CHEMICAL ANALYSIS AND BIOTEST IN THE ASSESSMENT OF NICOSULFURON SOIL POLLUTION

Sanja Lazić, Bojan Konstantinović, Dragana Šunjka, Nataša Samardžić, Mirjana Kojić, Slavica Vuković, Milena Popov, Milan Blagojević

Department of Environmental and Plant Protection, Faculty of Agriculture, University of Novi Sad, 21000 Novi Sad, Trg Dositeja Obradovića 8,Serbia
e-mail: draganas@polj.uns.ac.rs

Abstract
The assessment of soil pollution exposed to nicosulfuron can be conducted through chemical analysis, by determination of residue quantity, and biotests. In this research, the field dissipation study of nicosulfuron herbicide in soil under maize, was conducted in controled conditions. Nicosulfuron residue in top soil layers were determined by HPLC-DAD, while nicosulfuron extraction was performed by modified QuEChERS method. In five months period, from the first application at the manufacturer's recommended concentration, until harvest, nicosulfuron concentration decreased over time from 0.95 mg/kg to 0.12 mg/kg. In order to evaluate phytotoxicity of nicosulfuron on susceptible crops, *Triticum vulgare* L.and *Beta vulgaris* L.were used as test plants. Inhibitory action of nicosulfuron on the studied species was proved on the basis of the obtained results, by studying the morphological parameters, roots and above-ground seedlings lengths (mm), i.e. length of shoot.

Introduction
After herbicide application its remainings in the soil can prove beneficial during the season for control of later flushes of target weeds. However, their residues, found in the soil during the following year, can reduce yields of susceptible crops grown in rotation. The amount of herbicide that sorbs to the soil and the rate it can desorb back into the soil solution determines the overall phytotoxicity of the herbicide [1].
Sulfonylurea herbicides are used to control a variety of broad-leafed weeds and grasses in cereals and other row crops and for industrial weed control. They can be considered such "as new formulation pesticides" because of their high selectivity and low persistence in the environment [2]. These characteristics significantly reduce the amount of the applied chemicals to the field in comparison with conventional ones [3]. However, the application of low levels of these herbicides does not guarantee high levels of environmental tolerance. In some cases, sensitive plants suffer damage by dosages lower than 1% of the initial application rate [4]. Sulfonylurea herbicides, such as nicosulfuron, are more mobile in alkaline soils and in soils with lower organic matter content. Nicosulfuron is the most applicable sulfonylurea herbicide in maize for successful control of annual and perennial weeds.
The phytotoxic effect of nicosulfuron and its metabolites on dicotyledonous plants leads to a self-limitation in the re-planting period. After sowing, in 27-30 days interval since pesticide application, phytotoxic effects are obvious on cereals, sugar beet, canola and clover. The listed crops should not be sown on land previously treated by nicosulfuron before next spring, when the expected level of herbicide residues and their degradation products is <0.001 mg/kg [5].
Quality evaluation and the level of environmental pollution are established by application of different physical, chemical and biological methods. Usually, the assessment of soil contamination is based on the chemical analysis. These methods imply the use of corresponding

extraction and purification methods, as well as sensitive methods of pesticide residues determination, such as gas and liquid chromatography.
However, world wide, there is an increase in the use of biological methods that imply use of cultivated plants as test organisms, i.e. phytoindicators. Biological methods and tests by which soil quality is evaluated, have a great importance for agricultural production as they indicate potentialphytotoxical action of the applied pesticides. Use of cultivated plants as tests organisms is especially significant for biological tests in agricultural production.

Experimental

Field trial

The monitored field trial was carried out on a chernozem soil, according to OEPP standard methods for experimental design and data analysis. In mazie field, nicosulfuron (OD, 40 g/l) was applied at the recommended dose, at the corn growth stage of BBCH 12-18. Soil samples were collected before and immediately after the pesticide application, and every two weeks until harvest, from surface soil layer, of 0-30 cm.

Chemical analysis

For the chromatographic analysis, an Agilent 1100 Series system with DAD detector and Zorbax SB-C18 column (5 μm, 250mm ×3 mm internal diameter) were used. The mobile phases were composed of acetonitrile (A) and 0.1% (V/V) acetic acid in water (B), at a flow rate of 1.0 ml/min, using following gradient profile: 0–10 min linear from 52% to 47% (A).
For nicosulfuron extraction of 10.0 g of previously dried, milled and sieved soil samples were weighted and transferred into 50 ml polypropylene tube. Afterwards, 3 ml of deionized water and 10 ml of acidified acetonitrile were added. The tube was shaken and vortexed for a 1 min. A mix of buffered salts from separate pouches was added, shaken for 1 min and vortexed 1 min. The tube was placed in an ultrasonic bath for 10 min and centrifuged at 4000 rpm for 5 min. The supernatant was filtered through a 0.45 μm membrane filter and transferred into an autosampler vial for HPLC-DAD analyses [6].

Biotest

The method prescribed by ISTA rules (International rules for seed testing) for 2013 and the Regulations on the quality of seeds of agricultural plants [7] was used for the biotests. In laboratory bioassays, in controlled conditions the impact of different, previously by chemical analysis determined nicosulfuron doses, was studied on seed germination and the initial growth of wheat and sugar beet. On filter paper in Petri dishes 10 ml of nicosulfuron working liquid was added in doses close to those previously obtained by chemical analysis (0.96-0.07 μg/ml), and each Petri dish was filled with 10 seeds of wheat (*Triticum vulgare* L.) and sugar beet (*Beta vulgaris* L.). The trial was set up in four replications. Seeds were germinated for 10 days in thermostat at a temperature of 25±2 °C in dark. Morphological parameters, root length and seedlings above-ground parts (mm), i.e. length of seedlings of the above mentioned cultures were measured after incubation.

