Journal of Nutrition and Food Security
Shahid Sadoughi University of Medical Sciences
School of Public Health
Department of Nutrition
Nutrition & Food Security Research Center

eISSN: 2476-7425 pISSN: 2476-7417 JNFS 2020; 5(3): 248-258 Website: jnfs.ssu.ac.ir

The Effect of Different Doses of Tank-mixed Herbicides on Antioxidant Enzymes Activity of Soybean

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ARTICLE INFO

ORIGINAL ARTICLE

Article history:
Received: 2 Dec 2019
Revised: 22 Jun 2020
Accepted: 29 Mar 2020

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ABSTRACT

Background: Herbicide plays the main role in guaranteeing the quantity and quality of food. Soybean reactive oxygen species scavenging system eliminates the herbicides' side effects on the plant by activating antioxidant enzymes, which affects the yield. So, we investigated this procedure to reveal the effect of herbicides on food production. **Methods:** Experiments were carried out in two locations of Alborz province in 2014 during the growing seasons. Seven different tank-mixed herbicides were produced by mixing one triple mixture of imazethapyr+bentazon+sethoxydim active ingredient, three double mixture of imazethapyr+bentazon, imazethapyr+sethoxydim, and bentazon+sethoxydim, as well as single usage of imazethapyr, bentazon, and sethoxydim. Reduced herbicide rates were 100, 60, and 30% of the recommended dosage inducing soybean plots through leaf expanding phase. **Results:** Both herbicides and their reduced rates changed soybean yields through antioxidant enzymes' activity. Maximum soybean yield was registered at tank-mixed imazethapyr+bentazom+ sethoxydim which were induced at 33, 320, and 125 g active ingredient/hectare, respectively. The minimum activity of enzymes (superoxide dismutase 3.9 international unit) was also demonstrated in this research. Our data showed that when the herbicide rate was reduced from 100% to 30%, as a result, the label recommended soybean yield was reduced by just 17%, while superoxide dismutase activity was reduced too. The minimum yield was 1.2 ton/hectare of sethoxydim with 225 g active ingredient/hectare. **Conclusion:** Antioxidant enzymes were promoted to maximum activity by increasing the herbicide rate for scavenging the herbicide side effects. Tank-mixed herbicides, with reduced herbicide rates can eliminate poison residue in the environment and food chain while increasing weed control.

Keywords: *Reduced Rates; Herbicide; Tank-Mixed; Soybean; Enzyme*

Introduction

Soybean is the main legume and oil seed crop across the world that contains the main sources of animal protein food and is important for food chain production (Argaw, 2012). During food production, weeds and pest interference will reduce yield. Among numerous ways to control

This paper should be cited as: Fallah-Tafty S, Lak Sh, Mojaddam M, Naderi A, Abdollahian-Noghabi M. ***The Effect of Different Doses of Tank-mixed Herbicides on Antioxidant Enzymes Activity of Soybean.*** *Journal of Nutrition and Food Security (JNFS)*, 2020; 5 (3): 248-258.

