RESIDUAL IMPACT OF THE REDUCED AMOUNT OF MESOTRIONE AND TERBUTHYLAZINE FROM DIFFERENT PREPARATIONS

Maja Meseldžija, Milica Dudić, Miroslava Nežić, Ivana Ninić

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

Abstract
The aim was to determine the sensitivity of the pea plants to the soil residues by subjective evaluation and measuring the height after application of the reduced amounts of the herbicide combination of mesotrione and terbuthylazine. The experiment with tested herbicides was placed at the location of Žabalj (Serbia). After 14 days of application the average plant height was the highest (5cm) in 1/8X of the applied amount of mesotrione 50 g/l + terbuthylazine 326 g/l and then decreased with the increase in the amount, which also applies to the amounts of mesotrione 50 g/l + terbuthylazine 125 g/l, where the highest average height was 5.54 cm. The percentage of plants damage 14 days after treatment of mesotrione 50 g/l + and terbuthylazine in a higher amount 326 g/l was in the range of 38-79%, while after 21 days the percentage of damage was 60-89%. Preparation based on active substance mesotrione 50 g/l + terbuthylazine 125 g/l caused a percentage of damage to pea plants in the range of 30% (1/8X) to 85% (4X), but when peas were in the four trifoliate leaf stage 21 days after the treatment percentage of damage was 60-98%. All treated pea plants were dried after 25 days of setting up the experiment, except the control where the height of plants was 9 cm.

Introduction
The use of pesticides is directed at controlling target organisms, and despite this fact, it is not possible to predict the environmental fate of pesticides. The widespread use, mobility, persistence, and toxicity of pesticides can lead to their dissipation to all elements of the natural environment. Thus, the excessive use of pesticides is still a major problem affecting the quality of the environment [1]. Residual herbicides are useful for long-term weed control, but problematic when the same characteristic leads to injury crops planted in rotation or to natural vegetation in uncropped areas over a long period [2]. The persistence of herbicide depends on the climatic conditions as well as the physical and chemical properties of soil, and in particular on the soil content of organic matter, which limits the transport of active ingredients of herbicides to water [3]. Soil pH affects differently on carryover of the sulfonylurea, imidazolinone, triazolopyrimidine, and triazine herbicides [4]. Herbicide persistence is an important consideration in crop production since the residue of some herbicides can persist to the next growing season and may injure sensitive crops in the rotation [5]. Side effects of pesticides, including herbicides, are a problem that needs to be discussed, especially in the time of their increasing use in the EU countries. One method used to determine if herbicide residues injurious might exist in the soil is to conduct a bioassay. A bioassay is the measurement of a biological response by a living organism to determine the presence or concentration of a chemical in a substrate. This is a direct and simple method to determine if it is safe to seed or plant into areas treated with herbicides. Unlike a chemical assay, a bioassay is unique in that it measures plant susceptibility. Chemical detection identifies a substance, not biological activity [5].
Mesotrione is a member of the triketone family of herbicides [6], and provides pre- and post-emergence control of broadleaf weed species, primarily in maize [7]. Mode of action is to inhibit the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), which affects carotenoid

biosynthesis [8]. Mesotrione is commonly used in formulated preparation alone or in mixture with some other herbicides, atrazine, S-metolachlor, terbuthylazine, and glyphosate. In soil is easily degradable and its biological persistence is the shortest in clay soils, followed by heavy sandy loam and sandy loam types [9]. Mesotrione can be degraded by photolysis at the soil surface and can undergo microbial degradation as it translocates deeper in the soil. Some bacterial species, including *Bacillus sp*., *Pantoea ananatis*, *Bradyrhizobium sp*., and *Escherichia coli* can completely degrade mesotrione [7]. Studies reporting dissipation time of mesotrione usually showed a low persistence in soils, with a half-life time (DT50) ranging from 2 to 34 days [9]. Terbuthylazine is a herbicide belonging to the triazines family [10] and is used for weed control in pre- and post-emergent treatment of a variety of agricultural crops as well as roads, railways, and the industrial area [11]. This chemical interferes with the photosynthesis and in particular at the level of photosystem II. Terbuthylazine has a very high soil persistence, depending on the soil characteristics, it can be present up to 17 months after the field treatments [12]. Residence times of terbuthylazine in soil and aquifer sediments are likely to be long due to slow degradation under unfavorable environmental conditions [13]. In 2011, the European Food Safety Authority (EFSA) provided an extensive peer review of data concerning the environmental behavior and fate, ecotoxicology and toxicology, and risk assessment of terbuthylazine [14]. The European Commission based on these conclusions approved the inclusion of terbuthylazine in Annex I of Council Directive 91/414/EEC and its use only as a herbicide until December 2021 [15].
The aim of this study was to assess the persistence and phytotoxicity of mesotrione and terbuthylazine in the soil or to determine the effect of their residues on the growth and development of pea (*Pisum sativum* L.) on the basis of the obtained results.