Results and discussion

The results for determination of nicosulfuron in trace amount were presented in our previous paper [6]. The validation study performed according to SANCO/825/00 rev. 8.1 16/11/2010 [8], has fully met the criteria mentioned in the document.
The results of dissipation demonstrated a gradual and continuous decrease of nicosulfuron content in the soil. Herbicide concentration decreased over time from 0.95 mg/kg to 0.12 mg/kg,

in five months period. The half-life ($t_{1/2}$) of nicosulfuron in soil, calculated using results obtained in this study, was 6.93 days.

The amount of nicosulfuron residue obtained in the field dissipation study were used in biotests.Ten days after the treatment, wheat and sugar beet seedlings exhibited different levels of inhibition depending on the applied dose of nicosulfuron.

It was established that all studied doses of nicosulfuron applied in wheat significantly inhibit seedlings length in comparison to the control. In regard to the control, seedlings length was reduced by 86.89-91.37%(Table 1, Figure 1). In sugar beet, more significant inhibition in seedlings growth was recorded in the use of the highest dose of nicosulfuron, while the results of the remaining variants were statistically at the same significance level in comparison to the control, for shoot length (Table 1). Inhibition of growth of seedlings treated by different nicosulfuron doses was from 6.23 do 85.04%.

Table 1. The effect of nicosulfuron on seedling length *T. vulgare* L. and *B. vulgaris* L. after 10 days under laboratory conditions

nicosulfuron (a.i. µg/ml)	*Triticum vulgare* L. (cm)		*Beta vulgaris* L. (cm)	
	root	shoot	root	shoot
0.96	8.35[a]	12.97[a]	0.03[a]	0.09[a]
0.52	11.80[a]	18.25[a]	0.08[a]	0.17[a]
0.34	7.37[a]	13.35[a]	0.05[a]	0.11[a]
0.22	8.97[a]	14.02[a]	0.12[ab]	0.15[a]
0.07	13.10[a]	17.35[a]	0.04[a]	0.21[a]
Control	**116.00[b]**	**116.40[b]**	**0.18[b]**	**0.13[a]**

[*]Values followed by the same letter at the same significance level (95% confidence interval)

Figure 1. Effect of nicosulfuron from 0.52 µg/ml of the length of root and shoot *T. vulgare* L. after ten days (a); Length of root and shoot *T. vulgare* L. in control after ten days (b)

According to the results obtained in biotests, it can be concluded that the lowest amount of nicosulfuron remained in soil five months after application, determined by chemical analysis, can cause inhibitory effect on seedlings length *T. vulgare* L. (86.9%). However, the same concentration of nicosulfuron was significantly reduced only root length *Beta vulgaris* L. (77.8%), without influence on shoot length, in comparison to the control.

Conclusion

Based on the obtained results it can be concluded that there exists an inhibitory effect of the herbicide nicosulfuron residues on growth of wheat (*Triticum vulgare* L.) seedlings, compared to the control treated with distilled water. Wheat shows significant susceptibility to nicosulfuron residues in soil, and they can have negative effect on this culture. Moreover, the conducted laboratory assays showed that inhibitory action of nicosulfuron increase sugar beet root length. In relation to roots and seedlings length, seedlings of *T.vulgare* L., and smaller seedlings of *B. vulgaris* L. exibited higher susceptibility to the tested concentrations of nicosulfuron. In relation to roots and shoots length, seedlings of *T.vulgare* L., and smaller seedlings of *B. vulgaris* L., exibited higher susceptibility to the tested concentrations of nicosulfuron.

Acknowledgements

The authors appreciate funding from the Ministry of Education and Science of the Republic of Serbia (Project No. III43005) for supporting this study.

References

[1] B.G.L. Geisel Thesis for the Degree of Master of Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 2007.

[2] M. Benzi, E. Robotti, V. Gianotti, Giornate Italo-Francesi di Chimica, GIFC 2010, 26-27 Aprile 2010, Genova, Italy.

[3] H.M. Brown, Pesticide Science, 29 (1990) 263.

[4] E.M. Beyer, M.J. Duffy, J.V. Hay, D.D. Schlueter, in: P.C. Kearney, D.D. Kaufman (Eds.), Chemistry, Degradation and Mode of Action, Marcel Dekker Inc., New York, 1987.

[5] S. Lazić, D. Šunjka, I. Stojanović, S. Vuković, B. Konstantinović, M. Popov, 6^{th} International Scientific Agricultural Symposium “Agrosym 2015”, October 15 - 18, Jahorina, Bosnia and Hernzegovina, Proceedings, pp. 959 (2015).

[6] S. Lazić,D.Šunjka, N. Grahovac, 20^{th} International Symposium on Analytical and Environmental Problems, SZAB Kémiai Szakbizottság Analitikai és Környezetvédelmi Munkabizottsága, 22 September 2014, Szeged, Hungary, (2014) Proceedings, pp. 143.

[7] Official Gazette of SFRY", No. 47/87, 60/87, 55/88 and 81/89 and "Official Gazette of SRY", No. 16/92, 8/93, 21/93, 30/94, 43/96, 10/98, 15/2001 and 58/2002, ‘’Official Gazzete of RS”, No. 23/2009, 64/2010, 72/2010 and 34/2013.

[8] EU COMMISSION, Directorate General Health and Consumer Protection, SANCO/825/00 rev. 8.1 16/11/2010, Guidance document on pesticide residue analytical methods.