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Original Article

Study of Changes in Activity of Wheat Antioxidant Enzymes under Stress Residue of Imazethapyr Herbicide

Rasoul Fakhari[1*], Ahmad Tobeh[1], Mohammad Taghi Alebrahim[1], Mohammad Mehdizadeh[1], Hossein Karbalaei Khiavi[2]

[1]Department of Agronomy and Plant Breeding, Faculty of Agriculture and Natural resources, University of Mohaghegh Ardabili, Ardabil, Iran
[2]Plant Protection Research Department, Ardabil Agricultural and Natural Resources Research and Education Centre, AREEO, Ardabil, Iran

*Corresponding Author E-mail: r.fakhari@uma.ac.ir

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ABSTRACT

In order to investigate physiological and biochemical changes of wheat under stress residue of Imazethapyr herbicide (Imazethapyr), an experiment was conducted in a completely randomized design with four replications in greenhouse conditions at university of Mohaghegh Ardebili. The treatments consisted of five dose levels of herbicide Imazethapyr (0, 0.011, 0.022, 0.033 and 0.044 micrograms of herbicide) per kilogram of soil. The analysis of variance showed that the content of the main and auxiliary pigments of leaf, proline, sugar content, protein, activity of catalase enzymes, peroxidase, polyphenol oxidase, root and shoot dry weight were significantly affected by herbicide. The results showed the Imazethapyr herbicide stopped the production of valine leucine and isoleucine amino acids. As a result, the rapid reduction in the volume of these amino acids led to a reduction in protein synthesis in wheat plant. Therefore, the application of doses of 0.011, 0.022, 0.033 and 0.044 micrograms of herbicides decreased 14, 26, 44 and 47 percent of leaf protein content, respectively, compared with control treatment. Further, theapplication of these doses resulted in the reduction of 2.8, 5, 27 and 64% of activity of catalase enzyme, 3.5, 39, 49 and 52% peroxidase enzyme activity and 13, 24, 35 and 46% activity of polyphenol oxidase enzyme, compared with control treatment. The results revealed that Imazethapyr herbicide activates wheat antioxidant enzymes to reduce plant tolerance from damage caused by herbicide residues; therefore, it can be used as a marker or index of herbicide damage rate in physiological research.

Key words: Carotenoid, Catalase, Peroxidase, Proline

Introduction

Wheat is one of the most important crops in the world, accounting for about 17 percent of the world's agricultural land. It is the source of food supplies of about 40 percent of the world's population (Peng *et al.*, 2011), containing about 21% calories and 20% protein (Braun *et al.*,

2010). In Asia, soybean plants are usually planted with wheat in rotation (Sondhia, 2015). The area under cultivation and the available agricultural land is not much increased. Therefore, identifying the factors that reduce or increase the yield of this crop can be effective in developing a way to increase production per unit area. In this regard, pest control and especially weed control is of particular importance in increasing production efficiency. It is necessary to achieve the optimum level of suppression of weed growth using special management methods. In Iran, herbicides have become one of the largest agricultural technology tools. In addition, the significant increase over the last four decades in the production of crops is partly due to the use of these compounds. The herbicide of Imazethapyr is a systemic herbicide, a group of Imidazolinones that has been registered for soybean culture. This herbicide inhibits the production of acetylacetate synthase (ALS) or acetic acid syntase (AHAS), which inhibits the production of branched amino acids, leucine and isoleucine, causing plant deaths (Hoseiny Rad *et al*., 2011).

