

Cucumber susceptibility to simulated soil residues of clomazone

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SUMMARY

A laboratory bioassay was conducted to investigate the susceptibility of cucumber to the residual activity of clomazone in loamy and sandy soils. The effects of three different levels of soil moisture (20, 50 and 70% field capacity - FC) was also examined. Clomazone was applied in concentration series of 0.047-6 mg a.i./kg soil. Plants grew for 21 days, upon which period physiological parameters (content of carotenoids, chlorophyll *a* and chlorophyll *b*) were measured.

Clomazone caused leaf bleaching and reduction in pigments content in cucumber leaves in both types of soil at all levels of soil moisture, and the degree of change depended on application rates. Inhibition was higher in plants grown in the sandy soil, while the least sensitive were cucumber plants grown in both types of soil with 20% FC. The findings in this study showed moderate cucumber susceptibility to clomazone residues in loamy and sandy soils.

Keywords: Cucumbers; Clomazone; Residues; Pigments content

INTRODUCTION

Herbicide persistence is an important consideration in crop production since residues can potentially injure sensitive crops grown in rotation. Damage to high-value vegetable crops can result in substantial economic losses. Residues can cause great variability in plant growth and quality and, in worse-case scenarios, they can cause complete crop loss and significant economic cost to growers. The length of time over which herbicide residues persist in soil depends on the herbicide (method of degradation, water solubility and rate of application)

and the site characteristics (soil type, rainfall and temperature). The benefits of applying a persistent herbicide include season-long weed control and fewer pesticide applications.

Clomazone (IUPAC: 2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone) is used in row crops including soybean, tobacco, oil seed rape and some vegetable crops (Lee et al., 2004; Pesticidi u poljoprivredi, 2016). Clomazone is taken up by plant roots and emerging shoots and is transported with the transpiration stream in the xylem to plant leaves (Ferhatoglu & Barrett, 2006). This is the only commercial herbicide

known to inhibit carotenoid synthesis upstream from phytoene desaturase. Actually, clomazone is inactive, but its metabolite ketoclomazone inhibits DXS (1-deoxy-*d*-xylulose 5-phosphate synthase), the thiamine diphosphate-dependent enzyme that catalyzes the first step in the MEP (2-*C*-methyl-*d*-erythritol 4-phosphate) pathway (Müller et al., 2000; Ferhatoglu & Barrett, 2006). This results in impaired chloroplast development and pigment loss in susceptible plants. When carotenoids are absent and plants are exposed to light, singlet oxygen and triplet chlorophyll degrade chlorophyll and initiate membrane lipid peroxidation, causing a bleached appearance and white, yellow or light-green plants (Hess, 2000).

Soil factors influence clomazone activity. The adsorption and availability of clomazone have been highly and positively correlated with soil organic-matter content, and less highly correlated with clay content. Clomazone activity is the highest in sandy soils where organic carbon levels and cation exchange capacity are low (Gunasekara et al., 2009). The persistence of clomazone is significantly affected by environmental factors, such as moisture content, and the degradation process is therefore slower when precipitation, as well as temperature, are lower (Kirksey et al., 1996; Cumming et al., 2002). Clomazone is relatively persistent in soil, its half-life reported for field and laboratory experiments ranges from 15 to 153 days (EFSA, 2007). Due to slow clomazone dissipation in some soils, a potential risk of carryover injury to rotational crops is a concern. Many examples of vegetable injury from clomazone residues have been reported (Miller, 2003; Frost & Barnes, 2003; Nurse et al., 2006; Soltani et al., 2006).

There is a need for a simple method for detecting clomazone residues prior to planting sensitive crops in order to assist with crop rotation planning and to prevent crop failures and economic losses. Bioassays are often used to determine the presence of phytotoxic levels of herbicide residues in soil. Therefore, the aim of this study was to assess the susceptibility of cucumber,

as a potential following crop in rotation, to different amounts of clomazone residues in loamy and sandy soils of variable moisture in laboratory bioassays.

MATERIAL AND METHODS

Soils were sampled from 10 cm depth in areas with no history of herbicide use in the last ten years. The samples were air dried at room temperature and passed through a 3 mm sieve. Selected properties of the soils used in this study are listed in Table 1. Field capacity (FC) moisture content was estimated by determining the volume of water required to completely wet the air-dried soil to the bottom of a plastic vial. The soils received water until the wetting front reached the bottom of the vial but did not have free standing water at the bottom (Eliason et al., 2004).