Experimental

The study was conducted at locality in Žabalj (Serbia) on 19 July and lasted 28 days. For this experiment were used two different preparations Calaris pro (50 g/l mesotrione + 326 g/l terbuthylazine) and Tvister (50 g/l mesotrione and 125 g/l terbuthylazine), water, soil taken from the area where herbicides were never applied, pots and pea seeds. All treatment were set in three repetitions, including a control with soil not treated with herbicides. The preparations were applied in 6 different amounts each separately, where 500g of soil were treated with the tested amounts of herbicides, and by mixing the resulting mixture was equally distributed in pots, in which the pea was sown. The pots were in semi-controlled conditions, they had a constant light and they were daily watered. The preparations were applied in the recommended amount (X), reduced amounts (1/8X, 1/4X, 1/2X), as well as in two and four times higher amounts (2X and 4X). The evaluation was done after 14, 21 and 28 days after treatment. Efficacy treatment was assessed by a linear subjective scale of 0 - 100%, where 0% indicates that there are no changes in the plants, while 100% indicates the complete destruction of plants.

Results and discussion

Treatment results of the assessment by linear subjective scale damage as well as by measuring the height of the pea plants 14, 21 and 28 days after the treatment with herbicides are shown in Figures 1-3.

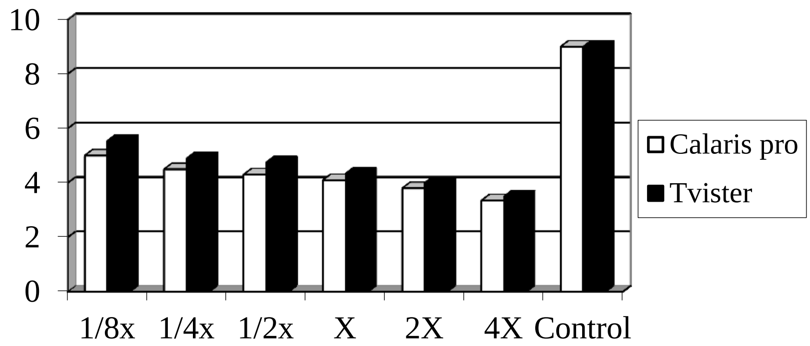

Figure 1. The average height of pea plants 14 days after treatment

Based on the results of the assessment 14 days after the application of the herbicide, the average plant height was the highest in 1/8X of the applied amount of mesotrione 50 g/l + terbuthylazine 326 g/l (5 cm), and then decreased exponentially by increasing the amount of the applied preparation, which also applies to the second preparation based on active substance mesotrione 50 g/l and terbuthylazine 125 g/l, where the highest average plant height was 5.54 cm. In the variant where 4 times higher amount than the recommended was used, the height of plants was 3.35 cm with the use of the mesotrione 50 g/l and terbuthylazine in a higher amount 326 g/l, and 3.5 cm for the application of the mesotrione 50 g/l + terbuthylazine 125 g/l. Non-treated plants had an average height of 9 cm.