weeds, using chemicals are the easiest ones, but they are highly dangerous for the environment and human beings. Recently, researchers used many ways to reduce herbicide wastes and residues in the environment, but tank-mixed and reduced herbicide rates were the most efficient ones (Barroso *et al.*, 2010). Long-term application of herbicides will induce herbicide resistant in weeds, which constraints food production and leads to poor quality and quantity productions (Délye *et al.*, 2013, Heap, 2014, Yuan *et al.*, 2007).Tank-mixed herbicides increase poison mode of action and enhance weeds control by changing herbicides' active ingredient (Hatzio and Penner, 1985, Zhang *et al.*, 1995). Furthermore, tank-mixed herbicide particles interact with each other in many synergic, additive, and antagonistic ways. When synergic effect is detected, reduction of the herbicide rate is recommendable. This advantage provides the farmers with the opportunity to reduce herbicide rates due to economic, technical, and environmental reasons. As a result, the poison drift and residue are reduced in the final productions, which guarantee sustainable food chain (Blackshaw *et al.*, 2006), Pannacci and Covarelli, 2009). Imazethapyr prohibits grass and broad leaf weeds' growth by inhibiting amino acid synthesis and accumulating in the meristemic plant body parts, which finally inhibit the plant growth. Imazethapyr recommended rate for soybean field is 100 g active ingredisents/hectare (g ai/ha) (Krausz *et al.*, 2001). Bentazon is a post emergence herbicide that exposes the plants to bentazon damaged by reactive oxygen species (ROS) and affects protein and cell membrane (Ahrens, 1994, Armel *et al.*, 2007, Hugie *et al.*, 2008, Powles and Yu, 2010). Bentazon activity is in chloroplast of broad-leafed weeds and its recommended rate for soybean field is 960 g ai/ha. (Han and Wang, 2002, Williams and Nelson, 2014, Zhu *et al.*, 2009). Sethoxydim, a post emergence systemic herbicide, impacts grass weeds' infestation within lipid synthesis inhibition mode of action and Sethoxydim recommended rate for soybean field is 375 g ai/ha. Tank-mixed herbicide improves weed control by manipulating the site of action and preventing the weed tolerance occurrence. Mixed herbicide modifies the enzymes' activity considering various sites of action. However, single herbicides trigger different enzyme activity and have different effects on soybean. They also detoxify the effects of herbicides in many ways such as closing stomatal as well as using hormones and antioxidant enzymes (Alexieva *et al.*, 2001, Caverzan *et al.*, 2016, Czarnocka and Karpiński, 2018, Mittler, 2002). Moreover, closed stomatal is in the front line of herbicide uptake prevention which induces accumulation of ROS produced by unused energy (Boulahia *et al.*, 2016, Jiang and Yang., 2009, Pan *et al.*, 2017, Zhang *et al.*, 2014). By closing stomatal, Carbon dioxide cannot take apart in photosynthesis since sunlight is absorbed and electron chain continues working dramatically towards ROS production. Finally, these reactions can damage the cell wall, DNA, and proteins (Bailly, 2004, Bailly *et al.*, 1996, Foyer and Noctor, 2005, Mittler, 2017, Mühling and Läuchli, 2003, Tan *et al.*, 2006, Xu *et al.*, 2010, Yordanova *et al.*, 2004). Superoxide (O2) is the most dangerous ROS that harms cells in plants due to high oxidative ability (Jung, 2004, Triantaphylidès and Michel, 2009). Superoxide dismutase (SOD) catalyzes superoxide anion to hydrogen peroxide (H2O2) (Babior *et al.*, 1975, Galeshi *et al.*, 2009, Gill and Tuteja, 2010, Li *et al.*, 2014), where Ascorbate peroxidase (APX) (Kafi *et al.*, 2009, Wang *et al.*, 2004), Glutathione reductase (GR) (Ahmed *et al.*, 2002), Catalase (Airam *et al.*, 2009, Dubey, 2010), and Dehydro ascorbate reductase (DHAR) (Anjum *et al.*, 2014, Gupta *et al.*, 2001) transform H2O2 to H2O and O2. Activity of these 5 enzymes revealed soybean ability against side effects of herbicides and proposed its detoxification ability in consuming the energy of final yield that minimized the herbicides' residue in yield and reduced the environment harms. So, these antioxidant enzymes play the main role in producing safe foods during soybean production.

The aim of this study was to find the best herbicide component to promote minimum enzyme activity affecting food quantity and quality directly. Moreover, we aimed to characterize the best reduced herbicide dose against weeds' infestation.

Materials and Methods

Treatments characteristics: This study was carried out by DPX cultivar of soybean planted in ploughed field at different plots during 2014 in growing seasons. The experiments were carried out in two locations of Alborz province including a research farms in Islamic Azad University of Karaj and Sugar Beet Research Farm Institute at Kamalshahr. Each experimental unit had an area of about 18 m^2, which included 6 soybean rows with 50 cm lateral distance. Weed flora was different in each area showing a wide aspect of herbicide usage that affects yield. The soil texture in the first and second locations were loamy sandy and sandy loamy, respectively. This study composed of two main treatments, which include herbicides in 7 levels of single usage of imazethapye, bentazon and sethoxydim, double solution of imazethapyr + bentazon, imazethapyr + sethoxydim and bentazon + sethoxydim, and finally triple solution of imazethapyr + bentazon + sethoxydim as one solution. The second treatment included different herbicide rates containing the full recommended dosage of 100%, reduced to 60% and 30% of the label recommended dose. Exact dose calculation of each herbicide is proposed in **Table1**. These treatments were applied at leaf expanding growth stage of soybean by a backpack sprayer using flat nuzzle.

