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Persistence of pendimethalin residues in Swiss chard

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Abstract: The aim of this study was to determine the persistence of pendimethalin and its impact on Swiss chard, as the succeeding crop in the crop rotation, using the bioassay method, by means of the morphological parameters measurement. In applying the tested concentrations of pendimethalin from 0.2475 to 3.96 ppm, the inhibition of certain measured parameters of the Swiss chard tested plants was demonstrated: root growth suppression in the range of 3.91 to 55.47 %, root fresh weight reduction from 5.56 to 55.56 %, reduction of shoot fresh weight from 2.2 to 44.35 % and the reduction of fresh weight of the whole plant in the range of 2.5 to 46.17 %. Inhibition of the fresh root weight a the most sensitive parameter in relation to the applied rates of the herbicide, was demonstrated, while the shoot fresh**-**weight is a parameter that was not sensitive enough. Significant growth inhibition of all measured parameters was observed at pendimethalin concentrations above 0.99 ppm.
Key words: pendimethalin, inhibition, persistence, chard**.**

Introduction

Pendimethalin is a selective herbicide of the dinithroaniline class that plants absorb by roots and leaves. It inhibits the formation of microtubules by which the

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cell division is ceased, after which plants dye immediately after germination (Janjić, 2005). Unlike other compounds, these compounds do not accumulate in plants and their organs. They are leached fast from the soil or are degraded in the process of denitration to the non-toxic final products (Xu et al., 2007). Pendimethalin degrades in the soil relatively slowly and this depends on the soil properties and climatic conditions (DT-50 = 72 to 172 days). Degradation in the soil is done by oxidation of the 4-methyl group to carboxylic acid, dealklisaton of the amino groups and by the reduction of the nitro group (Kramer and Schirmer, 2007; Chopra et al., 2015).

The herbicide pendimethalin can be applied in three different ways (Kramer and Schirmer, 2007): 1) it can be incorporated prior to sowing, 2) after sowing but prior to emergence and 3) prior to transplanting or early after emergence in order to suppress annual grass and broad-leaved weeds in cereals, vegetables, planted fruits and lawns. The objective of this study was to observe persistence of pendimethalin by using the bioassay method, as well as its effects on Swiss chard as a succeeding crop in the crop rotation.

Materials and methods

The trial with the herbicide pendimethalin (*N*-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine), preparation Zanat, intended for the commercial use (330 g a.i./l) was carried out under controlled conditions with the non-treated soil from experimental plots with degraded calcareous chernozem of the Maize Research Institute, Zemun Polje. Table 1 presents soil properties.

Table 1. Properties of calcareous chernozem in the location of Zemun Polje.

0-20-cm depth	Hygroscopic moisture %	**Fractions**					
		Sand 1.0-0.005 mm		Silt 0.05-0.002 mm		Clay <0.002 mm	
	3.97	3.15		60.31		36.54	
	Chemical properties						
	% CaCO3	pH		% Humus	% Nitrogen	Easy accessible P_2O_5 mg/100g	Easy accessible K_2O mg/100g
		in H_2O	in KCL				
	3.97	8.23	7.53	4.46	0.212	4.19	21.69
	Water and physical properties						
	Bulk density g/cm^3	Density g/cm^3	Porosity %	Capacity		Wilting moisture %	Accessible water %
				Field capacity %	Air %		
	1.41	2.7	47.77	38.85	8.92	17.42	21.43

Soil is low permeable with a coefficient of filtration of $6.83x10^{-5}$ cm/s at the 0-20-cm depth. Swiss chard was used in the trail as a test plant. Prior to performing the trial the following series of solutions of pendimethalin of certain concentrations (calculated per 1 kg soil) was prepared: 0.2475 ppm; 0.495 ppm; 0.99 ppm; 1.98 ppm and 3.96 ppm. The concentration of 3.96 ppm of active substance corresponds to the maximum concentration applied in the field, plastic- or glass-house and corresponds to the amount of 6 l of preparation per hectare. The trial was performed in four replications and each replication in three different pots (200 ml in volume) with the appropriate control treatments.