Imazethapyr in soybean is preferably used in post-emergence and in the spring and depending on the type of soil it can stay active on the soil for at least four months. It also affects newly grown plants. However, the relatively long shelf life of this herbicide in the soil will increase the length of the weed control period. Therefore, the damage to the crops in the next alternation such as wheat, as one of the important consequences of using this herbicide has drawn the attention of farmers and experts in recent years. The stress of herbicide residues leads to the production of reactive oxygen species (ROS) (Reade *et al*., 2002). Free radicals such as O^{-}_{2} super oxides, H_2O_2 hydrogen peroxide and OH-hydroxyl radicals that are caused by plants in response to stress can cause damage to the DNA, lack of protein structure, chlorophyll depletion and Lipids of peroxidation (Fruest and Norman, 1991). Free radicals act both as a marker in addition to damage to plant cells and activate the existing living defense responses to the stresses applied (Arora *et al*., 2002). In plant tissues, two catalase and ascorbate peroxidase enzymes play an important role in eliminating hydrogen peroxide (H_2O_2). The catalase enzyme can convert H_2O_2 in the cell into O_2 and H_2O without needing a reducing agent (Damanik, 2012). The role of ascorbate peroxidase (APX) as an antioxidant is also the transformation of H_2O_2 molecules into water molecules. In this regard, several studies have been carried out on different herbicides, which in addition to their results, has been shown to change the antioxidant activity of plants against the use of herbicides in different sources (Wang *et al*., 2004; Song *et al*., 2000; Peixoto *et al*., 2006; Song, 2007). In a trial, an increase in the dose of chlorothoruron herbicide increased the activity of the enzyme catalase and the ascorbate peroxidase enzyme in wheat (Song, 2007). In a trial aimed at the effects of Tribenuron-methyl herbivores on rye and oat plants, it was found that with increasing herbicide doses, the activity of the enzyme catalase increased (Gar'kova *et al*., 2011). Researchers have acknowledged that increased catalase activity in plants has been responding to H_2O_2 accumulation in plant tissues. In other experiments, increased activity of catalase in response to herbicides of paracuat (Štajner *et al*., 2004), glyphosate (Apel *et al*., 2004) and Norflurasol (Jung, 2003) has been reported. In an experiment on the effect of granular herb on wheat, corn, rye and oat plants, it was found that ascorbate peroxidase (APX) increased the activity of the enzyme ascorbate peroxidase (APX) by increasing herbicide (Gar'kova *et al*., 2011). Plant response to herbicide stress depends on various parameters such as herbaceous species, herbicide mechanism, environmental conditions, plant growth stage, tissue under the influence of herbicide. In a probe into the effects of Tribenuron-methyl herbicide on wheat, corn, rye and oat plants, it was reported that in wheat and rye plants, the activity of the enzyme superoxide dismutase (SOD)

decreased with increasing herbicide, but in corn, the amount of enzyme activity (SOD) increased with increasing dosage of herbicides (Gar'kova *et al*., 2011). In this experiment, it was determined that the highest activity of SOD enzyme was in corn while the least activity was related to wheat plant. Starch is one of the most important products of the photosynthesis cycle in plants made during the day with carbon fixation and converts starch into soluble sugars during the night to stabilize the metabolism cycle (Stettler *et al*., 2009). The results of the experiments showed that Imazethapyr herbicide increased the amount of starch and soluble sugars in chickpea (Royuela *et al*., 2000; Gaston *et al*., 2002). Another study reported that Imazethapyr reduced starch content and increased glucose levels in soybeans (Scarponi *et al*., 1996). In the report, the use of glyphosate herbicides increased the amount of proline in the bean plant (El-Taybe and Zaki, 2009). Various reports have revealed that the reduction of chlorophyll content against the use of herbicides can be considered as an important indicator in the discussion of herbicide residues on periodic products (Yang *et al*., 2005; Wang *et al*., 2004; Song, 2007). In the experiment by Yin (Yin *et al*., 2008) on evaluating doses (2, 5, 3, 5, 10 and 20 mg/kg soil) of Isoproturon herbicide, it was found that the application of herbicide to wheat plant significantly reduced its chlorophyll content so that even at the lowest herbicide dose, chlorophyll content decreased by 11% compared with control treatment. Since herbicide Imazethapyr is one of the most important and widely used herbivores in soybean culture due to the effect of herbicide residues on crop rotation with wheat in Iran and especially in Moghan region, this study was conducted to investigate the response of the physiological effects of wheat to the herbicide residue.

Experimental

Materials and methods

In order to study the physiological and biochemical changes of wheat under stress residue of Imazethapyr herbicide, an experiment was conducted in a completely randomized design with four replications in greenhouse conditions at university of Mohaghegh Ardebil. The treatments consisted of five dose levels of herbicide Imazethapyr (0, 0.011, 0.022, 0.033 and 0.044 micrograms of herbicide) per kilogram of soil (equivalent of 0, 25, 50, 75 and 100 percent of the recommended herbicide for soybean cultivation). The choice of the doses used in the experiment was based on the application of 1 liter of commercially available Imazethapyr herbicide with SL 10% formulation for soybean. The test soil was selected from a field that had not previously been sprayed with any herbicide with its physical and chemical properties as shown in Table 1. After air drying the soil samples and separating rocks and additional materials in it, soil samples were transferred to pots with a diameter of 15 cm. Then, a stock solution of 1000 mg/L of herbicide was prepared from its dissolution in water, and the remaining doses for the herbicide were prepared by dilution of the mother solution. Next, using the pipette, the calculated amount of herbicide solution was picked and mixed with the soil, and poured onto the soil surface of the pots. It was then completely mixed with the top layer of the potting soil. After that, 10 seeded wheat cultivars of *N*-80-19 Fall were cultivated uniformly at appropriate depth at each pot after sterilization. During the experiment, controlled conditions were carried out at a temperature of 15 to 20 °C and a cycle of 16 hours of light and 8 hours of darkness and irrigation of the pots. One week after emergence, the plant was thinned into five plants per pot. After 30 days, seedlings were removed from the crown and transferred to the laboratory. Measurement of leaf proline content was done using Bates *et al*., (1973) method.