Sampling assay: Soybean yield was harvested 95 days after planting and the collected samples were weighted. To survey the enzymes' activity, leaf samples were collected 3 days after herbicide application. Immediately, they were frozen by liquid nitrogen. Later, the samples were extracted by a pestle from the ice cooled crasher using 4 ml of 0.05 M Na2Hpo4/NaH2Po4 (pH 7.0) buffer containing 0.2 µM Ethylene Diamine Tetracetic Acid (EDTA) and 1% Poly Vinil-Pyrolidone (PVP). The homogenates were centrifuged at 4^{o}C for 20 min at 15000 rpm (Zhang *et al.*, 2005). Supernatants were collected and used for enzymes' activity assay.

Ascorbate peroxidase (APX) assay: Ascorbate peroxidase activity was measured according to Nakano and Asada procedure, which depended on decreasing absorbance at 290 nm, while the ascorbate was oxidized. Reaction mixture composed of 50 µM/l Na-phosphate buffer (pH 7.0), 50 µM/l ascorbate, 0.1 µM/l EDTA, 1.2 µM/l H2O2, and 0.1 ml of enzyme extract in a final assay volume of 1 ml. Concentration of oxidized ascorbate was calculated by coefficient of 2.8 µM/l/cm. Each unit of APX included reduction of 1 µM/ml/min ascorbate oxidized (Nakano and Kozi, 1981).

Catalase (CAT) assay: CAT extract (20 ml), was added to reaction component, which included 750 ml hydrogen peroxidase (H2O2) and 750 ml of 100 µM phosphate buffer (pH 7.0). Later, this solution was adjusted to 3 ml with sterile distilled water. Finally, the absorbance was read at 240 nm.

Glutathione reductase (GR) assay: GR activity was measured as Foyer and Halliwell assay. Container consisted of 25 µM Na-phosphate buffer (pH 7.8), 0.5 µM GSSG, 0.12 µM NADPH, and 0.1 ml enzyme extract in a final assay volume of 1 ml. NADPH oxidation intercepted at 340 nm. Activity was calculated with extinction coefficient of NADPH (6.2 µM/cm). Each unit of GR included reduction of 1 µM/ml/min glutathione (Foyer and Halliwell, 1976).

Dehydroascorbate Reductase (DHAR) assay: DHAR was measured by reducing 0.7 ml phosphate buffer (pH 7.0), 20 µM/l of reduced glutathione (GSH) in phosphate buffer (pH 7.0), 2 µM/l DHA, and 0.1 ml crude enzyme. Freshly prepared DHAR, kept on ice, was added to the reaction mixture in covette. Reduction of DHAR to ASA was monitored by increase of absorbance at 290 nm, taking 2.8 µM/l/cm as the absorbance coefficient (Krivosheeva *et al.*, 1996).

Superoxide dismutase (SOD) assay: Activity of SOD was calculated by the photoreduction of Nitortetrazolium Blue Chloride (NBT). Reaction solution contained 100 µM phosphate buffer (pH 7.0), 0.1 µM EDTA, 13 µM methionine, 75 µM Nitrotetrazolium Blue Choloride, 2 µM riboflavin, and adequate supernatant. Riboflavin was added to solution as lazy component and then the reaction started by a 15 watts' florescent lamp. After the reaction ended, the reaction product was measured at 560 nm. Isoenzymes of SOD were separated on 10% none-denaturing PAGE at 4°C. Finally, the same volume of each sample was loaded to this solution. These extracts were electrophoresed and SOD activity was calculated by monitoring according to Demirevska-Kepova procedure (Demirevska-Kepova *et al.*, 2004).

