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# Sensitivity of oilseed rape (*Brassica napus* L.) to soil residues of imazethapyr herbicide

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## Abstract

Imazethapyr is one of imidazolinone herbicides that may leave residual activity in the soil for extended periods causing injury and yield reduction of susceptible crops in rotation. An experiment was conducted in order to study the sensitivity of oilseed rape (*Brassica napus* L.) to imazethapyr soil residues. Experimental type was completely randomized design with three replications. Treatments included Imazethapyr simulated concentrations residuals in soil (0.002, 0.004, 0.008, 0.012, 0.02 and 0.04 mg. $kg^{-1}$soil). Shoot and root biomass production was measured 30 days after emergence. Oilseed rape responses to imazethapyr residues was fitted with sigmoidal 3 and 4 parametric equations to the root and shoot biomass data as a function of the herbicide residue concentrations and was used to calculate the doses for 50% inhibition of root and shoot growth ($ED_{50}$). Results showed that the oilseed rape shoot and root dry matter were significantly affected by increasing imazethapyr soil residue ($p<0.01$). This reduction in root dry matter was severe than shoot dry matter, where root $ED_{50}$ (0.0019 mg.$kg^{-1}$soil) was less than shoot dry matter (0.0025 mg.$kg^{-1}$soil). Considering the high sensitivity of oilseed rape to imazatepara residues, attention is needed to provide oilseed rape cultivation in fields with a history of imazateaper application.

**Keywords:** Dry matter, Herbicide, Imidazolinone, Pesticide, Sensitivity

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## Introduction

Weed interference with agricultural plants is considered as one of the most important factors affecting crop yields and therefore weed control in agricultural land is of great importance. Persistence of herbicides in soil could be useful due to increasing the effectiveness control of weeds which alternately grow in fields but damage to sensitive crops in rotations (Mehdizadeh et al., 2016; Melo et al., 2016) and also their inhibitory effects on soil microorganisms is a negative consequence of this phenomenon (Sebiomo et al., 2011; Sathiyavani et al., 2015). Hence, understanding the factors and mechanisms that determine the persistence and degradation of herbicides, by providing management strategies, could be used to ensure the health of agro ecosystems. Herbicides with active residues in the soil are compounds that, due to their toxic residues, affect the growth of the plant in the next seasons (Mehdizadeh and Gholami-Abadan, 2018). Imidazolinone are one of the groups of herbicides that are easily absorbed by the root and foliage of the plants and accumulate in the meristem tissues and reduce the growth, the formation of necrosis and chlorosis, and finally the death of plant. Imazethapyr is one of the imidazolinone herbicides that are widely used for control of broad-leaved weeds in many crops (Webster et al., 2018). This herbicide inhibits the acetylacetate synthase enzyme, which plays an important role in the biosynthesis of branched-chain amino acids, valine, leucine and isoleucine (Zabalza et al., 2007). Imazethapyr is a relatively high persistant herbicide in the soil and its half-life has been reported for about 60 to 90 days in agricultural soils (Vencill, 2002). The persistence of these herbicides in the environment may increase the duration of weed control period, but it can cause damage to non-target organisms and sensitive crops in the rotation and also endangers human and environment health. Gaston et al. (2002) reported that with increasing imazethapyr concentration in soil, growth of root meristems, dry weight and fresh weight, and chickpea yield significantly decreased. Wiatrak et al. (2009) evaluate the performance of imazapic simulated residues on cotton plant and observed that growth, yield quantity and quality of cotton were significantly affected by the herbicide residues. Moyer and Esau (1996) found that sugar beet was affected by imazethapyr herbicide even one year after application. Alister and Kogan (2005) reported that the residues of imazapyr herbicides with imazaquin and imazethapyr reduced the yield of barley, oats, chickpeas, sugar beet and tomatoes one year after application.

Residues of herbicides in the soil could be measured by analytical or bioassays methods. Analytical methods are very specific and sensitive and just quantifying the total amount of herbicide residues in the soil (Grimalt and

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Dehouck, 2016; Janaki et al., 2018). However, these methods are costly, time-consuming, requiring complex equipment and organic solvents for the extraction process (Sondhia and Singh, 2018). Bioassay is a relatively simple and inexpensive method that can measure toxic portions of the herbicides residues in the soil. Therefore, it seems that the use of analytical methods such as high performance liquid chromatography (HPLC) and plant bioassay method complement each other and can greatly help researchers to identify and quantify herbicide residues in the soil (Mehdizadeh et al., 2017). In this regard, Szmigielska and Schoenau (1999) reported that the use of plant bioassay method was more sensitive than ion exchange extraction method in evaluating the imazethapyr residues in soils. They detected very low concentrations of herbicide by bioassay method. Since the oilseed rape has been used as a reference plant to evaluate herbicides residues, this experiment was conducted to evaluate its sensitivity to simulated concentrations of imazethapyr herbicide in soil using bioassay method.

