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Investigation of Tank-Mixed and Reduced Rate of Imazethapyr, Bentazon and Sethoxydim on Soybean Antioxidant Enzymes Activity

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ABSTRACT

BACKGROUND: Soybean antioxidant enzymes activity can change within different herbicide and herbicide active ingredient rates application but it is not corroborating to all herbicides and doses, hence more survey will help to identify herbicide effect on soybean antioxidant enzymes.

OBJECTIVES: Goals was to estimate five antioxidant soybean enzyme activities during herbicide tank-mix under different reduced rate exposure.

METHODS: Completely randomized factorial design with 3 replications was used to survey data variance and simple mean comparison used to compare treatment effect on enzymes activity. Herbicide treatment were apply at soybean v2 growth stage by backpack sprayer with v type nuzzle. There were two main treatment consist of herbicide treatment in 7 level composed of single exert of Imazethapyr, Bentazon and sethoxydim, doubled solution of Imazethapyr + Bentazon, Imazethapyr + sethoxydim and Bentazon + Sethoxydim and tripled solution, consist of Imazethapyr + Bentazon + sethoxydim active ingredients. Second treatment was herbicide different rates in 3 levels, where it was compose of: full herbicide dose (equals to 100% of producer recommended dose), reduced to 60% of recommended active ingredient per acre and reduced to 30% of recommended active ingredient per acre.

RESULT: Minimum SOD activity registered at imazethapyr + bentazon + sethoxydim which it was 1.9 iu that induced at reduced rate of 10, 96 and 37 gr a.i ha^{-1} respectively. In contrast ascorbate peroxidase increased dramatically at bentazon treatment over 960 gr a.i ha^{-1}, which it raises from 1.2 i.u in control to 7.2. Lowest APX activity demonstrated at imazethapyr + bentazon + sethoxydim which used as reduced rate of 30% of full recommended dose of each solution that it fit to 10, 96 and 37 gr a.i ha^{-1} respectively. Maximum APX activity that registered 7.2 i.u recorded at bentazon in full rate of 960 gr a.i ha^{-1}. Sethoxydim at full rate of 375 gr a.i ha^{-1} induced maximum CAT activity, where registered 5.2 i.u, which in compare of control treatment in raised 10 times. Accordance to other enzymes, minimum CAT activity obtained at reduced to 30% of label recommended of imazethapyr + bentazon + sethoxydim treatment.

CONCLUSION: Throughout all treatment by reducing herbicide rates enzymes activity diminished, similarly tank-mixed herbicide lower enzymes activity which it led to promote using reduced herbicide rate on soybean.