Data analysis: Data obtained by different methods were analyzed by SAS software to determine the treatment effect on enzymes' activity. Completely randomized factorial design was used with three replications to analyze data variance and to determine its significant treatment effect on yield and enzymes. To investigate the effect of both treatments (herbicide and doses) on enzymes, the mean comparisons' method was used based on DUNCAN procedure.

Results

Analysis of variance revealed that both main treatments (herbicide rates and herbicide) had significant effect on soybean yield. In other words, antioxidant activity varied by changing herbicides at different rates and affected the final yield (**Table 2**). The maximum soybean yield was registered at tank-mixed Imazethapyr + Bentazon + Sethoxydim treatment within all rates. The findings showed that the maximum rate was 3.7 ton/hectare (t/ha) at full recommended rate of 100% (including 33 g Imazethapyr + 320 g Bentazon + 125 g Sethoxydim active ingredients as a one solution) and 3.1 t/ha when herbicide rate was reduced to 30% of the label recommended rate (which included 10 g Imazethapyr + 96 g Bentazon + 33 g Sethoxydim active ingredients as one solution) (**Table 3**). Minimum yield registered during single herbicide treatment was 2.2 t/ha for imazethapyr, 2 t/ha for bentazon, and 1.2 t/ha for Sethoxydim (**Table 4**). The maximum activity of enzymes registered at all herbicide treatments induced a full rate of 100%, where the minimum enzymes' activity was demonstrated at minimum herbicide rate of 30% (**Table 5**) during tank mixed treatments (**Table 4**). All enzymes' activity units were international unit in one gram of the sample international unit/gram (iu/g) (which was explained at material and methods' section) and soybean yield was t/ha.

Table 1. Single and tank-mixed herbicide treatment rates calculations

Active ingredients rate	Treatments (Herbicide & Herbicide Rates)										
	IBS100	**IBS60**	**IBS30**	**IB100**	**IB60**	**IB30**	**IS100**	**IS60**	**IS30**	**BS100**	
Imazethapyr (g)	33	20	10	50	30	15	50	30	15	480	
Betazon (g)	320	192	96	480	288	144	187	112	56	187	
Sethoxydim (g)	125	75	37	-	-	-	-	-	-	-	
Active ingredients rate	**BS60**	**BS30**	**I100**	**I60**	**I30**	**B100**	**B60**	**B30**	**S100**	**S60**	S30
Imazethapyr (g)	288	144	100	60	30	960	576	288	375	225	112
Betazon (g)	112	56	-	-	-	-	-	-	-	-	-
Sethoxydim (g)	-	-	-	-	-	-	-	-	-	-	-

IB = Imazethapyr + Bentazon, IS = Imazethapyt + Sethoxydim, BS = Bentazon + Sethoxydim, IBS = Imazethapyr + Bentazon + Sethoxydim, 100 = Full recommended rate of herbicide, 60= Reduced to 60 percent of the label recommended rate, 30 = Reduced to 30 percent of label recommended rate.

Table 2. Analysis of variances of soybean yield and enzymes activity (SOD, APX, CAT, GR and DHAR)

Source of variance	DF	Yield	SOD	APX	CAT	GR	DHAR
Location	1	0.24[a]	0.31[a]	0.30[a]	0.36[a]	0.30[a]	0.11[a]
Block×Location	4	0.04[a]	0.44[a]	0.40[a]	0.62[a]	0.19[a]	0.03[a]
Herbicide	6	10.9[a]	33.6[a]	14.5[a]	18.0[a]	5.4[a]	0.8[a]
Herbicide dose	2	5.86[a]	105.0[a]	17.4[a]	8.0[a]	2.1[a]	0.3[a]
Herbicide×Herbicide dose	12	0.15[a]	1.1[a]	0.7[a]	0.07[a]	0.04[a]	0.001[a]
Location×Herbicide	6	0.01[b]	0[b]	0.001[b]	0[b]	0[b]	0[b]
Location×Dose	2	0.01[b]	0[b]	0[b]	0[b]	0[b]	0[b]
Location×Herbicide×Dose	12	0.008[b]	0[b]	0.001[b]	0[b]	0[b]	0[b]
Error	80	0.009	0.001	0.001	0.001	0.009	0.0001
CV		3.6	0.8	0.92	0.99	3	2.3