The sugar solution was measured by phenol sulfuric method (Dubois *et al.*, 1956). Catalytic activity was measured by the method of Comak and Horst (1991). Khan method (Khan, 1975) was used to measure the polyphenol oxidase enzyme. The absorbance of the enzymatic extract was read at 410 nm. The amount of protein was measured by the method of Bradford (1976). Statistical analysis of the data was done using SAS software and Excel charts were used to analyze the test results.

Table 1. Physical and chemical properties of the soil from the experimental field

Organic carbon (%)	soil Texture	Salinity (ds/m^{-1})	K (ppm)	P (ppm)	N (%)	Clay (%)	Silt (%)	Sand (%)
0.78	Sandy-loam	0.388	320	4.5	0.064	8	32	60

Results and discussion

The main and auxiliary pigment content

The results of analysis of variance showed that the content of the main and auxiliary pigments of the wheat under the influence of Imazethapyr herbicide increased significantly (Table 2). Therefore, the changes in the content of the main and auxiliary pigments showed that the increase of herbicide dose reduced the content of chlorophyll a, chlorophyll b and carotenoid in wheat (Figures 1, 2 and 3). The results showed that wheat plant had a low level of resistant to the trace residues of imazethapyr herbicide; however the content of the main and auxiliary pigmentation of the leaf severely decreased with increasing the herbicide residues. These results indicate a high sensitivity of wheat to the residues of imazethapyr (Figure 1, 2 and 3). The results showed that the application of doses of 0.03, 0.022, 0.033 and 0.044 micrograms of herbicides reduced the content of chlorophyll a, 15, 17, 21 and 24%, chlorophyll content by 27, 32, 35 and 37% respectively. b, and 42, 57, 59 and 61% of leaf carotenoid content were compared to the control (Figures 1, 2 and 3). In a study aimed at examining the residues of Pendimethalin, Pretilachlor, Triasulfuron, Ethoxysulfuron, Pyrazosulfuronethyl, Carfentrazone-ethyl and 2,4-D on wheat plant, we showed that the remnants of these herbicides reduced the chlorophyll content of the wheat plant (Zahan *et al.*, 2018). In another experimentonthe effects of doses of 0.01 to 10 mL of Glyphosate herbicide, the doses were found to reduce the chlorophyll content of chickpea and wheat leaves (Basantani *et al.*, 2011). The results of this study showed that the use of herbicide reduced the chlorophyll a content by 26%, the chlorophyll b by 27% and the carotenoid by 63% compared with the Compared with no herbicide application treatment (Maria *et al.* 2014).

Table 2. Analysis of variance of wheat traits under the influence of Imazethapyr herbicide in soil

S.O.V	df	Mean Squares			
		Chlorophyll a content	Chlorophyll b content	Carotenoidcontent	Sugar content
Imazethapyr dose	4	0.041**	0.060 **	0.043**	0.406**
Error	15	0.002	0.003	0.002	0.017
C.V	-	10.57	8.24	13.02	13.76

**, *, ^{ns}: insignificant and significant difference in level of 1 and 5%, respectively

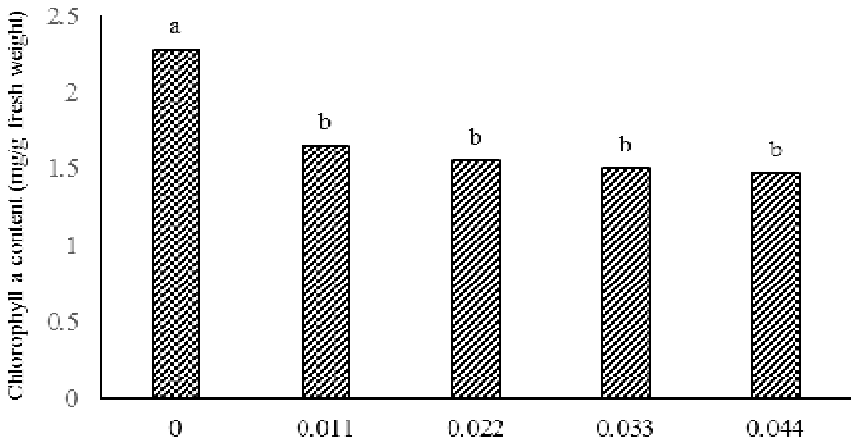


Figure 1. Response of chlorophyll a content wheat plant to the residues dose of Imazethapyr in soil