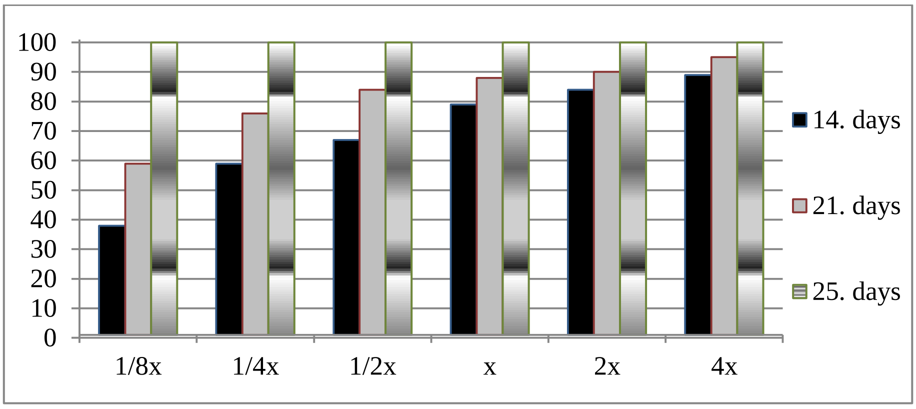

Figure 2. The percentage of plants damages 14, 21, and 28 days after application of mesotrione 50 g/l + terbuthylazine 326 g/l

From fig.2 it can be seen that 14 days after treatment with herbicides mesotrione 50 g/l + terbuthylazine 326 g/l the percentage of plants damage was in the range of 38%, with the application of 1/8X and up to 79% where four times higher amount was applied. In control, pea plants were in the 2 leaf phase. Phytotoxic effect on plants was manifested as bleaching and leaf chlorosis. When the peas were in the four trifoliate leaf stage, 21 days after treatment the increase in damage was determined and also the progressive effect of the herbicide. The percentage of damage was 59% with the application of 1/8X, and with the increase in the amount an increase in damage was also found (60-89%).

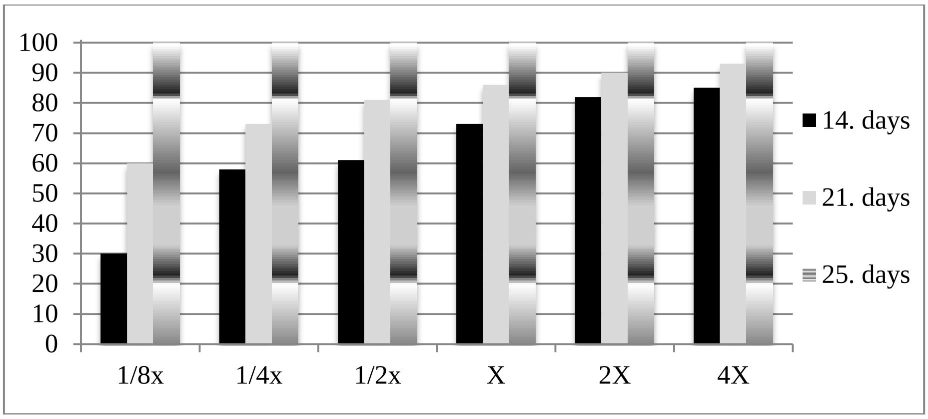

Figure 3. The percentage of plants damages 14, 21, and 28 days after application of mesotrione 50 g/l + terbuthylazine 125 g/l

The percentage of plants damage were in the range of 30% (a 1/8X application) and up to 85% where four times higher amount was applied. On the second assessment (21 days after treatment) the increase in damage was determined and also the progressive effect of the applied herbicides. Applying 1/8X amount of herbicides mesotrione 50 g/l + terbuthylazine 125 g/l, the percentage of damage was 60%, and with the increase in the amount, an increase in damage was also found (73-98%).

Conclusion

In this study, all applied amounts of both preparations showed phytotoxic effects on pea plants. The average plant height was the highest in 1/8X of the applied amount of mesotrione 50 g/l + terbuthylazine 326 g/l (5cm), and then decreased exponentially by increasing the amount, which also applies to the mesotrione 50 g/l + terbuthylazine 125 g/l where the average height was 5.54 cm. The percentage of plants damage 14 days after treatment was in the range of 38-79%, while after 21 days the percentage of damage was 60-89%. Herbicides mesotrione 50 g/l + terbuthylazine 125 g/l caused a percentage of damage to pea plants in the range of 30% (1/8X) to 85% (4X), while after 21 days percentage of damage was 60-98%. After 25 days of setting up the experiment, all treated plants were dried, except for control plants that had a height of 9 cm.

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