Cucumber seeds (Jazzer F1, Enza Zaden) were used in the assay. Technical grade clomazone (Shenzhen, China, 95% pure) was obtained from Galenika Fitofarmacija, Serbia. A series of solutions containing clomazone at concentrations of 0.047, 0.094, 0.19, 0.38, 0.75, 1.5, 3 and 6 mg a.i./kg soil was prepared. For each treatment a sample of sifted soil (250 g) was measured and placed in a thin layer on a plastic tub. From the prepared solutions of each herbicide 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 3 bars. Control samples remained untreated with the herbicide. After herbicide application, the soil was hand-mixed and transferred to pots, which were then planted with cucumber seeds. In addition, the soils were wetted to moisture content equivalent to the specified percent of FC (20, 50 and 70% FC). Plants grew for 21 days in a climate chamber with the following conditions: photoperiod 14 h/10 h, temperature 26°C/day and 21°C/night, humidity 60-70% and light intensity 300 $\mu\text{E}/\text{m}^2\text{s}$. Throughout the experiment, soil moisture was constantly maintained at the defined FC levels.

Table 1. Selected properties of soils used for the study of clomazone phytotoxicity

Soil (type)	Soil (location)	pH	Texture			
			Humus	Sand	Silt	Clay
%						
Loam	Zemun polje	7.17	3.96	49.80	33.40	16.80
Sand	Tavankut	7.63	0.91	91.44	1.32	7.24

Several physiological parameters: contents of carotenoids, chlorophyll *a* and chlorophyll *b*, were measured as indicators of phytotoxicity. The content of pigments was determined by cutting fresh shoots (leaf slices until total weight of 0.1 g) and performing extraction with 3 ml of *N,N*-dimethylformamide (DMF). The solutions were made in the dark at 4 °C over 24 hours. After that period, extract absorption was measured by visible spectroscopy at 480 nm wavelength for carotenoids, 664 nm for chlorophyll *a* and 647 nm for chlorophyll *b* (spectrophotometer LKB Biochrom Novaspec II 4040, Great Britain). A formula according to Wellburn (1994) was used to calculate the concentration of pigments (mg/ml), and it was then converted to mg/g fresh leaf weight.

The effects of clomazone concentrations on these parameters were evaluated by the analysis of variance followed by Tukey's HSD test at the 5% level of significance. Statistical analysis was made in StatSoft 8.0. The data were used for a regression analysis to estimate the EC₅₀ (i.e. the effective concentration of clomazone that reduced pigments content by 50%) using the software package BIOASSAY97 (Onofri, 2005).

RESULTS AND DISCUSSION

The inhibiting effect of clomazone on pigments (carotenoids, chlorophyll *a* and chlorophyll *b*) content in cucumber varied depending on the investigated soils. In plants grown in loamy soil with 20% FC, concentrations ≤ 1.5 mg a.i./kg soil caused little or no inhibition of all measured parameters. Statistically significant reduction in the content of pigments (52.6–83.7%) was detected for the two highest concentrations of clomazone (Figure 1-3). The reduction in pigments contents in loam containing 50% FC was below 29% for the concentrations ranging from 0.047 to 1.5 mg a.i./kg. The concentrations of 3 and 6 mg a.i./kg caused 56.3–76.5% reduction, compared to control plants. Under moisture conditions of 70% FC, a significant reduction (32.3–62.3%) in chlorophyll *b* content was detected at concentrations ≥ 0.75 mg a.i./kg. However, greater reductions in carotenoids (44.1–45.8%) and chlorophyll *a* (44.7–50.1%) were revealed for the higher (3 and 6 mg a.i./kg) application rates (Figure 1-3).