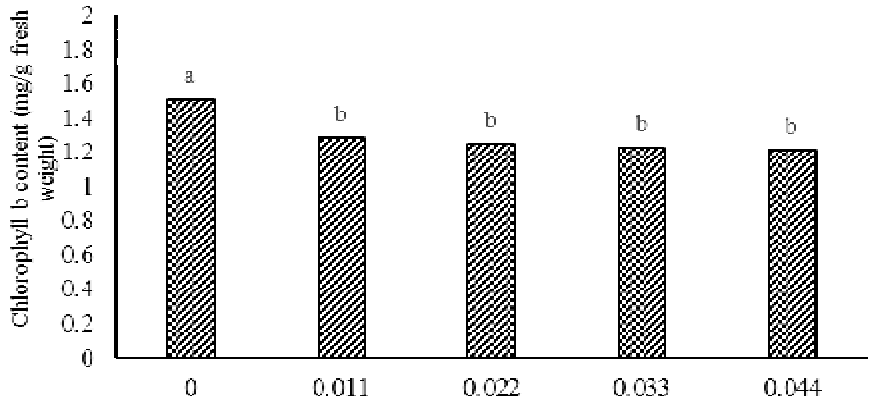


Figure 2. Response of chlorophyll b content wheat plant to the residues dose of Imazethapyr in soil

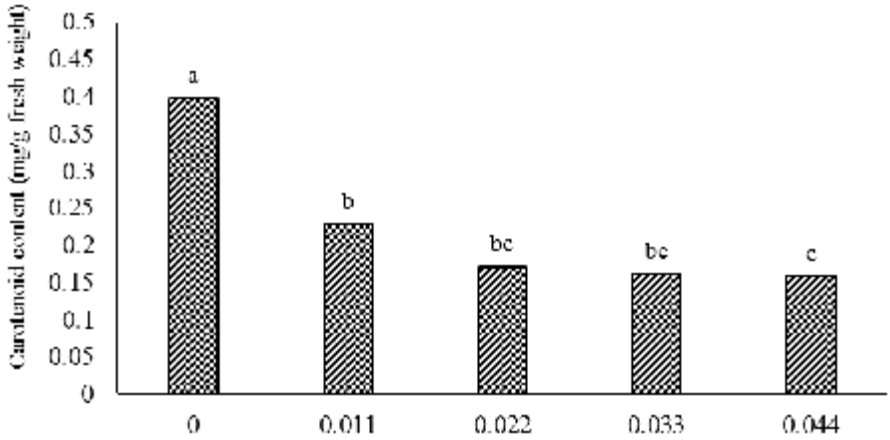


Figure 3. Response of carotenoid content wheat plant to the residues dose of Imazethapyr in soil

Soluble sugars content

The results showed that the effect of Imazethapyr herbicide on the content of soluble wheat leaves was significant (Table 2). Therefore, with increasing dosage of Imazethapyr herbicide, the trait significantly reduced the decreasing trend in the emergence stage (Figure 4). The results of the comparison showed that the application of doses of 0.11, 0.022, 03.03 and 0.044 micrograms of weed grass reduced 56, 71, 77 and 78 percent of the content of leaf soluble sugars compared with the contro Compared with no herbicide application treatment (Figure 4). There are various reports of changes in the antioxidant enzymes of plants due to the use of imidazolinone group herbicides (Pang *et al*., 2003; McCourt *et al*., 2005). In an experimental study, the use of Imazethapyr herbicide reduced the amount of soluble sugars in Arabidopsis thaliana leaves (Qian *et al*., 2011). As Singh *et al*., (2010) concluded from their research, the increase in the amount of damage to the oat plant resulted from increasing the dose of Trifluralin herbicide. This result was also supported by the increase in the herbicide Imazethapyr cache. This finding is consistent with the research by Parish *et al*. (Parrish *et al*., 1995) regarding chickpea and barley plants. The researchers reported that as the amount of sulfosulfuron herbicide residues in the soil increased, the damage to both plants planted with wheat increased.

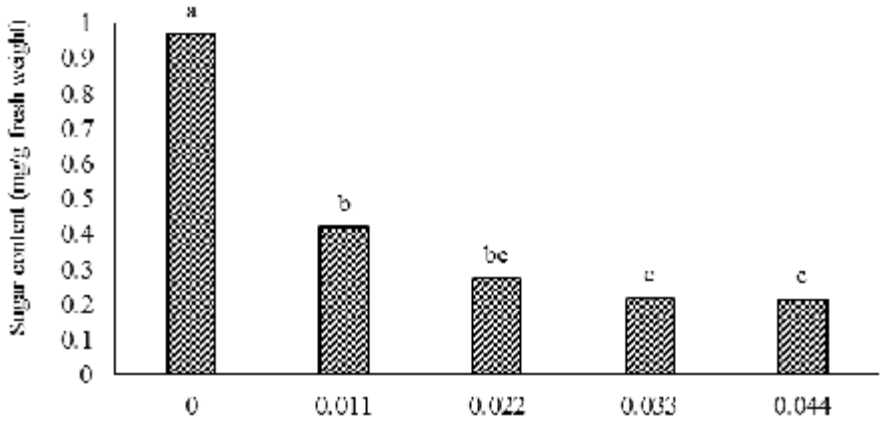


Figure 4. Response of sugar content wheat plant to the residues dose of Imazethapyr in soil