The procedure of bioassay started with measuring of 1 kg of the sieved soil for each concentration, followed by measuring of 10 ml of a previously prepared herbicide solution of a certain concentration for the soil treatment. The application is done by a thin layer chromatography sprayer attached to a compressor, hence the complete amount of the herbicide was sprayed by a constant pressure of 1.2 bars and evenly spread over the whole sample soil area. After the application, the soil was first manually mixed, and then in the rotary blender at 60 rpm for 5 minutes. The treated soil was arranged in the 200-ml pots. Six seeds of each test plant were sown and watered with 48 ml tap water (up to field water capacity), and then placed in the growth chamber. Test Swiss chard plats were grown in the chamber for 14 days, on the average, under the following control conditions: 1) 6/8 h day/light regime, 2) temperatures: 22°C (day) and 18°C (night), 3) air humidity: 50% and 4) light intensity: 300 $\mu E/m^2s$. Plants were watered each day with 10 ml water in order to maintain the appropriate soil moisture. After taking out, roots were rinsed under running tap water, and then kept in water for a certain period, in order to remove of soil particles. Immediately prior to measuring, plants were wiped and dried between sheets of cotton paper.

The following parameters were measured in the experiment: root length, fresh weight of both roots and shoots. The initial diameter was determined for the each plant individually, and then the means were calculated. The final value was a mean for all replications. Remaining two parameters were measured as a cumulative value for all plants of one replication, and final value was a value for all replications. Parameters related to the weight are mean values obtained from the relation of the total weight of all plants in a given treatment and the number of plants. Based on obtained results, achieved inhibitions of measured parameters were estimated. Results of bioassay were read on Swiss chard plants after 13 days. Seeds were not additionally dressed, which resulted in sporadic occurrence of fungi of genera *Pythium* and *Fusarium.* The average values were calculated for each pot, each replication and a total mean value for each treatment and control.

The regression $\mathbf{y = a + b*x}$ was used to describe the dependence of changes in measured parameters on changes in pendimethalin concentrations:

y – effect, i.e. % of inhibition for a measured parameter.

a – average level of inhibition in the pendimethalin concentration equals null.
b – regression coefficient (also determines the slope of the regression line), i.e. how much is an average change in % inhibition if pendimethalin concentration increases by 1 ppm.
x – pendimethalin rate.

Based on the regression equation, the ED_{50} values were estimated for all measured parameters. The statistical package Microsoft Office Excel 2003 was used for the estimation of regression equations and graphic presentation of the results.

Results and Discussion

Based on obtained results (Table 2) it is observable that the higher herbicide concentration was the lower germination was, and also the growth of Swiss chard was inhibited.

Table 2. Results - calculated mean values of the measured parameters by the specified concentrations of pendimethalin.

Concentrations	Root length (mm)	Root fresh weight (g)	Shoot fresh weight (g)	Plant fresh weight (g)
Control	32	0.018	0.093	0.111
0.2475 ppm	30.75	0.017	0.09025	0.10725
0.495 ppm	27.5	0.0145	0.0875	0.102
0.99 ppm	26	0.01325	0.084	0.09725
1.98 ppm	20	0.01075	0.06725	0.078
3.96 ppm	14.25	0.008	0.05175	0.05975

Etiolation or whitening of Swiss chard leaves occurred at the lowest herbicide concentrations. Swiss chard plants in the soil with higher herbicide concentrations were subjected to the attack of soil pathogens. Inhibition of the following parameters of Swiss chard test plants was expressed when pendimethalin was applied in the concentration ranging from 0.2475 to 3.96 ppm: reduction in root growth in the range from 3.91 to 55.47 % (Figure 1), reduction in fresh root weight (5.56-55.56 %. Figure 2), reduction in fresh shoot weight (22-44.35 %, Figure 3) and reduction in fresh weight of the whole plant (2.5-46.17 %, Figure 4).

Figure 5 presents the effect of pendimethalin on inhibition of all measured parameters in Swiss chard. In relation to the applied herbicide rates, inhibition of fresh weight of root was the most sensitive parameter. Based on the regression equation, ED_{50} values were estimated for all measured parameters (Table 3).

Table 3. ED_{50} of measured parameters.

MEASURED PARAMETERS	**ED_{50} (ppm)**
Root length	3.2921
Root fresh weight	3.17668
Shoot fresh weight	4.29494
Plant fresh weight	4.0886

Measurements of length and fresh weight of roots and shoots show that the fresh weight of roots was the most sensitive parameter with the strongest inhibitory effect by the herbicide.