KEYWORDS: *Herbicide, tank-mix, reduced rate, antioxidant enzymes, soybean.*

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1. BACKGROUND

Soybean is the main legume and oil seed crop across the globe (Argaw, 2012). Within seed production weed interfere can lower yield quantity and quality, hence weed defiant play the main role in seed production (Pinke *et al*., 2016; Barroso *et al*., 2010). Application herbicides with single site of action or use one herbicide to control weed flora has low strength to hamper weed spectrum thereby this will promote herbicide resistance and tendency to constraint yield production that leading poor yield (Heap, 2014; Delye *et al*., 2013; Yuan, 2007). Thus tank-mixed herbicide will led to multiplying herbicide site of action that it induces rich incessant herbicide control by differing herbicide active ingredient (a.i) mode of actions (Zhang *et al*., 1995; Hatzios and Penner, 1985). Moreover tank-mix herbicides can interact each other in synergic, additive or antagonistic ways (Green, 1989). In addition positive herbicide interaction (synergic or antagonistic) will expand weed flora control throughout multiple herbicide modes of actions that it allow farmer to reduce herbicide rates without yield loss. Not only tank mixed herbicide, but also reduced herbicide rate can disrupt herbicide tolerance (Blackshaw *et al*., 2006; Pannacci and Covarelli, 2009). Imazethapyr: It prohibitive broad and some grass leaf spectrum weeds growth within inhibition of amino acid synthesis specially ALS that it led to kill sensitive weeds through systemic xylem and phloem translocate procedure within root and leaf uptake and finally accumulate in the meristemic region of plant and inhibit plant growth. It recommended rate through soybean cultivation is 100 gr.ha-1 active ingredient (a.i) and it consisted on both post and pre emergence weed control (Krausz *et al*., 2001). Bentazon: Is post emergence, contact (none systemic) herbicide of benzothiadiazole family and it scavenging weeds by inhibition of photosynthesis chain throughout impact on quinon B (site of action). plant exposed to this herbicide do not die because of photosynthesis impact, as sunlight still absorb through cells but it doesn't take apart in photosynthesis thus contributes to the formation of the Chl. triplet, which leads to the formation of ROS that damage proteins and membranes of plants (Ahrens, 1994; Hugie *et al*., 2008; Armel *et al*., 2007; Powles, 2010). Bentazon spectrum activity is within chloroplast of broadleaf weeds. Bentazon proper rate in soybean field is 960 gr.ha^{-1} a.i (Han and Wang, 2002; William *et al*., 2009; Martin *et al*., 2014). Sethoxydim: Is systemic and post emergence herbicide of cyclohexanedione family and it impact on grass weeds infestation consisted on lipid synthesis inhibition mode of action in contrast it infest to acetyl coA carboxylase and prohibitive its activity that finally it led to weeds death. Sethoxydim label recommended dose is 375 gr.ha^{-1} a.i. and it does not control broad leaf weeds. Tank-mixed herbicide will increase weed control as a result of manipulating sites of action and prohibition of weed tolerance occurrence. Tank-mixed herbicide can change antioxidant enzymes activity. Otherwise each herbicide composition

could trigger different enzyme activity in contrast of different effect on soybean. Soybean detoxify herbicide side effect interference in different procedure throughout all plant body such as closing stomatal, hormones and antioxidant enzymes (Alexieva *et al*., 2001; Mitller, 2002; Czarnocka and Karpinski, 2018; Caerzan *et al*., 2016). Moreover closing stomatal is the front line of herbicide uptake prevention where as it induce accumulation of reactive oxygen species (R.O.S), which they produce by accumulated energy (Pan *et al*., 2017; Jiasng and Yang, 2009; Zhang *et al*., 2014; Boulahia *et al*., 2016). As a result of this procedure closed stomatal doesn't allow co2 to absorb and participate in photosynthesis chain (Gong *et al*; Luna, 2004). In contrast electron transfer chain by receiving sun light work dramatically that it increase free electones in chloroplast, where they join to oxygen and produces ROS resulting damage to DNA, cell wall, protein and other cell parts (Bailly, 1996 and 2004; Mittler, 2017; Muhling and launchi, 2003; Xu *et al*., 2010; Yordanova *et al*., 2003; Yong *et al*., 2006; Foyer and Noctor, 2005). The most dangerous ROS for cells is superoxide (O_2), its oxidative ability is too much thus harm all plant body (Jung, 2004; Triantaphylides and Havaux, 2009). Moreover, the degree of damage by ROS depends on the balance between the product of ROS and its removal by this antioxidant scavenging mechanism (Azooz *et al*., 2009). In soybean antioxidant enzyme system adjusts ROS activity and it plays the main role (Gechev *et al*., 2002). There is lot confirming survey, which affirmatively consists on this hypothesis that whenever antioxidant enzymes reduced in contrast ROS will rise dramatically in soybean which it effect on yield (Rao *et al*., 2006; Torres *et al*., 1997; Bailly *et al*., 1996; Chiu *et al*., 1995). First stage of detoxification of superoxide consisted on superoxide dismutase enzyme activity which it catalysis superoxide anion to hydrogen peroxide (H_2O_2) that in further chain reactions, will consume by other enzymes and their side effect to soybean will detoxify (Gill and Tuteja, 2010; Li *et al*., 2014; Biaber *et al*., 2004; Galshi *et al*., 2009). SOD main activity occurs at chloroplast, mitochondria and cytosol (Mitller, 2002). Ascorbat Peroxidase (APX) consumes former hydrogen peroxide which produced by SOD and transforms it to H2O and O_2 (Kafi *et al*., 2012; Wang *et al*., 2004). APX main activity is through chloroplast and sytosol (Dabrowska et al., 2007). Catalase cooperate incessantly beside APX as second resistant line in peroxysomes and mitochondria and change two molecule of hydrogen peroxide to water and oxygen (Dubey, 2011; Sairam *et al*., 2009). Glutathione Reductase (G.R) scavenge hydrogen peroxidase throughout chloroplast and produce water and oxygen similar to APX at second line of struggle against ROS infestation toward cells (Ahmad *et al*., 2002; Rasoli *et al*., 2011). Dehydro Ascorbate Reductase (D.H.A.R) participate in modulation of hydrogen peroxide level to water and oxygen indirectly which it cause its lower activity in comparison to other enzyme. D.H.A.R regenerates ascorbate which is substance