Compatibility metabolites

The results indicated a significant effect of Imazethapyr herbicide on the amount of leaf proline (Table 2). The results showed that from 0 to 0.022 micrograms of application of herbicide, proline content of leaf did not decrease significantly, however, the amount of proline was decreased significantly at 0.033 µg. So the application of doses of 0/011, 0.022, 0.033 and 0.044 micrograms of herbicide decreased 3, 7, 19 and 25 percent of proline leaf content, respectively (Figure 5). It can be concluded that this decrease in the amount of proline in the seedling was due to the stress induced by wheat as a result of the absorption of herbivorous residues by seed lings and the increased production of free radicals after stress in wheat. Proline loss in plant tissue is likely to occur due to decreased protein production under stress conditions caused by herbicide. In the experimental study, the use of Imazethapyr herbicide reduces the amount of amino acids by 15% in Arabidopsis

thaliana (Qian *et al.*, 2011). Proline in plants is known as an antioxidant system against oxidative stress (Radwn *et al.*, 2007; Okuma *et al.*, 2004; Molinari *et al.*, 2007; Demiral and Turkan, 2004; Khedr *et al.*, 2003) because this enzyme is responsible for the removal of oxygen molecules as an antioxidant (Hemaprabha, 2013). In a test with application of 0.5, 1 and 2 times the recommended dose of bentazon herb on peanut, the use of herbicides increased the content of proline in the leaf of this plant whereas with the increase of herbicide dose, the content of proline decreased (Khalaf *et al.*, 2011). Research on the effect of herbicide oxyfluorfen (960 g ha-1) on rice plant revealed that this herbicide caused proline accumulation in plant tissues (Langaro *et al.*, 2017). The researchers believed that the increase in proline was to remove ROS molecules from plant cells, thereby activating the plant's defense system (Molinari *et al.*, 2007). Past investigations showed that Imazethapyr herbicide had a significant effect at 1% probability level on wheat leaf protein content (Table 2). The results of different doses of Imazethapyr herbicide showed that the highest mean content of wheat leaf protein was related to control treatment and the lowest leaf protein content was related to the application of 0.044 micrograms per kilogram of herbicide (Figure 6). The results showed that application of doses of 0.11, 0.22, 03.03 and 0.044 micrograms of herbicides reduced 14, 26, 44 and 47 percent of leaf protein content, respectively (Figure 6). In stress conditions, proteins are stopped and the plant begins to decompose proteins, which results in the accumulation of amino acids in the plant tissue. Since protein molecules exist in all the components of plant cells and play an important role, any reduction in protein levels will cause heavy damage to the growth and development of the plant (Langaro *et al.*, 2017). In the early stages of seedling growth and emergence, the protein breakdown into amino acids is carried out to synthesize new enzymes, cell components, or transfer to the seedling seeding axis of the bud, and almost all of the protein storage of the seed will be used to grow the seed buds (Ashton, 1976). Therefore, it is likely that the Imazethapyr herbicide in the soil after being dissolved in water tends to penetrate into the seeds and plant tissues, which ultimately affects seed emergence and wheat seedling growth (Hoseiny Rad *et al.*, 2011). Imazethapyr is known to be a potent inhibitor of StolectatSynthetase. The phytotoxic effects of this herbicide can stop the production of valine, leucine and isoleucine amino acids, resulting in a rapid reduction in the volume of these amino acids, which in turn will decrease protein synthesis in the seedling (Shaner, 1984). Reducing wheat seedling protein levels suggests that this herbicide prevents protein synthesis in wheat seedling stage. These results are in line with the results of experiments on the effects of butachlor herb on wheat and corn (Nemat Alla *et al.*, 2008), oxyfluorfen on rice (Langaro *et al.*, 2017) and paraquaton Azollamicrophylla (Sood, 2012). In another report with Imazethapyr doses on wheat, it was found that wheat germinating protein content decreased with increasing herbicide (Hoseiny Rad *et al.*, 2011). Another experiment showed that the effects of Bentazone, Imazethapyrand Imazamox on the protein content of Trifoliumresupinatum in the first year were not significant while in the second year, the use of these herbicides had a significant effect on the protein content of this plant (Celen *et al.*, 2006). Antimicrobial synthesis inhibitors (ALS) also interfere with the synthesis of DNA and cell division, which reduces the growth of the roots and stems of the affected plant (Zabalza *et al.*, 2004). The thickening of nucleic acid and the lack of chromosomal separation in Imazethapyr test on Bean (El-Nahas, 2000), Trowbridge and Isopterone on wheat (Kumar *et al.*, 2010) and atrazine on Allium cepa (Jabee *et al.*, 2008) have been proven.

