozidaze i proteaze i mikrobiološku biomasu fosfora. Ogled je postavljen u laboratorijskim uslovima na dva poljoprivredna zemljišta koja se razlikuju po svojim fizičko-hemijskim karakteristikama. Primenjene su koncentracije od 0,3, 0,6, 3,0 i 30,0 mg a.s/kg zemljišta. Uzorci za analizu uzeti su 3, 7, 14, 30 i 45 dana nakon primene nikosulfurona.

Dobijeni rezultati su pokazali da je nikosulfuron značajno smanjio broj celulolizatora kod oba tipa zemljišta, kao i vrednost mikrobiološke biomase fosfora u peskuši. Stimulativno delovanje ovog herbicida zabeleženo je kod enzima β-glukozidaze i proteaze kod oba tipa zemljišta kao i kod mikrobiološke biomase fosfora u ilovači. Na brojnost proteolitičkih mikroorganizama nikosulfuron nije ispoljio nikakav uticaj.

Ključne reči: Zemljište; fosfor; nikosulfuron; mikroorganizmi; biomasa