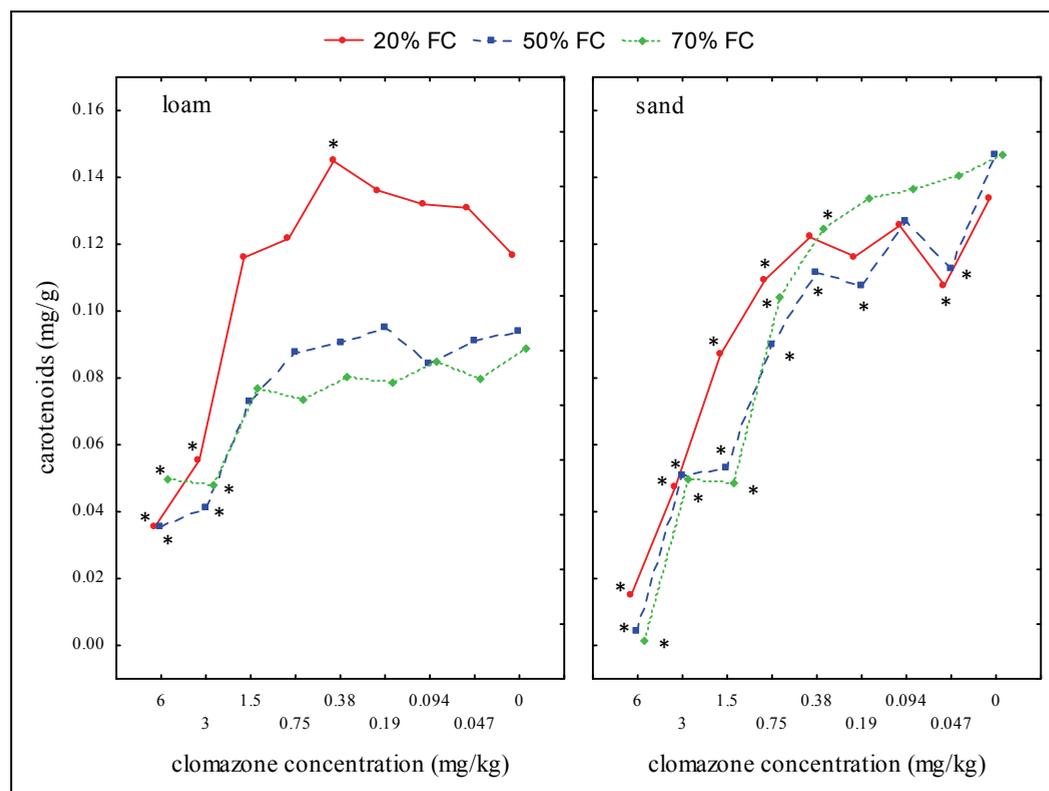


Figure 1. Changes in carotenoids content in cucumber leaves affected by clomazone residual activity in loamy and sandy soils with different soil moisture percentage

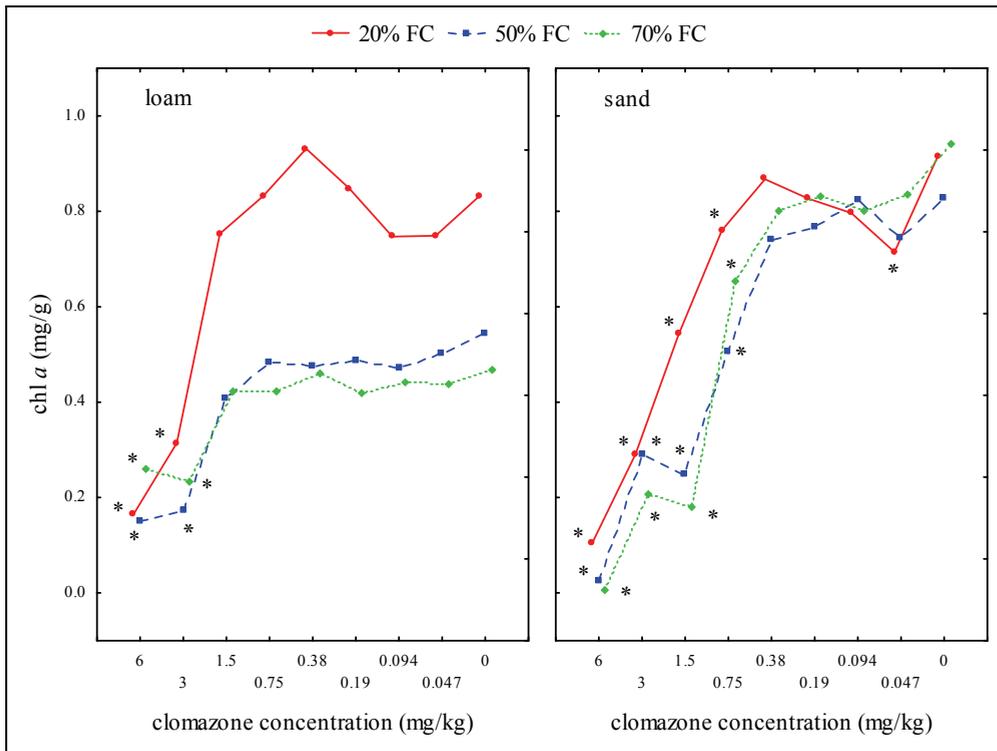


Figure 2. Changes in chlorophyll *a* content in cucumber leaves of affected by clomazone residual activity in loamy and sandy soils with different soil moisture percentage