Table 3. Analysis of variance of wheat traits under the influence of Imazethapyrherbicide in soil

S.O.V	df	Mean Squares						
		Prolinecontent	Protein content	Catalase content	Peroxidase content	Polyphenol oxidase content	Root dry weight	Shoot dry weight
Imazethapyr dose	4	0.019*	259.476**	0.164*	13.148**	12.460**	0.092**	0.054**
Error	15	0.071	41.848	0.034	2.047	1.889	0.003	0.003
C.V	-	12.08	15.66	15.46	13.98	12.62	2.44	1.52

**, *, ns: Insignificant and significant difference in level of 1 and 5%, respectively

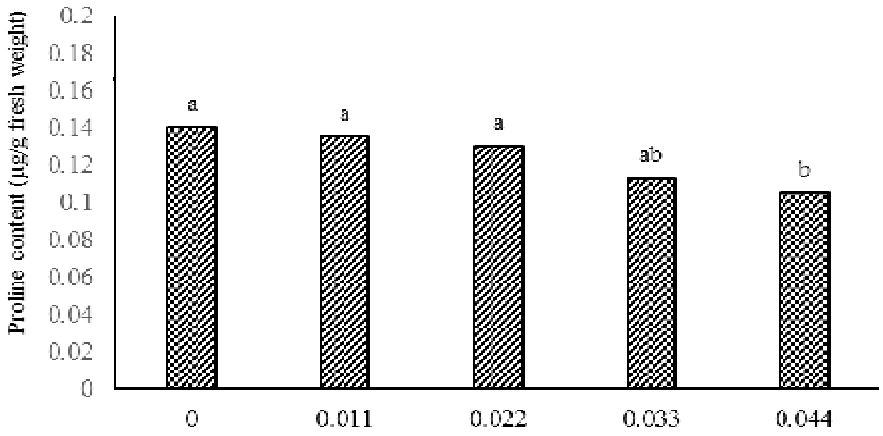


Figure 5. Response of proline content wheat plant to the residues dose of Imazethapyr in soil

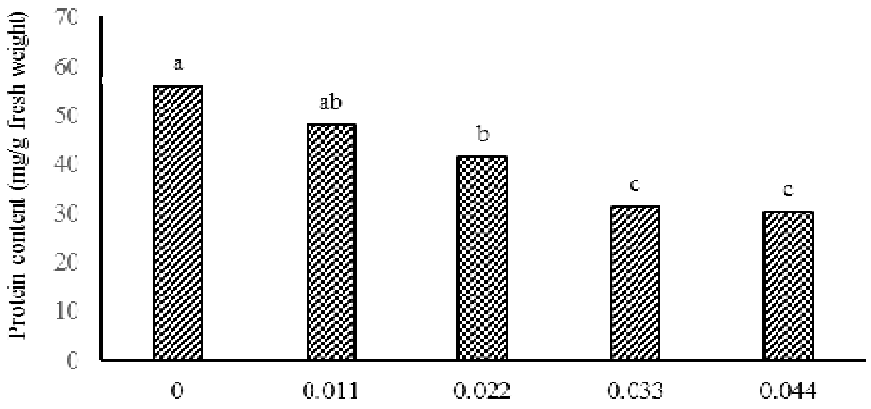


Figure 6. Response of protein content wheat plant to the residues dose of Imazethapyr in soil

Activity of antioxidant enzymes

The results of theanalysis of variances showed that the effects of Imazethapyr herbicide had a significant effect on activity of catalase enzyme at 1% probability level (Table 1). According to Figure 7, it was observed that the activity of the catalase to 0.22 mg/g of germicidal application of the herbicide activity of the catalase enzyme was subtle, but with