[a:] significant at 5% and 1% probability level, [b:] Non-significant, SOD: Superoxide dismutase, APX: Ascorbate peroxidase, CAT: Catalase, GR: Glutathione reductase , DHAR:Dehydroascorbate Reductase

Table 3. Mean of both location by induction treatment on soybean yield and enzym activity (SOD, APX, CAT, GR and DHAR)

Variables	**Treatments (Herbicide & Herbicide Rates)**										
Enzymes activity (iu)	**IBS100**	**IBS60**	**IBS30**	**IB100**	**IB60**	**IB30**	**IS100**	**IS60**	**IS30**	**BS100**	**BS60**
Superoxide dismutase	3.9	3.5	1.9	6.1	5.1	3.1	5.2	4.5	2.7	4.7	4.1
Ascorbate peroxidase	3.9	3.7	3.5	4..1	3.8	3.6	4.7	4.1	3.7	5.1	4.5
Catalase	2.4	2	1.7	2.7	2.2	1.9	3.1	2.6	2.4	3.5	3
Glutathione reductase	0.4	0.3	0.2	0.6	0.5	0.3	0.8	0.6	0.4	1.1	0.9
Dehydroascorbate Reductase	0.2	0.1	0.07	0.3	0.2	0.1	0.4	0.3	0.2	0.5	0.4
Soybean yield (t/ha)	3.7	3.5	3.1	3.6	3.3	2.8	3.2	2.7	2.4	3.0	2.9
Anzymes activity (iu)	**BS30**	**I100**	**I60**	**I30**	**B100**	**B60**	**B30**	**S100**	**S60**	**S30**	**Control**
Superoxide dismutase	2.2	8.8	6.9	4.7	7.5	5.7	3.7	8.1	6.4	4.1	0.50
Ascorbate peroxidase	3.9	5.9	5.1	4.2	7.2	6	4.9	6.5	5.7	4.6	1.20
Catalase	2.7	4.2	3.9	3.2	4.7	4.3	3.7	5.2	4.8	4.1	0.50
Glutathione reductase	0.6	1.4	1.1	0.8	2.1	1.8	1.5	1.8	1.5	1.2	0.15
Dehydroascorbate Reductase	0.3	0.6	0.5	0.4	0.8	0.7	0.6	0.7	0.6	0.5	0.02
Soybean yield (t/ha)	2.7	2.7	2.3	1.7	2.6	2.1	1.4	1.7	1.1	0.7	3.8

IB = Imazethapyr + Bentazon, IS = Imazethapyt + Sethoxydim, BS = Bentazon + Sethoxydim, IBS = Imazethapyr + Bentazon + Sethoxydim, 100 = Full recommended rate of herbicide, 60= Reduced to 60 percent of the label recommended rate, 30 = Reduced to 30 percent of label recommended rate.

Table 4. Mean comparison of the main effect of herbicide treatment on soybean yield and enzymes activity (SOD, APX, CAT, GR, and DHAR)

Herbicide Treatment		**Soybean yield (t/ha) and antioxidant enzymes activity (i.u)[a]**				
	Yield	**SOD**	**APX**	**CAT**	**GR**	**DHAR**
Imazethapyr + Bentazon + Sethoxydim	3.5 A	3.1 G	3.7 G	2.1 G	0.35 G	0.15 G
Imazethapyr + Bentazon	3.2 B	4.8 D	3.8 F	2.3 F	0.51 F	0.22 F
Imazethapyr + Sethoxydim	2.8 C	4.1 E	4.2 E	2.7 E	0.65 E	0.32 E
Bentazon + Sethoxydim	2.8 C	3.7 F	4.5 D	3.1 D	0.91 D	0.43 D
Imazethapyr	2.2 D	6.8 A	5.1 C	3.8 C	1.1 C	0.53 C
Bentazon	2 E	5.6 C	6.1 A	4.2 B	1.8 A	0.72 A
Sethoxydim	1.2 F	6.2 B	5.6 B	4.7 A	1.5 B	0.63 B

[a]: Within each column, means with the same letter do not have difference and are grouped as one bunch according to the Duncan test ($P \leq 0.05$), SOD: Superoxide dismutase , APX: Ascorbate peroxidase , CAT: Catalase , GR: Glutathione reductase , DHAR: Dehydroascorbate Reductase.

