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Scientific paper

Maize, Sunflower and Barley Sensitivity to the Residual Activity of Clomazone in Soil

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SUMMARY

Sensitivity of maize, sunflower and barley to clomazone residues in loamy soil was assessed in the study using bioassay. Clomazone was applied at a series of concentrations from 0.12 to 12 mg a.i./kg of soil. After 14 days, morphological (shoot height, fresh and dry weight) and physiological (content of carotenoids, chlorophyll *a* and chlorophyll *b*) parameters were measured. The results showed that morphological parameters are not valid indicators of clomazone sensitivity. Based on the results showing inhibition of the physiological parameters, I_{50} values were calculated and used to estimate the difference in sensitivity between the species tested. Sunflower was the most sensitive species, while the difference in sensitivity between maize and barley was not significant.

Nomenclature: clomazone (2-(2-chlorbenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one), maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.), barley (*Hordeum vulgare* L.)

Keywords: Clomazone; Maize; Sunflower; Barley; Bioassay; Chlorophylls; Soil

INTRODUCTION

Clomazone is a selective systemic herbicide from the isoxazolidinone group. It is absorbed by root and shoot, transported ascending through xylem to the top of the plant reaching the leaves by diffusion. Mode of action of clomazone is based on the inhibition of isopentyl pyrophosphate (IPP) formation, which is a common precursor of all isoprenoids (including photosynthetic pigments, carotenoids, electron carriers (plastokinons), tocopherol and hormones (gibberellins)). Clomazone inhibits the activity of the enzyme deoxy-D-xylulose-5-phosphate synthase (DXS), which together with DXR (deoxy-D-xylulose-5-phosphate reducto-isomerase) catalyzes the process of DXP (deoxy-D-xylulose-5-phosphate) and MEP (4 methylerythritol phosphate) formation as it intermediates between G3P (glyceraldehyde 3-phosphate) and IPP in plastids (Ferhatogly and Barrett, 2006). Clomazone is listed among the inhibitors of carotenoid biosynthesis (HRAC) whose most important role is to prevent the active forms of oxygen from breaking down chloroplast membranes in a process of lipid peroxidation (Duke et al., 1985; Young, 1991; Böger and Sandmann, 1993). Herbicides that inhibit enzymes in the process of carotenoid biosynthesis cause characteristic symptoms on sensitive plants known as the bleaching effect.

In Serbia, clomazone is used to control broad-leaved and some grass weeds in soybean, tobacco, oil seed rape and some vegetable crops (Janjić and Elezović, 2010). It can be applied either prior to sowing with obligatory incorporation, or after sowing and before weed emergence, or as a foliar herbicide. Regarding its physiochemical properties,clomazone is relatively water soluble (1100 mg ml/l at 25°C) and its vapor pressure is 19.2 mPa at 25°C. Clomazone fumes can cause leaf bleaching on sensitive plants and therefore shallow incorporation is recommended when applied in practice (Thelen et al., 1988; Tomlin, 2003).

Clomazone has weak mobility in soil, depending on the content of organic matter and clay, but also on the presence of plant cover on soil surface (Loux et al., 1989a; Mills et al., 1989; Mills and Witt 1989; Curran et al., 1992; Mervosh et al., 1995; Antonius, 2000). The persistence of this compound depends on its availability, which is determined by the intensity and degree of adsorption. Previous studies point to a strong correlation between the adsorption and the content of organic matter in soil, but also to a significantly lower correlation between adsorption and clay content. Also, clomazone persistence is significantly affected by environmental factors, particularly by moisture content, hence the degradation process is slower in soils with low organic matter content and lower precipitation and temperature, (Loux et al., 1989a, 1989b; Kirksey et al., 1996; Gunasekara et al., 2009). The influence of tillage on the persistence of this herbicide is not consistant, while application method may reduce the loss of clomazone fumes from soil surface and plant remains (Mills and Witt 1989; Ahrens and Fuerst 1990; Curran et al., 1991, 1992). Degradation half-life (DT_{50}) of clomazone in soil, depending on these conditions, ranges from 5 to 117 days (Loux et al., 1989a; Mills et al., 1989; Gallaher and Mueller 1996; Kirksey et al., 1996; Cumming et al., 2002).