the increase of herbicide dose from 0.22 µg, the activity of the catalase enzyme greatlyreduced. The application of doses of 0.11, 0.022, 03.03 and 0.044 micrograms of herbicides reduced the catalase enzyme activity by 2.8, 5.2, 27 and 64 percent, respectively (Figure 7). The results showed that peroxidase enzyme activity was also significantly affected by Imazethapyr herbicide at 1% level (Table 2). The results showed that increasing imazethapyr concentration resulted in a significant increase in peroxidase enzyme activity; however, the activity of this enzyme was completely inhibited at 0.044 µg of imazethapyr (Figure 8). The application of doses of 0.11, 0.022, 03.03 and 0.044 micrograms of herbicides increased the activity of enzyme peroxidase by 3.5, 39, 49 and 52 percent, respectively (Figure 8). The results showed that the effect of Imazethapyr herbicide on the activity of wheat polyphenol oxidase enzyme was significant (Table 2). Therefore, with increasing dosage of Imazethapyr herbicide residues, traits were significantly reduced (Figure 9). The application of doses of 0.11, 0.022, 03.03 and 0.044 micrograms of herbicides reduced 13, 24, 35 and 46 percent activity of polyphenol oxidase activity of wheat, respectively (Figure 9). In a test, the effect of Bentazone (photosynthesis inhibitor in photocisteemic 2), Penoxsulam (acetylacetate synthesis inhibitor) and Haloxyfopbutyl (acetyl coenzyme carboxylase inhibitor inhibitor) on rice plant, it was determined that the use of bentazone and pinocylamreduced catalase activity, increased H_2O_2 levels and increased lipid oxidation (Nohatto *et al*., 2016). In a trial on the effect of Bentazone and Penoxsulam herbicides on rice plant, the use of herbicides reduced the activity of catalase activity, based on which researchers have suggested these herbicides as inhibiting the enzyme activity (Abedi and Pakniyat, 2010). In experiments of (Yan *et al*., 2008) on the effects of Isoproturon on wheat growth, it was reported that by concentrations of 2, 3.5, 5, 10, and 20 mg/kg soil at concentrations of 2 Up to 5 milligrams of catalase, the activity of wheat increased, while in the more concentrations of herbicides, the activity of this enzyme decreased. They stated that by increasing the concentration of herbicide, the catalase enzyme was suppressed, which was probably due to the high sensitivity of the enzyme to the weed concentration, or the herbicide. By increasing the production of ROS, catalase activity was suppressed. In an experimental study, the use of Imazethapyr herbicide reduced the activity of the enzyme catalase to about 33.9% of the control in Arabidopsis thaliana (Qian *et al*., 2011).

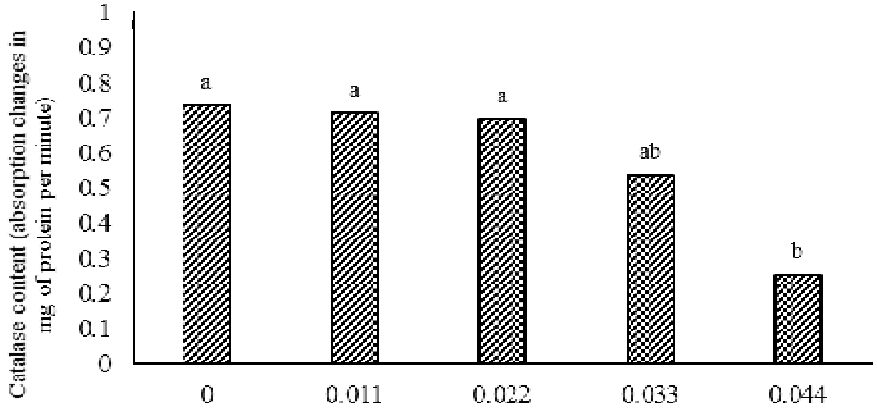


Figure 7. Response of catalase content wheat plant to the residues dose of Imazethapyr in soil

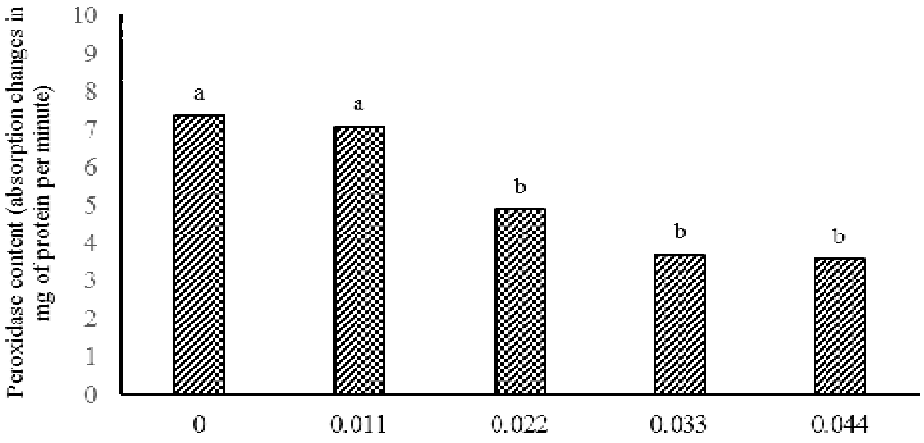
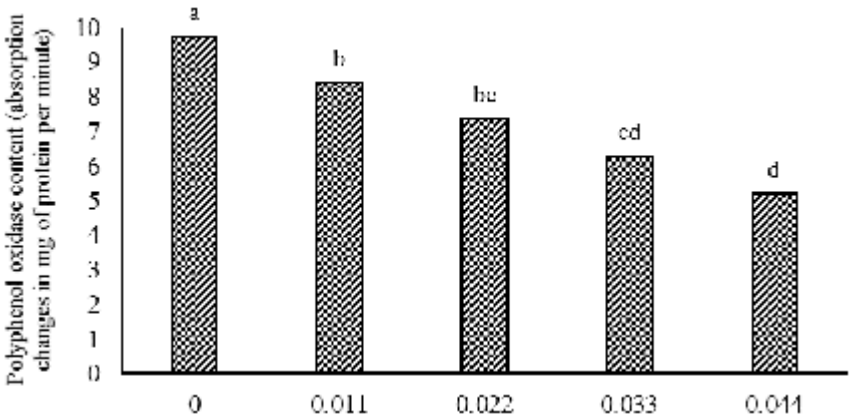