**Materials and Methods**

A greenhouse experiment was conducted during 2016 in a completely randomized design with 3 replications. The treatments consisted of different concentrations of imazethapyr in soil (0, 0.002, 0.004, 0.008, 0.012, 0.02 and 0.04 mg. $kg^{-1}$soil), which the effects of these concentrations on root and shoot biomass of oilseed rape were evaluated. For this purpose, after preparation of a soil with equal ratio of sand, soil and leaf mould, the aqueous stoke solution of 1000 ppm of imazethapyr was prepared and the remaining concentrations were prepared by dilution of stoke solution. Then, by using the syringe pipette, the calculated amount of herbicide solution was mixed up with the soil and poured onto the soil surface of the pots and after evaporation of the solvent (water) was completely mixed with the upper layer of the soil. Then 10 seeds of oilseed rape (Hyola cultivar) were planted at appropriate depth in every pot. During the experiment, the pots were irrigated uniformly. One week after the emergence of plants, five plants were thinned in each pot. After 30 days the plants were harvested and separately dried in an oven at 60 °C for 48 hours. Then, the root and shoot dry weight was measured. The data was subjected to analysis of variance using the MSTAT-C software. Mean comparisons were performed using Duncan Multiple Range Test (DMRT) set at 0.05. Oilseed rape response of dry weight of roots and shoot per pot (Y) were described by a three parameter log-logistic regression model as a function of imazethapyr doses, x:

$$Y = \frac{d}{1+\exp\{b(\log(x)-\log(e))\}}$$

Where $d$ is the upper asymptote (maximum biomass per pot), which is close to the untreated control, $b$ denotes the slope of the curve around the $e$, which denotes the dose that inflicts a 50% biomass reduction relative to $d$.

**Results and Discussion**

Based on the findings of experiment, the response of plant roots and shoot biomass to the simulated imazethapyr residues follows the logistic model which is accordance with results from other studies (Halloway et al., 2006; Santin-Montanya et al., 2006). The results showed that the imazethapyr residues in concentrations of 0.002-0.04 mg. $kg^{-1}$ soil significantly reduced the oilseed rape root and shoot biomass (P<0.01) (Table 1).

According to the results, damage amount of root and shoot dry weight increased significantly with increasing imazethapyr concentration in soil, so that at the concentration of 0.002 mg.$kg^{-1}$ soil, approximately 54% of root biomass and 45% of shoot biomass were reduced, while, at 0.122 mg. $kg^{-1}$ soil, the root and shoot losses of oilseed rape were 100%.

**Table 1.** Analysis of variance of oilseed rape root and shoot dry matter to imazethapyr residues in soil.

| Variation Resources | Df | Shoot Dry Matter | Root Dry Matter |
|---|---|---|---|
| Herbicide doses | 6 | 4181.71** | 4040.24** |
| Error | 14 | 5.887 | 3.716 |

** Significant difference at P<0.01

**Table 2.** Mean comparison of rapeseed root and shoot dry matter in different levels of imazethapyr residues.

| Herbicide doses (mg. $kg^{-1}$ soil) | Shoot Dry Matter (% to control) | Root Dry Matter (% to control) |
|---|---|---|
| 0 | $100^{a}$ | $100^{a}$ |
| 0.002 | $54.6^{b}$ | $46.3^{b}$ |
| 0.004 | $39.2^{c}$ | $31.2^{c}$ |
| 0.008 | $18.4^{d}$ | $12.5^{d}$ |
| 0.012 | $0^{e}$ | $0^{e}$ |
| 0.02 | $0^{e}$ | $0^{e}$ |
| 0.04 | $0^{e}$ | $0^{e}$ |

*Significant differences are denoted by different letters within each column at P<0.05 according to Duncan's Multiple Ranges Test.

**Table 3.** Parameters estimated by 3-parameter sigmoidal logistic equation to rapeseed root and shoot dry matter to imazethapyr residues in soil.

| Traits | B | D | $ED_{10}$ | $ED_{50}$ | $ED_{90}$ |
|---|---|---|---|---|---|
| Shoot Dry Matter | 1.256(0.033)* | 99.577(6.12) | 0.000608(0.0002) | 0.0025(0.0004) | 0.0106(0024) |
| Root Dry Matter | 1.216(0.028) | 99.808(4.50) | 0.000446(0.0002) | 0.0019(0.0003) | 0.0086(0.0016) |

* Standard error

In biodegradation studies of herbicide residues in soil, $ED_{10}$, $ED_{90}$ and especially $ED_{50}$ are used to determine the sensitivity of plants to herbicide residues in soil. According to the results, the $ED_{50}$ parameter for root biomass (0.0019 mg. $kg^{-1}$ soil) was lower than the shoot biomass (0.0025 mg $kg^{-1}$ soil), which indicates greater sensitivity of the root to residues of this herbicide in the soil (Table 3).