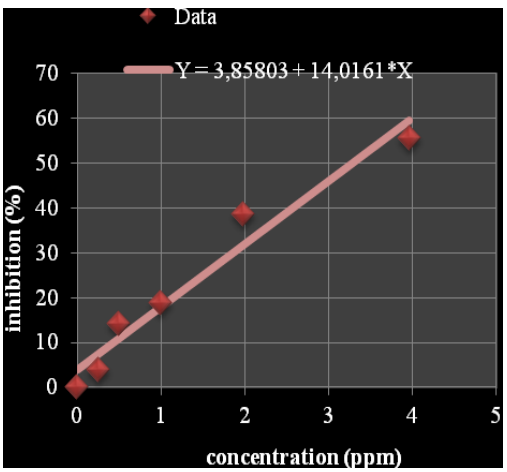

Figure 1. The impact of pendimethalin on inhibition of Swiss chard root length. ED_{50}= 3.2921 ppm

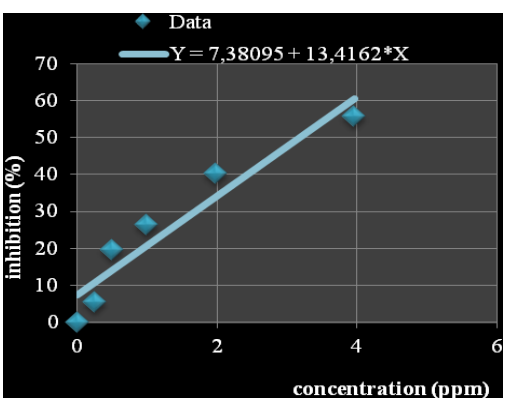

Figure 2. The impact of pendimethalin on inhibition of Swiss chard root fresh weight. ED_{50}= 3.17668 ppm

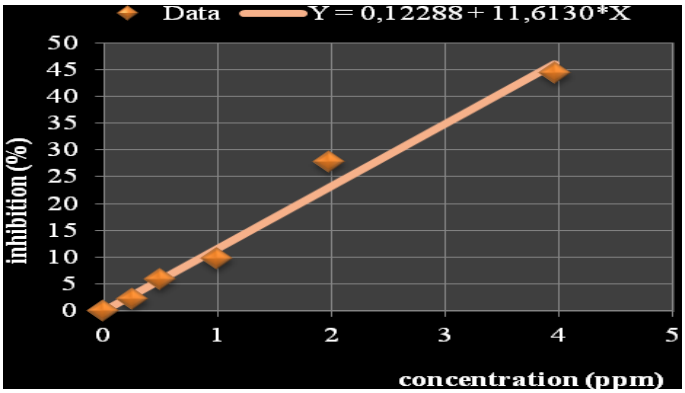

Figure 3. Influence of pendimethalin on inhibition of Swiss chard shoot fresh weight. ED_{50}= 4.29494 ppm

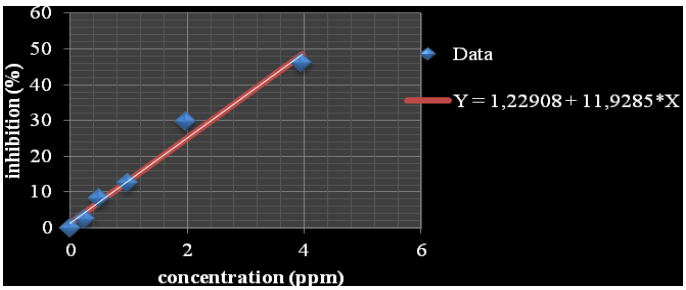

Figure 4. The impact of pendimethalin on inhibition of Swiss chard plant fresh weight. ED_{50}= 4.0886 ppm

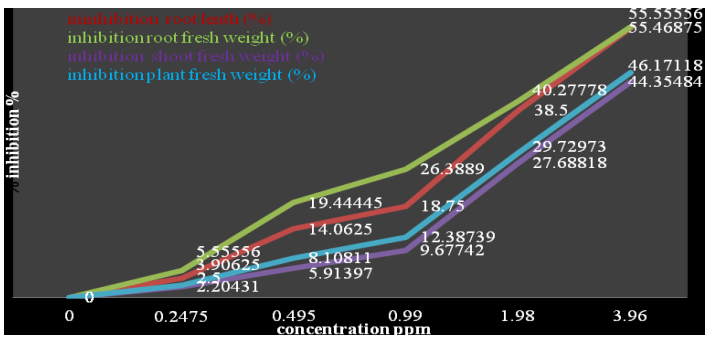

Figure 5. The impact of pendimethalin on inhibition of measured parameters in Swiss chard.

The significant increase in inhibition of all measured parameters is observed for the pendimethalin concentrations above 0.99 ppm, while lower concentrations had smaller effects on the reduction of measured parameters. Inhibition of the fresh weight of shoots showed to be the most unreliable parameter, especially with concentrations below 0.99 ppm, when a significantly slower increase of inhibition with increased pendimethalin concentrations was observed. At the same time, the fresh weight of shoots had the greatest ED_{50} value (4.29 ppm). The root length was a relatively reliable parameter, particularly for the concentrations above 1.98 ppm and ED_{50} values for this parameter. According to

the stated, it is observable that the fresh weight of roots was the most sensitive parameter, although the root length provided reliable enough response.