Studies on the mobility and persistence of herbicides in soil usually tend to determine the fate of herbicides and the consequences that their residues cause to the following crops in rotation. For a quantitative analysis of residues, instrumental methods are mainly used, as well as methods with isotopic compounds. However, the only valid and reliable method to determine bioavailability of herbicide residues is bioassay, which requires the use of sensitive test species, and specific and measurable response (Günther et al., 1993; Streibig and Kudsk, 1993).

Former studies on damage caused by clomazone residues to the following crops in rotation have involved several plant species, but their results varied, and were often inconsistent even for a same species. Possible phytotoxic effects resulted from soil properties, meteorological conditions in a year after application, but also from a clomazone application rate and a period elapsed from herbicide application to the following crop sowing (Gallandt et al., 1989; Loux et al., 1989a; Ahrens and Fuerst 1990; Monks and Banks 1991; Krausz et al., 1992).

Although clomazone has been used in our country for a long time, there are no experimental data available regarding its persistence in soil, which indicates a risk for the following plants in rotation under our agroecological conditions. Therefore, the aim of this bioassay study was to assess the sensitivity of maize, sunflower and barley as potentially following crops in rotation to different amounts of clomazone residues in loamy soil.

MATERIAL AND METHODS

Technical grade concentrate of clomazone (Shenzhen, China), 95% purity, obtained from Galenika Fitofarmacija enterprise was used in the assay. The seeds

of maize (PR35F38, Pioneer), barley (525 Novi Sad, Institute of Field and Vegetable Crops Novi Sad) and sunflower (Krajisnik, Institute of Field and Vegetable Crops Novi Sad) were used in the assay. Loamy soil (Table 1) was collected from a Putinci site previously untreated with herbicides and dug out from 10cm depth. The soil was cleaned from surface and underground plant remains and sifted through a 3 mm sieve.

The following morphological parameters were monitored as phytotoxicity indicators: shoot height, and fresh and dry weight of shoots, while physiological parameters included the contents of carotenoids, chlorophyll *a* and chlorophyll *b*.

Pigment content was determined by dimethylformamide (DMF) extraction. Leaf slices (5 mm in diameter) were taken from intact leaves to make total weight of 0.1 g and transferred into glass tubes with 3 ml of DMF. The extraction was carried out in the dark at 4°C for 24 hours. After this period, extracts absorption was recorded on a spectrophotometer (LKB Biochrom Novaspec II 4040) at 480 nm wavelength for carotenoids, 664 nm for chlorophyll *a* and 647 nm for chlorophyll *b*. The formula developed by Wellburn (1994) was used to calculate pigment concentration (mg/ml), and then a conversion of pigment content (mg/g fresh leaf weight) was conducted.

Table 1. Physical and chemical properties of loamy soil samples

Chemical properties							
$CaCO_3$	pH		C	Humus	N	P_2O_5	K_2O
%	H_2O	KCl	%	%	%	mg/100 g	mg/100 g
5.60	7.64	7.17	2.30	3.96	0.246	46.0	65.0
Soil texture							
Sand						Dust	Clay
Large (mm)		Small (mm)		Total (mm)		0.02-0.002 mm	<0.002 mm
2-0.2		0.2-0.02		2-0.02			
1.53		48.27		49.80		33.40	16.80

A series of clomazone concentrations was prepared for the bioassay. For each concentration a sample of sifted soil (250 g) was measured and a thin layer of it placed on a plastic tray sized 23 x 18 cm. From previously prepared solutions of each concentration, 3 ml were pipetted and transferred into a thin-layer chromatography sprayer, which was connected to a compressor. The soil was treated uniformly over the surface, under constant pressure of 1.3 bars. This way, a series of soil samples with rising concentrations of clomazone: 0.12, 0.25, 0.50, 1, 2, 4, 6, 8, 10 and 12 mg a.i./kg of soil, was obtained, and the 6 mg a.i./kg concentration was the label rate for field application at 0.75 L/ha (480 clomazone g/L). Immediately after application, the soil was hand-stirred, transferred into pots, planted with seeds of the tested plant species and watered to reach 50% of its field water capacity. Simultaneously, control variants (for all three plant species) were prepared and grown. The experiment was set in four replicates. For each plant species, the experiment was performed twice.

The plants were grown for 14 days in a chamber under controlled conditions of day/night light regime (14h/10h) and temperature (26°C daytime and 21°C nighttime). During the experiment, soil moisture was constantly maintained at 50% of field water capacity.