Figure 8. Response of peroxidase content wheat plant to the residues dose of Imazethapyr in soil



Figiure 9. Response of polyphenol oxidase content wheat plant to the residues dose of Imazethapyr in soil

Dry root and shoot dry weight

The results showed that the effect of Imazethapyr herbicide on root and shoot dry weight was significant (Table 2). Therefore, with increasing dosage of Imazethapyr herbicide residues, the traits significantly reduced the trend (Figures 10 and 11). The application of doses of 0.03, 0.022, 03.03 and 0.044 µg of herbicide reduced 14, 21, 30 and 42% of dry weight of root of wheat, respectively (Figure 10). Also, the application of these doses reduced 8, 11, 16 and 24 percent of the root dry weight of the wheat, respectively (Figure 11). (Yin *et al*., 2008) reported concentrations of 2, 3.5, 5, 10, and 20 mg/kg soil of Isoproturon on wheat growth, at concentrations 10 to 20 mg herbicides, the wheat biomass decreased sharply. In their experiment, wheat root biomass also decreased by 44% compared to control treatment. In another experiment, residues of Pendimethalin, Quizalofop, Imazethapyrand Imazamox herbicides used in soybean were not effective on germination percentage, plant height and subsequent dry weight (Wilcut, 1998). Experimental results showed that residues of Pyrazosulfuronused in rice plant caused damage to germination traits and root and shoot length of cucumber (PI, 2015).

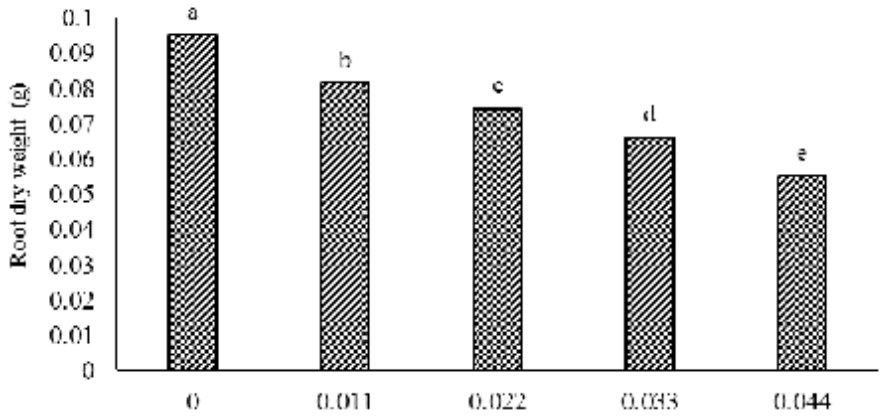


Figure 10. Response of root dry weight wheat plant to the residues dose of Imazethapyr in soil

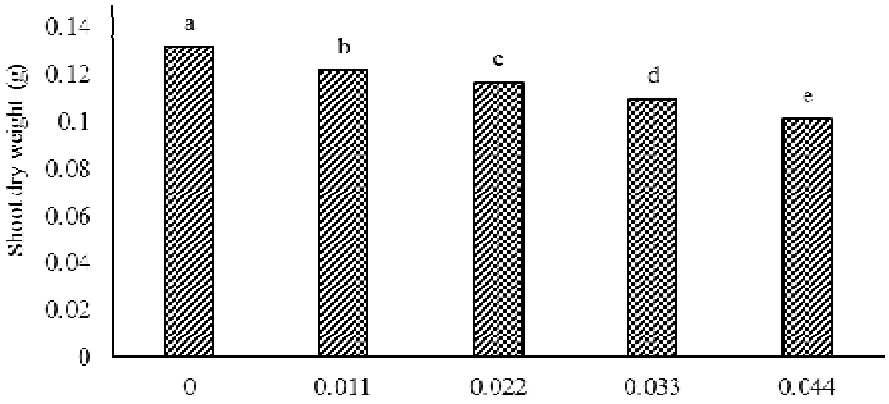


Figure 11. Response of shoot dry weight wheat plant to the residues dose of Imazethapyr in soil

Conclusions

According to these results, it could be concluded that imazethapyr herbicide had a negative impact on wheat growth parameters and the higher concentrations of this herbicide could result in a significant increase in peroxidase enzyme activity in stress conditions. These findings may explain the wheat tolerance mechanism against herbicides side effects and the wheat could be used as a biomarker or a bio indicator for assessment of imazethapyr phytotoxicity and bioassay researches.

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