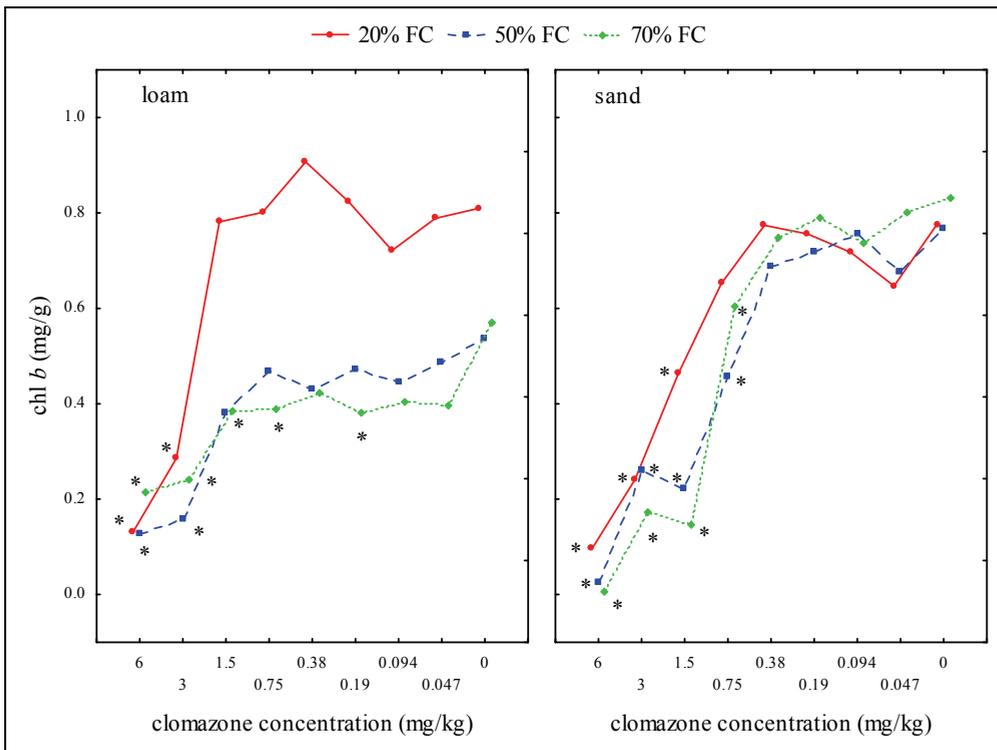


Figure 3. Changes in chlorophyll *b* content in cucumber leaves affected by clomazone residual activity in loamy and sandy soils with different soil moisture percentage

Cucumber grown in sandy soil, at all three levels of moisture, showed greater susceptibility to clomazone residues since the measured parameters were inhibited at all concentrations. The reduction in carotenoids, chlorophyll *a* and *b* was below 30% for the concentrations ≤ 0.75 mg a.i./kg soil. The concentration of 1.5 mg a.i./kg in this type of soil with moisture maintained at 20% FC reduced pigments content in a range of 34.8-40.7%. On the other hand, at the higher moisture levels, the same concentration resulted in 63.8-70.9% reduction in soil with 50% FC, while it was even more prominent (67.1-82.2%) at 70% FC.

The highest clomazone concentration (6 mg a.i./kg) reduced the content of pigments by over 85% as follows: in soil with 20% FC (87.6-88.9%), 50% FC (96.7-97%) and 70% FC (99-99.2%).

Inhibition of the measured parameters of cucumber, in the present study, was followed by shoot injury, expressed as foliar chlorosis (bleaching) and necrosis of tissue, symptoms characteristic of clomazone injury (Figures 4 and 5). In many trials with different herbicide rates and soil types, injuries from clomazone residues on sensitive rotational vegetable crops have been noticed.