The effect of clomazone concentrations on the assessed parameters was evaluated using the F-test at significance level of 5%. Further statistical analysis and graphics were processed using StatSoft 6.0. I_{50} values for the measured physiological parameters were calculated using a nonlinear regressional equation.

RESULTS

Clomazone influence on height, fresh and dry weight of shoots

A significant reduction in shoot fresh weight was detected for maize plants at the clomazone concentration of 10 mg a.i./kg, while significant differences were registered at concentrations ≥ 6 mg a.i./kg (Figure 1) for the other two parameters (shoot height and dry weight).

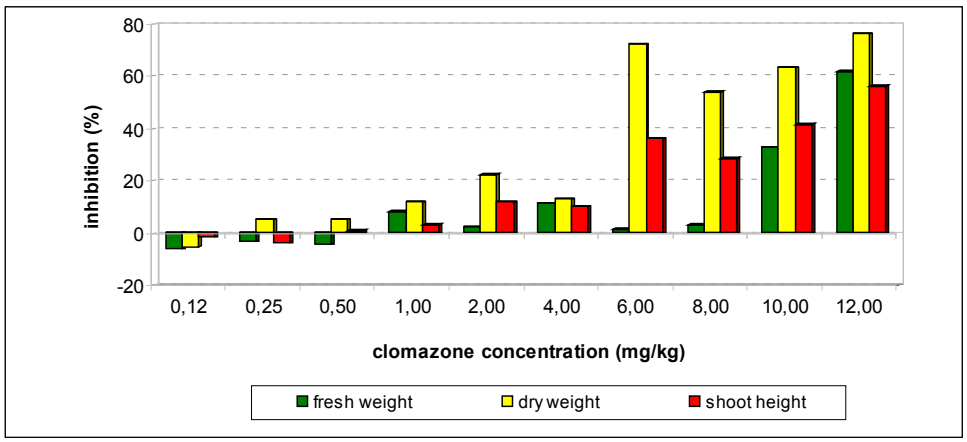


Figure 1. The effect of clomazone on the inhibition of morphological parameters of maize

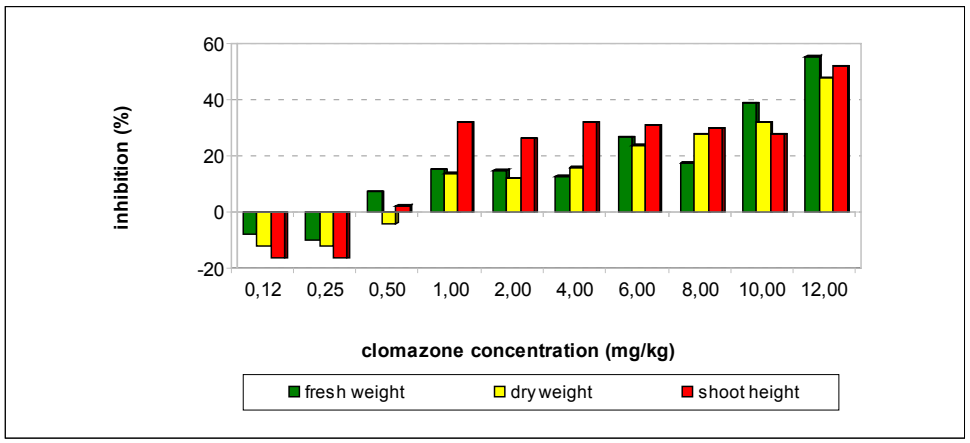


Figure 2. The effect of clomazone on the inhibition of morphological parameters of sunflower