Table 5. Mean comparison of the main effect of herbicide doses on soybean yield, enzymes activity (SOD, APX, CAT, GR, and DHAR) .

Herbicide dose	Soybean yield (t/ha) and antioxidant enzymes activity (iu)[a]					
	Yield	SOD	APX	CAT	GR	DHAR
100%	2.9 A	6.3 A	5.3 A	3.7 A	1.2 A	0.52 A
60%	2.6 B	5.2 B	4.7 B	3.3 B	1.0 B	0.43 B
30%	2.2 C	3.2 C	4.1 C	2.8 C	0.76 C	0.33 C

[a]: Within each column, means with the same letter do not have difference and are grouped as one bunch according to the Duncan test (P≤ 0.05), SOD: Superoxide dismutase , APX: Ascorbate peroxidase , CAT: Catalase , GR: Glutathione reductase , DHAR: Dehydroascorbate Reductase.

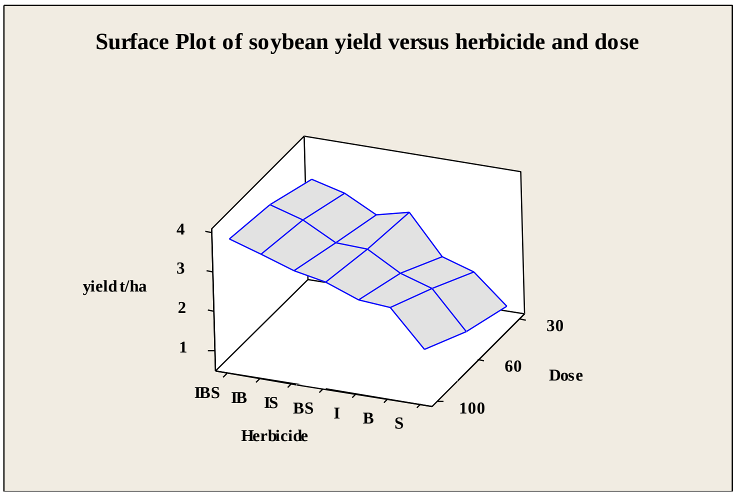

Figure 1. Interaction of herbicides and doses on soybean yield

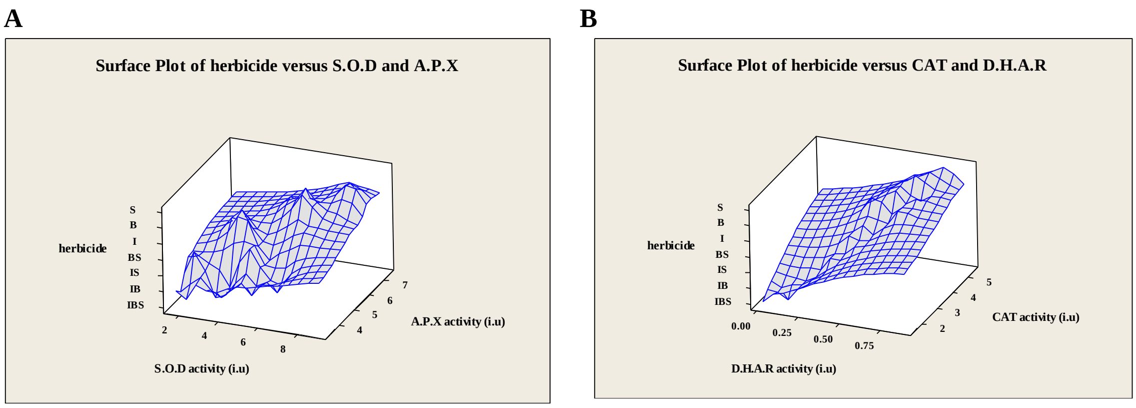

Figure 2. Effect of different herbicides on SOD, APX activity (A) and DHAR, CAT activity (B)