Figure 4. Bleaching of cucumber due to clomazone residues in loamy soil with 70% FC

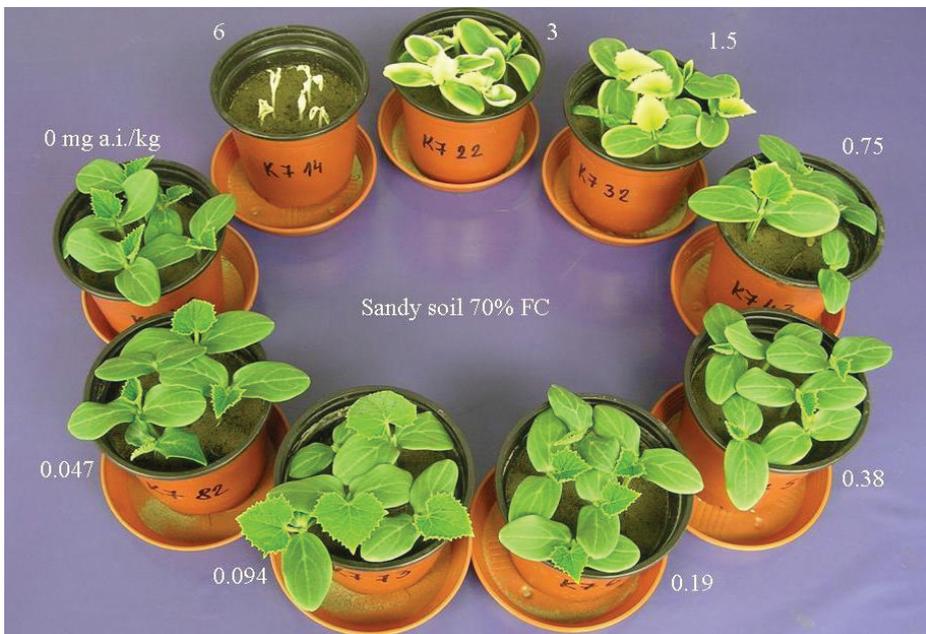


Figure 5. Bleaching of cucumber due to clomazone residues in sandy soil with 70% FC

The results are in correlation with a previous study by Scott et al. (1994) who found that different concentrations of clomazone caused inhibition of the synthesis of total chlorophyll and carotenoids in tomato and pepper seedlings. Also, Nurse et al. (2006) reported bleaching injury in transplanted tomato plants at all doses tested in field experiments. The injuries were observed as bright, white leaf chlorosis. Similarly, Barth et al. (1995) recorded a decrease in pigments content in pumpkin leaves coupled with significant deterioration of fruit quality, despite the fact that clomazone is registered for pumpkin protection in the USA. Later, Keinath and DuBose (2000), as well as Harrison Jr. and Keinath (2003) reported injuries of pumpkin and squash plants, whereby damage rate primarily depended on the plant cultivar.

Overall, there is a lack of literature data comparable with the results obtained in our present study as different methods were used for investigating the effects of clomazone residues on cucumber susceptibility. While our study was carried out in the laboratory, most researchers have conducted tests in the field where clomazone had been applied in the preceding year. Thus, Miller (2003) tested clomazone in green pea and its residual effects on several rotational crops (spinach, strawberry, potato, and pickling cucumber) in a three-year trial. He concluded that rotational crops were not affected by the herbicide applied the preceding year, but he pointed out that the medium texture and moderate organic matter content of soil in the study, as well as the moist maritime climate favouring microbial degradation, reduced the probability of herbicide carryover. Similar conclusions were reported by Pornprom et al. (2010) who monitored the effects of residues of clomazone, applied in the preceding year in soybean crop, on cucumber early establishment and growth. The authors did not report any phytotoxic effects, and so concluded that this vegetable can be grown in rotation with soybean. However, it is important to emphasize that the experiments were conducted in early and late rainy seasons in northern Thailand.

As it is known that clomazone activity is highly influenced by soil factors, a full factorial analysis of variance of all three factors (soil type, soil moisture and clomazone concentration) was conducted in this study. The results showed that all of these factors had significant impact on pigments contents (carotenoids: $F=9.25$, $p=0.000$; chlorophyll *a*: $F=8.38$, $p=0.000$; chlorophyll *b*: $F=9.31$, $p=0.000$). Regression analysis was used to determine the dependence of cucumber pigments content on different clomazone concentrations, soil type and levels of soil moisture, and the EC_{50} values were calculated from

that analysis as indicators of plant sensitivity. Considering carotenoids, chlorophyll *a* and *b* content, the EC_{50} values were significantly lower in sandy soil than in loamy soil, while the least sensitive were cucumber plants grown in both types of soil with 20% FC (Table 2).