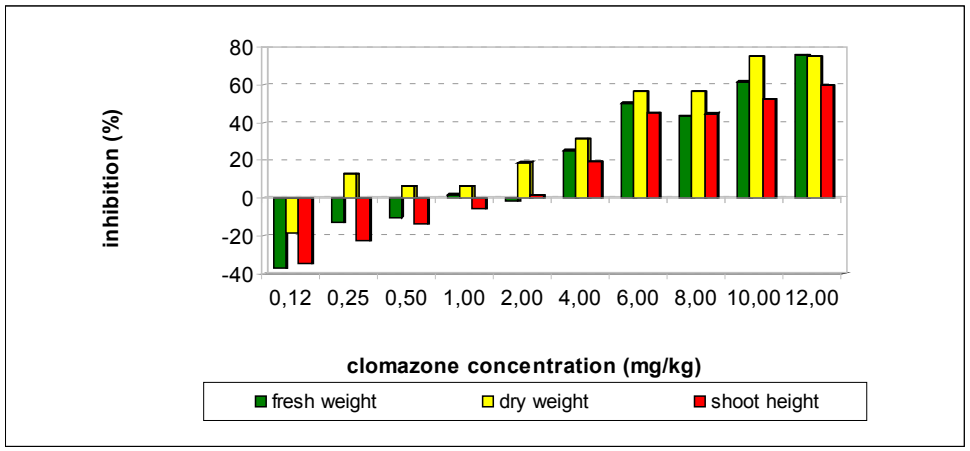
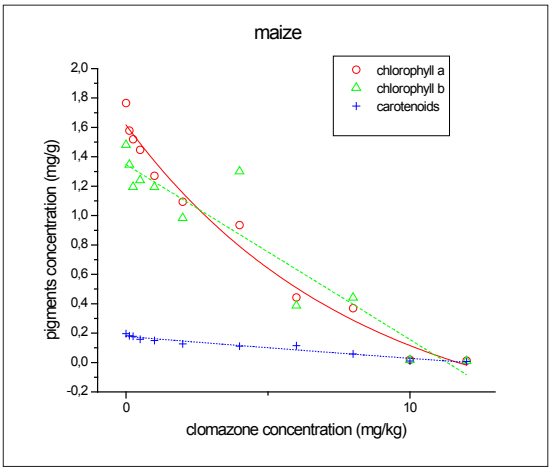
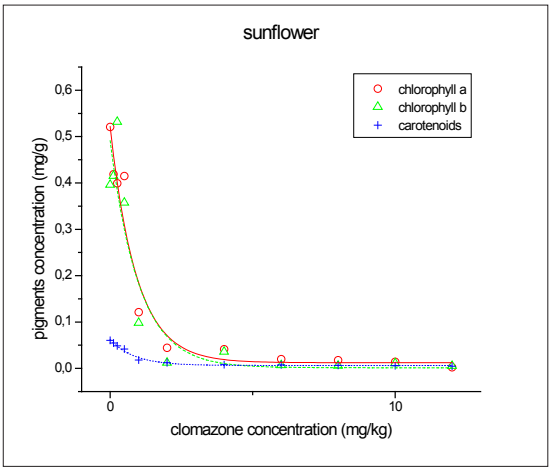


Figure 3. The effect of clomazone on the inhibition of morphological parameters of barley





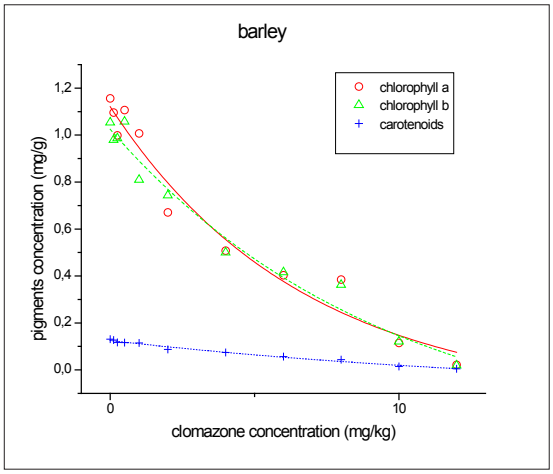


Figure 4. The effect of clomazone on pigment (mg/g) content in maize, sunflower and barley leaves

The inhibition of all three parameters was negligible in sunflower plants at clomazone concentrations ranging from 0.12 to 8 mg a.i./kg. A statistically significant reduction in shoot fresh weight was found at concentrations ≥ 10 mg a.i./kg, while dry weight and shoot height were significantly inhibited only when the highest clomazone concentration (12 mg a.i./kg) was applied (Figure 2).

Inhibition of the measured morphological parameters (Figure 3) was negligible in barley plants at concentrations from 0.12 to 2 mg a.i./kg (<10%), and slightly significant (18.92-25.22%) when clomazone was applied at 4 mg a.i./kg concentration. Statistically significant differences in all three parameters were found at concentrations ≥ 6 mg a.i./kg.