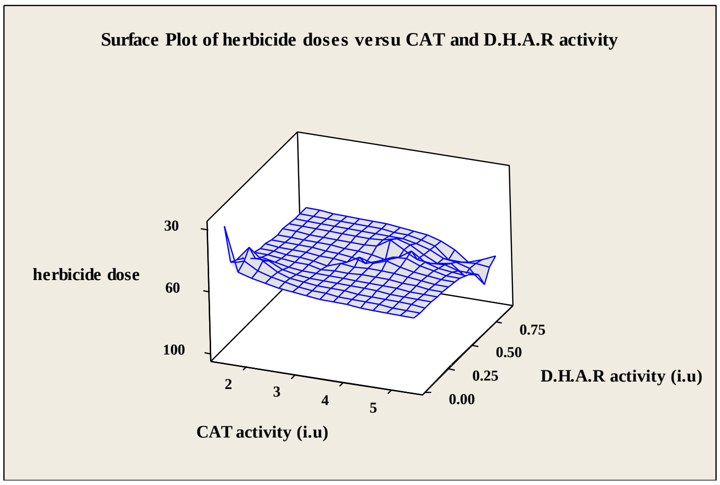

Figure 3. Effect of different herbicide doses on CAT and DHAR activity

Discussion

Many researchers confirmed constant soybean yield while tank-mixed herbicide was used with lower doses (Auskalnis and Kadzys, 2006, Barros *et al.*, 2005, Boström and Håkan, 2002, Walker *et al.*, 2002, Zhang *et al.*, 2000). However, we are faced with scarcity of information regarding soybean production using herbicide tank-mixing (Imazethapyr, Bentazon and Sethoxidym). This demonstrates reduction of imazethapyr rates by 23 g ai/ha (from 33 g ai/ha in full rate to 10 g ai/ha in the reduced rate), bentazon by 224 g ai/ha (from 320 g ai/ha in full rate to 96 g ai/ha in reduced rate), and sethoxydim by 88 g ai/ha (from 125 g ai/ha in full rate to 37 g ai/ha in reduced rate). When they are used as one tank-mixed solution, herbicide synergic effect will occur and weed control spectrum will increase (due to the manipulating herbicide sites of action that increase soybean yield besides less herbicide utilization) (**Table 3**). Simultaneous increase of herbicide rates raised enzymes' activity in all 5 antioxidants (**Table 5**), which was also confirmed by another researcher (Merve and Burcu, 2012). Long-term application of full rates of single herbicides will consume soybean energy and affect the yield negatively. Many researchers confirm this hypothesis (Boulahia *et al.*, 2016, Jiang and Yang., 2009, Pan *et al.*, 2017, Zhang *et al.*, 2014). For instance, tank-mixed herbicide superoxide dismutase activity reduced 2 iu (from 3.9 iu to 1.9 iu) when imazethapy + bentazon + sethoxydim rate diminished from 100% to 30%. This findings was also confirmed by some researchers saying that reducing herbicide rate will minimized the SOD activity (Alexieva *et al.*, 2001, Caverzan *et al.*, 2016, Czarnocka and Karpiński, 2018, Mittler, 2002). This result confirms our former hypothesis proposing that the lower enzymes' activity leads to higher soybean yield. In contrast, when single herbicides were used, the minimum soybean yield was registered (when sethoxidym doses was 375 g ai/ha soybean yield reduced to 1.7 t/ha). During this single component treatment, maximum fluctuation was observed in soybean yield; it reduced from 1.7 t/ha at 375 g ai/ha sethoxydim rate to 0.7 t/ha at 112 g ai/ha at reduced rate. This result reported by Rosales revealed that single component herbicide induced the maximum yield fluctuation(Rosales-Robles *et al.*, 2005). Besides, the highest antioxidant enzymes activity was registered at single component herbicide usage (including imazethapyr, bentazon, and sethoxydim). This incident is due to the effect of high active ingredients of each herbicide, when used as single components (**Table 1**), on soybean. It finally promotes soybean antioxidant enzyme system to eliminate herbicide side effects. For instance, SOD activity rose from 3.1 iu at triple tank-mixed herbicide to 6.2 iu, when sethoxydim was applied as a single herbicide (**Table 4**). Each enzyme participates in specific herbicide usage; for instance, APX maximum activity was at bentazon (**Figure 2A**) usage, which was due to the same work place of APX and bentazon (chloroplast and mitochondria). Simultaneously, the highest CAT activity (4.7 iu) and soybean yield reduction (1.2 t/ha in comparison to weed free treatment) (**Table 4**) were observed at single bentazon usage (269 g ai/ha) (**Figure 2B**). Moreover, the maximum GR activity (2.1 iu) and soybean yield reduction (1.3 t/ha) (**Table 3**) were seen at single usage of bentazon (260 g ai/ha). These results confirm the hypothesis saying that herbicides' treatment with one site of action increases antioxidant enzymes' activity that consumes the soybean energy and reduces its yield. As a result, single mode of action herbicides put a lot of stress on plant, which can have negative effects on yield and food chain security. This results were confirmed by a study(Knežević *et al.