Table 2. The EC_{50} for pigments contents in cucumber leaves

Soil type	FC (%)	$EC_{50} \pm SD$ (mg a.s./kg)		
		carotenoids	chlorophyll a	chlorophyll b
Loam	20	1.93 ± 0.31	2.28 ± 0.22	2.18 ± 0.30
	50	1.73 ± 0.14	1.70 ± 0.22	1.46 ± 0.33
	70	1.53 ± 0.22	1.83 ± 0.37	1.75 ± 0.31
Sand	20	1.73 ± 0.27	1.57 ± 0.27	1.61 ± 0.17
	50	0.87 ± 0.20	1.05 ± 0.15	1.02 ± 0.15
	70	1.15 ± 0.15	0.95 ± 0.14	1.00 ± 0.11

The results are consistent with clomazone properties regarding its adsorption and availability in soil, which is highly positively correlated with organic matter content and somewhat lower with clay content. The reduction in herbicide activity by soil organic matter results from the bonding of herbicide molecules by lipophilic organic matter surfaces. The highest clomazone activity is in sandy soils where organic carbon levels and cation exchange capacity are low, while its persistence is higher in soils with high organic matter content compared to ones with low organic matter content. Organic matter content is the most important soil property affecting pre-emergence herbicide activity and, therefore, almost all soil-applied herbicide recommendations are based in part on the percent of organic matter in soil (Kirksey et al., 1996; Antonious, 2000; Gunasekara et al., 2009; Pereira et al., 2016).

The effects of soil type on clomazone bioactivity were similar to those found in experiments conducted by Loux et al. (1989). Availability of clomazone in a wheat bioassay was greater in silt loam soil that contained 1.3% organic matter than in silty clay loam soil with 5.8% organic matter. In a field experiment, however, clomazone was more persistent in silty clay loam (EC_{50} was up to 3.5 times higher) than in silty loam soil. Also, Nurse et al. (2006) considered it possible that visual injury of transplanted tomato plants observed during that research was less severe at a site with loam soil (3.8-4.7% OM) versus a site with sandy loam (2.6% OM) because higher clay and organic matter contents caused a larger proportion of clomazone molecules to be adsorbed to clay particles and/or soil organic matter, which reduced

the effective dosage of the herbicide available in the aqueous soil solution in sandy soil

Considering the effect of moisture on clomazone bioactivity in soil, data obtained in this study clearly indicated that this herbicide is more available in soils with higher moisture content, having a greater impact on pigment content decrease. This is consistent with earlier data on soil characteristics and plant-available clomazone estimates where higher soil moisture, lower organic carbon and clay content ensured its more plant-available concentration in soil solutions. Ahrens and Fuerst (1990) conducted a two-year study on clomazone persistence in different soil types and under different meteorological conditions. They concluded that longer persistence, hence higher residual content after a period of time, would be obtained if clomazone was applied during a dry year followed by a year with moderate precipitation. Low soil moisture caused more prominent clomazone persistence, while increase in soil moisture in the next growing season led to an activation of its residues.

Based on the results of this study, and considering clomazone properties regarding its adsorption, persistence and availability in soil, cucumber showed a moderate susceptibility to residues of this herbicide in soil. However, this should be taken cautiously because differences in adsorption can vary substantially with changes in soil type and moisture content within growing seasons.

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Osetljivost krastavca na rezidualno delovanje klomazona

REZIME

Biotest metodom, u laboratorijskim uslovima, ispitivana je osetljivost krastavca na rezidualno delovanje klomazona u zemljištima tipa ilovača i peskuša pri različitim nivoima vlažnosti zemljišta (20, 50 i 70% poljskog vodnog kapaciteta – PVK). Klomazon je primenjen u seriji koncentracija od 0,047 do 6 mg a.s./kg zemljišta. Biljke su rasle 21 dan, a nakon tog perioda mereni su fiziološki parametri (sadržaj karotenoida i hlorofila a i b).

Klomazon je izazvao izbeljivanje listova i smanjenje sadržaja pigmentata u listovima krastavca u oba tipa i na svim nivoima vlažnosti zemljišta, a stepen ispoljenih promena je zavisio od koncentracije herbicida. Veći stepen osetljivosti ispoljile su biljke koje su gajene u peskovitom zemljištu, dok su na oba tipa zemljišta najmanje inhibicije utvrđene kada je vlažnost zemljišta održavana na 20% PVK. Na osnovu dobijenih rezultata, krastavac se može svrstati u grupu biljaka koje su umereno osetljive na prisustvo ostataka klomazona u zemljištima tipa ilovača i peskuša.

Ključne reči: Krastavac; Klomazon; Ostaci; Sadržaj pigmentata