Clomazone influence on pigment content

The application of several clomazone concentrations caused leaf bleaching and reduction in pigment content in all three tested plant species (Figure 4)

The lowest clomazone rate applied (0.12 mg a.i./kg) caused a reduction in chlorophyll *a* content in maize plants. But a reduction in chlorophyll *b* content was caused by the next higher concentration (0.25 mg a.i./kg), while carotenoid content reduction was detected at the clomazone rate of 0.50 mg a.i./kg. More significant reductions were registered regarding the content of chlorophyll *a* (74.92-99.28%) and chlorophyll *b* (73.83-99.27%) at concentrations ≥ 6 mg a.i./kg of clomazone, and carotenoid contents (70.34-96.34%) at clomazone rates ≥ 8 mg a.i./kg. Significant differences between treatments and the control for all three measured parameters were detected by the analysis of variance (Table 2).

A reduction in chlorophyll *a* content in sunflower plants was caused by the lowest clomazone concentration. The following higher concentration caused a reduction in carotenoid content but did not affect chlorophyll *b* content. However, when applied at rates ≥ 1 mg a.i./kg, clomazone reduced the content of all pigments

Table 2. Influence of different concentrations of clomazone in soil on physiological parameters (ANOVA)

Plant pigments	Plant species					
	Maize		Sunflower		Barley	
	$F_{(10,33)}$	p	$F_{(10,33)}$	p	$F_{(10,33)}$	p
chlorophyll *a*	498.93	<0.05	251.11	<0.05	196.30	<0.05
chlorophyll *b*	165.40	<0.05	155.80	<0.05	122.79	<0.05
carotenoids	162.31	<0.05	98.75	<0.05	175.04	<0.05

by over 70%, as follows: chlorophyll *a* (76.75-98.87%), chlorophyll *b* (75.19- 98.76%) and carotenoids (70.13-91.91%). The analysis of variance confirmed that differences between treatments and the control were statistically significant for all three parameters.

Barley plants did not respond by pigment content reduction to the three lowest clomazone concentrations, while significant reduction in chlorophyll *b* content was registered at 1mg a.i./kg of clomazone concentration. Greater reduction in chlorophyll *a* (41.94-98.13%), chlorophyll *b* (29.45-98.15%) and carotenoid (33.23-96.48%) contents was registered only at higher application rates (2-12 mg a.i./kg). The analysis of variance showed that differences between treatments and the control were significant for all three parameters.

The I_{50} values for each measured parameter and all three plant species were calculated by regressional analysis of the data obtained for plant pigment content depending on clomazone concentration. Suitability of this regressional model was evaluated by determination coefficient (R^2) (Table 3).

Table 3. I_{50} values and corresponding determination coefficients for all parameters of tested plant species

Plant pigments	Plant species					
	Maize		Sunflower		Barley	
	I_{50} (mg/kg)	R^2	I_{50} (mg/kg)	R^2	I_{50} (mg/kg)	R^2
chlorophyll *a*	4.06	0.98	1.14	0.82	4.72	0.96
chlorophyll *b*	4.71	0.95	1.46	0.82	5.07	0.98
carotenoids	5.10	0.97	1.59	0.76	4.65	0.97

The calculated I_{50} values, as well as the reductions in pigment contents, indicate that sunflower is slightly more sensitive than maize and barley, while no significant difference in sensitivity was registered between the latter two.

DISCUSSION

Fresh and dry weight of shoots and/or roots, as well as shoot and/or root height and length are the most commonly measured parameters in bioassays for evaluating crop sensitivity to herbicide residues in soil. In the case of highly sensitive plant species, the inhibition of these parameters is detected even at very low concentrations of a tested compound and it gradually increases with the increase in herbicide concentration. It enables detection of a regressional dependence between any given parameter and the applied herbicide concentrations. This test of maize, sunflower and barley sensitivity showed that inhibition of the measured morphological parameters was present only at higher concentrations of clomazone, which indicates that these species are not highly sensitive to its residual activity in soil. These results are in accordance with those reported by Gallandt et al. (1989), who opined that determination of a regressional dependence between measured parameters and clomazone concentrations could not be based on shoot biomass alone. Furthermore, the experiments conducted by Loux et al. (1989a) showed that inhibition of wheat root and shoot growth was not dependant on increasing clomazone concentrations, so that the parameter could not be used to develop an appropriate bioassay methodology. Scott et al. (1994) studied the effects of different concentrations of clomazone on the content of chlorophyll and carotenoids, as well as on height and fresh weight of shoots, using seedlings of tomato and pepper. Although clomazone caused a high inhibition percentage in the synthesis of total chlorophyll and carotenoids in that research, no significant change in height and fresh weight of the shoots of these plant species was registered.