Pendimethalin residues in the soil, determined by the bioassay under controlled conditions, can have phytotoxicity effects on Swiss chard (Shanmugasundaram and Kandasamy, 2003; Hatzinikolaou et al., 2004). Furthermore, it was determined that the increase in concentrations increased the expressed detrimental effect of this compound. At the concentration of 3.96 ppm, the following was observed: slight thickening of roots in the zone and a bit bellow the zone of the root collar, then narrowing of the leaf blade, accompanied by brittle leaflets that had become dark green. Furthermore, it was also observed that the higher concentrations of the herbicide resulted in a poorer formation of the secondary root system, inhibited growth and development of the whole plant, as well as a darker colour of leaf blades. In addition, it was noticed that the higher herbicide concentrations were the lower germination and more inhibited growth of Swiss chard were. Moreover, etiolation or whitening of leaves occurred even at the lower herbicide concentrations. Swiss chard plants in soil with higher herbicide concentrations were subjected to the attack of soil pathogens.

Applied herbicides maintain their properties and even their capacity to destroy weeds for a shorter or a longer period. This depends, first of all, on stability of herbicide molecules and this property is called persistence (Kramer and Schirmer, 2007; Sireesha et al., 2012). In certain cases, persistence provides herbicide activity in much longer period of time. Nevertheless, it can, at the same time, present danger for succeeding crops in the crop rotation that are susceptible to the effects of an appropriate herbicide or for humans and domestic animals if there are residues in final products intended for nutrition. Herbicide persistence is not an invariable. After four months of the herbicide application in the full amount of 6 l/ha, which corresponds to the concentration of 3.96 ppm, a half of this concentration, i.e. 1.98 ppm, will still remain in the soil, which is a lethal concentration for the majority of crops in the crop rotation, as well as for weeds susceptible to effects of this herbicide. After eight months, the concentration of this herbicide in the soil will be approximately 0.8 ppm, which is still lethal for very susceptible plant species. This concentration will be about 0.37 ppm after 16 months, which is an amount that inhibits measured parameters in Swiss chard, with the maximum inhibition of root fresh weight of 12.34 % (Xu et al., 2007). Under certain conditions, persistence will increase with the increased rate of the applied herbicide. However, there is not linearity between these two phenomena.

Arora and Tomar (2008) have proven in their studies that observed parameters, such as height, fresh and dry weight of the plant, were significantly reduced under effects of pendimethalin even up to 75 days after its application. The plant height was lowered by 31.0, 29.5, 22.7, 22.0 and 8.5 % in comparison with the control in the course of 15, 30, 45, 60 and 75 days, respectively, after pendimethalin had been applied. Moreover, a similar trend in decreasing of fresh and dry weight was observed. Previous studies suggest that pendimethalin

remains persistent in the soil cultivated with various crops up to 75 days after its application, and after this period there were no detected residues in the soil (Hatzinikolaou et al., 2004; Xu et al., 2007).

Conclusion

According to gained results on inhibition of all measured parameters it can be concluded that Swiss chard is very susceptible to observed compound. Besides, measured parameters differed in their sensitivity. The most sensitive parameter was fresh weight of roots, then root length, while shoot fresh weight was not a sufficiently sensitive parameter. Therefore, sowing of Swiss chard will be profitable in a certain plot if it is performed at least two years after the last application of pendimethalin in the full concentration (3.96 ppm).

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PERZISTENTNOST OSTATAKA PENDIMETALINA U BLITVI

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Rezime

Cilj ovog rada je bio da se utvrdi perzistentnost pendimetalina i njegov uticaj na blitvu kao narednu gajenu biljku u plodoredu korišćenjem biotest metode, merenjem morfoloških parametara. Pri primeni ispitivanih koncentracija pendimetalina od 0,2475 do 3,96 ppm ispoljena je inhibicija određenih merenih parametara blitve kao test biljke: redukcija rasta korena u rasponu od 3,91-55,47 %, redukcija sveže mase korena 5,56-55,56 %, redukcija sveže mase izdanka 2,2-44,35 % i redukcija sveže mase cele biljke u intervalu od 2,5-46,17 %. Kao najosetljiviji parametar, u odnosu na primenjene doze herbicida, pokazala se inhibicija sveže mase korena, a sveža masa izdanka predstavlja parametar koji nije dovoljno osetljiv. Značajni porast inhibicije svih merenih parametara uočava se pri koncentraciji pendimetalina iznad 0,99 ppm.

Ključne reči: pendimetalin, inhibicija, perzistentnost, blitva.