Bresnahan et al. (2000) Found that the residue of imazethapyr herbicide in very low concentrations (0.5 to 3 μg. $kg^{-1}$ soil) also resulted in damage to different crops. Other studies also highlighted the high sensitivity of oilseed rape to herbicides residues in the soil. Mehdizadeh (2016) reported that oilseed rape is very suitable for use in bioassays for evaluating the side-effects of triasulfuron at low concentration rates. Mansoori et al. (2008) observed that residues of sulfosulfuron herbicides in the soil resulted in toxicity and reduced oilseed rape yield in rotation with wheat. They reported that increasing sulfosulfuron application rate from 42 to 52 g a.i. $h^{-1}$ increased oilseed rape yield losses from 13.5 to 17.5 percent. It seems that in evaluating the imazethapyr herbicide residues, the use of root biomass parameters of sensitive plants such as oilseed rape, can be very accurate and effective due to the inhibitory effects of herbicide on cell division of root meristemic areas in susceptible plants. Therefore, Szmigielska et al. (2008) have used the mustard root bioassay as an appropriate biological indicator in evaluating the flucarbazone herbicide residues. Since roots are directly exposed to the herbicide residues, it seems to be more sensitive than shoots in response to herbicide residues. So that in this study, the sensitivity of oilseed rape root biomass was greater than that of the shoot biomass (Tables 2, 3 and Figure 1).

In bioassay studies of herbicides residues in soil, root growth of plants is one of the most important indicators in assessing the sensitivity of plant species to herbicide residues and determining the probable amounts of their residues. In this regard, Halloway et al. (2006) reported that the sensitivity of lentil root growth to metsulfuron methyl residues was an appropriate index for determining the possible residues of this herbicide in agricultural soils. They reported that, despite the inability of analytical methods to detect device the herbicide residues, lentil root bioassay test was a suitable measure for the detection of metsulfuron methyl residues. Szmigielska et al. (2008) found that mustard root bioassay was more effective than analytical methods in determining the flucarbazone herbicide residue in soil. They reported that mustard root bioassay had more than 88% of the acceptable results in determining the potential flucarbazone residues. The results of this study indicate that there is a difference in the sensitivity of oilseed rape root and shoot biomass to imazethapyr residues in the soil, suggesting that the evaluation of imazethapyr bioassay using oilseed rape root biomass could be used as an appropriate indicator for determining the residues of this herbicide.

## Conclusion

In general, based on the findings of this study, application of imazethapyr even in very low concentrations (0.002 mg. $kg^{-1}$ soil), it has a high potential for damage to oilseed rape plant, and it is possible to use oilseed rape, and especially the root biomass of this plant as a suitable parameter for determining the residues of this herbicide in agricultural soils. Hence, limitation in crop rotations could be one of the most important problems associated with the application of imazethapyr in soil. Although other factors can influence the persistence of herbicides in the soil, but according to the results of this study, it seems very important to consider the non-sensitive crops in rotation in fields that previously treated with imazethapyr. In this regard, it is recommended to perform analytical methods for determining the concentration of imazethapyr residues after harvest and comparing results with bioassay methods.

## Conflict of interest

The author confirms that there are no known conflicts of interest associated with this study.

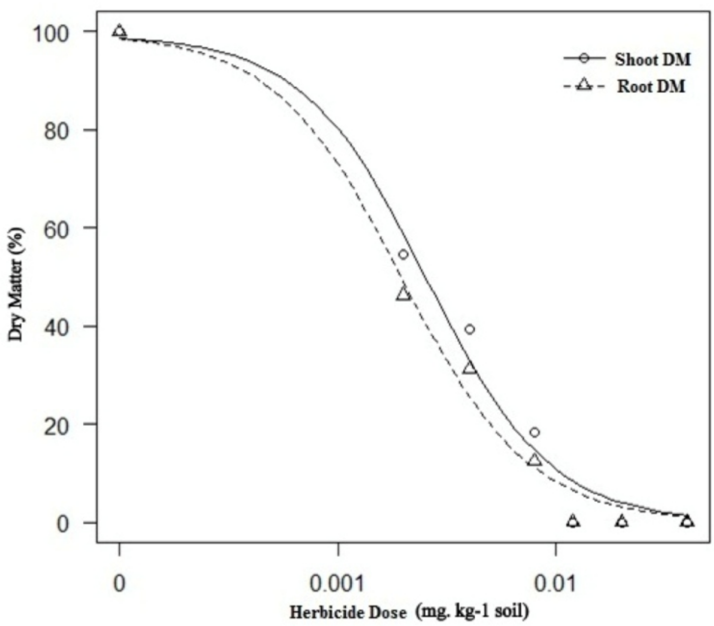


**Figure 1.** Rapeseed root and shoot dry matter response to imazethapyr residues in soil.

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