of APX enzyme (Gupta *et al*., 2001; Candalios, 2002; Anjum *et al*., 2014). Thus this two enzyme activity increase and decrease consistently. Thereby it's important to investigate both herbicides treatment and their spectrum rate to identify these enzymes activity because they reveal soybean ability of tolerates and detoxification of herbicide side effects.

Study objectives: The objectives of this study were to exert different effect of herbicide (single and mixed herbicides) on soybean antioxidant enzyme, consisted on S.O.D, A.P.X, and CAT. G.R, D.H.A.R activity and identifying how soybean behaves in presence of different herbicide mixture and their different doses.

2. OBJECTIVES

Goals were to estimate five antioxidant soybean enzyme activities during herbicide tank-mix under different reduced rate exposure.

3. MATERIALS AND METHODS

The experiment was laid out with DPX Soybean planted in ploughed field at different plots during 2014 growing season at two location of Karaj province of Iran, including Islamic Azad University research farm at Mahdasht and sugar beet institute research farm at Kamalshahr. Each experimental unit was a single plot with $18m^2$ area, which consisted of 6 soybean row with 50 cm space between each furrow. Each location has different weed flora, which at 1st location dominant weeds were: cocklebur, pigweed, and water hemp and land squarter. In contrast at 2^{nd} location bind weed, land squarter and purslane were prevailing weeds. Soil texture of first place was loamy sandy where second place has sandy loamy with presence. Completely randomized factorial design with 3 replications were used to survey data variance and simple mean comparison used to compare treatment effect on enzymes activity .Herbicide treatment were apply at soybean v2 growth stage by backpack sprayer with v type nuzzle. There were two main treatment consist of herbicide treatment in 7 level composed of single exert of Imazethapyr, Bentazon and sethoxydim, doubled solution of Imazethapyr + Bentazon, Imazethapyr + sethoxydim and Bentazon + Sethoxydim and tripled solution, consist of Imazethapyr + Bentazon + sethoxydim active ingredients. Second treatment was herbicide different rates in 3 levels, where it was compose of: full herbicide dose (equals to 100% of producer recommended dose), reduced to 60% of recommended active ingredient per acre and reduced to 30% of recommended active ingredient per acre. Imzethapyr recommended rate was 100 gr a.i ha^{-1} where it was 960 gr a.i ha^{-1} for bentazon and 375 gr a.i ha^{-1} for sethoxydim. Exact calculation of each herbicide treatment (specially doubled and tripled solution treatment) has shown in table 1.