For a reliable and valid bioassay, and for confirmation of the sensitivity of tested plant species to certain herbicides, it is necessary to monitor morphological parameters, as well as those that occur as a result of the herbicide mode of action. Having in mind the mode of action of clomazone, the effect of applied concentrations on pigment contents (chlorophyll *a* and *b*, and carotenoids) was assessed.

The data show that all three tested plant species responded with pigment content reduction even at low concentrations of clomazone. These three plant species may therefore be considered as good indicators of clomazone presence in soil, yet without an effect on growth and development. Based on such response, the tested plants can be categorized as moderately sensitive to clomazone. Sunflower showed high sensitivity regarding the lowest I_{50} value and the reduction in all pigments was over 70% when clomazone was applied at the rate of 1 mg a.i./kg. Maize and barley displayed somewhat lower sensitivity (I_{50} values were at the same level for these two but three times higher than the I_{50} for sunflower). Maize plants expressed a significant decrease in the content of all pigments (> 70%) at the clomazone

concentration of 6 mg a.i./kg, while significant decrease in the content of all pigments (> 60%) was registered in barley plants at 8 mg a.i./kg concentration. The data are in agreement with the I_{50} values calculated for pigments in all three plant species, indicating that it is sufficient in sensitivity assessment to measure only one of these parameters (content of carotenoids - depending on the mode of action).

The available literature offers no data to be appropriately used for comparison with the results in this study. The fact is that no damage has been detected on sunflower and barley plants so far. However, data on maize are numerous and their common feature is that visible damage (chlorosis) may occur early in vegetation, but without any effect on yield (Gunsolus et al., 1986; Mills et al., 1989; Mills and Witt 1989; Curran et al., 1991, 1992; Monks and Banks, 1991; Walsh et al., 1993). These facts support the results of our study regarding low sensitivity of sunflower, maize and barley to clomazone. As for grass species (closely related to barley), wheat and oat have been reported to show similar sensitivity, manifested as a chlorosis type of damage (Gallandt et al., 1989). In field experiments with wheat as the following crop in rotation, either plants were not damaged at all, or damage was found only in the early stages of crop development but without any effect on yield (Gunsolus et al., 1986; Loux et al., 1989b; Ahrens and Fuerst, 1990, Krausz et al., 1992, 1994; Miller, 2003). Similar data have been reported for oats (Gunsolus et al., 1986; Walsh et al., 1993). Many researchers emphasize the fact that data on the sensitivity of various crops to clomazone vary depending on organic matter content in different soils, which directly affects the adsorption, mobility and persistence of clomazone, but also its bioavailability. In a wheat bioassay study, Loux et al. (1989a) found the EC_{50} value to be up to 3.5 times higher in soil that contained 5.8% organic matter, as compared to the EC_{50} value in soil having 1.3% organic matter. Similar results were reported by Gallandt et al. (1989) using the same test plant in experiments with soils which contained 1.6 and 2.3% organic matter, although the differences were not considerable. As the results in this study indicate lower bioavailability of clomazone, we believe that, although this herbicide has longer persistence in soils with higher organic matter contents, phytotoxicity to sunflower, maize and barley should not be expected when the crops are sown one year after clomazone application. This raises a question of sensitivity of these species in soils that have different properties, as found in various production areas of our country.

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Osetljivost kukuruza, suncokreta i ječma na rezidualno delovanje klomazona u zemljištu

REZIME

U radu je ispitivana osetljivost kukuruza, suncokreta i ječma na rezidualno delovanje klomazona u zemljištu tipa ilovače, metodom biotesta. Klomazon je primenjen u seriji koncentracija 0,12-12 mg a.s./kg zemljišta. Nakon 14 dana rasta biljaka mereni su morfološki (visina, sveža i suva masa izdanka) i fiziološki parametri (sadržaj karotenoida, hlorofila *a* i hlorofila *b*). Konstatovano je da morfološki parametri nisu pouzdano merilo osetljivosti na klomazon. Prema ostvarenim procentima inhibicije za merene fiziološke parametre izračunate su vrednosti I_{50}, a na osnovu njih utvrđene su razlike u osetljivosti ispitivanih biljnih vrsta. Najosetljiviji je bio suncokret, dok se kukuruz i ječam nisu međusobno značajno razlikovali.

Ključne reči: Klomazon, kukuruz, suncokret, ječam, biotest, černozem