*, 2003). During doubled component herbicide usage, the yield raised in comparison to the single mode of action herbicide, but it was lower compared with the triplet mode of action herbicide. For instance, when Imazethapyr + Bentazon, imazethapyr + Sethoxydim, and bentazon + Sethoxydim solutions induced soybean, the yield raised to 3.2, 2.8, and 2.8 t/ha, respectively in comparison to single herbicides (**Table 4 and Figure 1**). During yield survey among double component herbicide solution, the maximum soybean yield was registered at Imazethapyr+Bnetazon (**Figure 1**), where minimum enzymes' activity occurred was 6.1 iu for SOD,4.1 iu for APX, 2.7 iu (**Figure 2A**) for CAT, 0.6 iu for GR, and 0.3 iu for DHAR (**Table 4 and Figure 2B**). Herbicide reduced rates during doubled component herbicide solution had more positive effects on yield but were not reliable in single component herbicide solutions since yield was unstable due to the higher activity of enzymes (**Figure1**) (Dogan, 2005, Fanadzo *et al.*, 2010). It was also proposed that herbicide rates were more reliable during tank mixed herbicide induction. In contrast, the maximum yield stability was observed in reduced herbicide rates of Imazethapyr + Bentazon + Sethoxydim treatment. In the following, Imazethapyr + Bentazon (at full recommended rate of 50 gr ai/ha Imazethapyr and 480 g ai/ha Bentazon) was ranked the second with regard to the best yield record of reduced herbicide rates in which the soybean yield was 3.7 t/ha. Results also showed that reducing herbicide rate to 30% of the lable recommended soybean yield remained as 3 t/ha (Walker *et al.*, 2002) (**Table 3 and Figure 1**). The imazethapy + bentazon + sethoxydim and Imazethapyr + Bentazon can be recommended to reduce the usage rate with secure yield besides the minimum herbicide residue in yield which guarantee the food chain health.

Conclusion

According to the findings, tank-mixed herbicides trigger less ROS that lowers priority of soybean antioxidant enzyme activity and increases the yield. It also reduces herbicide residue, which guarantees secure food production. Moreover, herbicides rates can be reduced and fixed yield quantity and quality can be guaranteed by manipulating modes of actions proposed by tank-mixed herbicides. As a result, sustainable secure food production programs can be designed and poison erosion can be prevented.

Author contributions

Fallah-Tafty S and Lak S designed the study; Fallah-Tafty S and Mojaddam M conducted the experiments. Fallah-Tafty S, Abdollahian-Noghabi M, and Naderi A analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

All authors declared no conflict of interests.

Acknowledgements

The authors thank all the individuals who were directly or indirectly associated with this experiment. This research was based on a PhD thesis with 23250208972001 ID number conducted in Department of Agronomy, Islamic Azad

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