Sampling assay: Soybean leaf samples collected 2 days after herbicide application and then they froze immediately with liquid nitrogen after that extracted with pestle in ice-colded trasher with 4ml of 0.05M Na_2Hpo_4/NaH_2Po4 (PH 7.0) buffer that contained 0.2 mM ethylenediaminetetracetic acid (EDTA)

and 1% polyvinil- 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 assay: APX activity was measured according to Nakano and Asada (1981). This procedure was depends on decreasing absorbance at 290 nm, where ascorbate was oxidized. The reaction mixture contained 50 mM Na-phosphate buffer (PH 7.0), 50 mM ascorbate, 0.1 mM EDTA.Na_2. 1.2 mM H_2O_2 and 0.1 ml of enzyme extract in a final assay volume of 1ml. Concentration of oxidized ascorbate calculated by coefficient of 2.8 mM^{-1} cm^{-1}. One unit of GR was reduction of 1mmol ml^{-1} ascorbate oxidized min^{-1}.

Glutathione Reductase assay: Activity was measured according to Foyer and Halliwell (1976) procedure. The assay medium contained 25 mM Na-phosphate buffer (PH 7.8), 0.5 mM GSSG, 0.12 mM NADPH.Na_4 and 0.1 ml enzyme extract in a final assay volume of 1ml. NADPH oxidation was followed at 340 nm. Activity was calculated using extinction coefficient of NADPH (6.2 mM^{-1} cm^{-1}). One unit of GR was reduction of 1 mmol ml^{-1} glutathione min^{-1}.

Superoxide Dismutase assay: SOD activity was determined by following the photo reduction of Nitortetrazolium Blue Chloride (NBT). Reaction solution was contain of 100 mM phosphate buffer (PH 7.0), 0.1 mM EDTA, 13mM methionine, 75 µM Nitrotetrazolium Blue Chloride, 2mM riboflavin and adequate mass of the supernatant. The lasy component that adds to solution was riboflavin then reaction started by placing tubes under 15watt fluorescent lamp. By removing reaction tube from light source reaction will finished. Reaction product measured at 560 nm Isoenzymes of SOD were separated on 10% none-denaturing PAGE at 4^{o}c. Then same volume of 40µg each lane loaded. These extract electrophorased and SOD activity determined by visualling according Demiverska-Kepora *et al* (2004).

Catalase activity assay: CAT extract (20µl) added to reaction mixture contain of 750 µl hydrogen peroxidase (H_2O_2), 70mM and 750 µl of phosphate buffer (PH 7.0) 100mM. then adjusted to 3ml with sterile distilled water. Then absorbance read at 240nm (Margonis *et al.,* 2007).

Table 1. tank-mixed and single herbicide treatment dose calculations

Treatments (Herbicide and Herbicide Rates)											
	A.I	IBS100	IBS60	IBS30	IB100	IB60	IB30	IS100	IS60	IS30	BS100
*	I	33gr	20gr	10gr	50gr	30gr	15gr	50gr	30gr	15gr	480gr
A.I	B	320gr	192gr	96gr	480gr	288gr	144gr	187gr	112gr	56gr	187gr
Rate	S	125gr	75gr	37gr	--	--	--	--	--	--	--

* A.I=active ingredients gr= grams I=imazethapyr B=betazon S= sethoxydim, IB= imazethapyr+bentazon IS= imazethapyt + sethoxydim, BS= bentazon + sethoxydim and IBS=imazethapyr+bentazon+sethoxydim. 100= full recommended rate of herbicide 60= reduced to 60 percent of label recommended rate 30= reduced to 30 percent of label recommended rate.

Continue table 1.

Treatments (Herbicide and Herbicide Rates)											
	BS60	BS30	I100	I60	I30	B100	B60	B30	S100	S60	S30
*** A.I**	288gr	144gr	100gr	60gr	30gr	960gr	576gr	288gr	375gr	225gr	112gr
Rate	112gr	56gr	--	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--	--	--	--

* A.I=active ingredients gr= grams I=imazethapyr B=betazon S= sethoxydim, IB= imazethapyr+bentazon IS= imazethapyt + sethoxydim, BS= bentazon + sethoxydim and IBS=imazethapyr+bentazon+sethoxydim. 100= full recommended rate of herbicide 60= reduced to 60 percent of label recommended rate 30= reduced to 30 percent of label recommended rate.

Dehydroascorbate reductase assay

to measure DHAR, a reaction mixture containing phosphate buffer (PH 7.0) 0.7 ml, reduced glutathione (GSH) 20 $m.mol.l^{-1}$ 0.1 ml in phosphate buffer (PH 7.0), 2 $m.mol.l^{-1}$ DHA 0.1 ml, and crude enzyme 0.1 ml was used. DHA was freshly prepared and kept on ice until it was added to reaction mixture in the cuvette to prevent its fast oxidation at room temperature. The reduction of DHA to ASA was monitored by the increase in absorbance at 290nm, taking 2.8 $(mmol/l)^{-1}$ cm^{-1} as the absorbance coefficient (krivosheeva *et al.,* 1996).

4. RESULT AND DISCUSSION

Analysis of variance (Table 2) demonstrates that both main treatment (herbicide and rate) had significant effect in 1% probability at all measured enzymes. It corroborate that by changing herbicide treatment antioxidant activity will change definitely (by 99% probability). Similarly, antioxidant enzyme activity changed during herbicide rate treatment exertion (at 99% probability).

Table 2. Analysis of variances of superoxide dismutse (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and dehidroxyascorbate reductase (DHAR)

S.O.V	df	SOD	APX	CAT	GR	DHAR
Location	1	0.31 **	0.3 **	0.36 **	0.3 **	0.11 **
Block	4	0.44 **	0.4 **	0.62 **	0.19 **	0.033 **
Herbicide	6	33.6 **	14.5 **	18 **	5.4 **	0.8 **
Herbicide dose	2	105 **	17.4 **	8 **	2.1 **	0.3 **
Herbicide × herbicide dose	12	1.1 **	0.7 **	0.07 **	0.04 **	0.001 **
Location × herbicide	6	0 ^{ns}	0.001 ^{ns}	0 ^{ns}	0 ^{ns}	0 ^{ns}
Location × dose	2	0 ^{ns}	0 ^{ns}	0 ^{ns}	0 ^{ns}	0 ^{ns}
Location × herbicide × dose	12	0 ^{ns}	0.001 ^{ns}	0 ^{ns}	0 ^{ns}	0 ^{ns}
Error	80	0.001	0.001	0.001	0.009	0.0001
C.V		0.8	0.92	0.99	3	2.3

^{ns}, * and ** indicates non-significant and significant at 5% and 1% probability level respectively.

According to none significant interaction between location and treatments (location × herbicide, location × herbicide rate and location × herbicide × herbicide rate) all table and discussions just arranged and interpret by mean of

both locations at one obtained data. Maximum activity of SOD as a front line of scavenging ROS constraint registered in Imazethapyr single spray over soybeans where it applied at full rate (100 gr ha^{-1} a.i thereby 8.8 i.u SOD activity, table3). This incident can be due to the same activity place of both herbicide active ingredient and SOD enzyme which they present at sytosol and mitochondria, also imazethapyr full rate can cause soybean stomatal closure, which it induce ROS activity that finally led to increase SOD activity. This result confirmed by Caverzan *et al* (2019) which demonstrate, imazethapyr induces ROS activity and accumolation that finally it led to increase antioxidant activity. As visual evidence in this research imazethapyr had no plant injury on soybean it can be due to proper stomatal reaction to herbicide molecols which they react incessant and close immediately. Bentazon at 960 gr a.i ha^{-1} rate recorded minimum SOD activity equals to 7.5 i.u, which in compare to control treatment (weed free soybean SOD activity) it raised 11.2 times. This tiny activity of SOD among single herbicide treatment consisted on different place activity of SOD and bentazon site of action, as a result they have poor adverse interference. In addition bentazon has low active ingredient power per acre, thus it cause less soybean stomatal closure which it led to reduce ROS production and activity. It confirmed by William *et al* (2009), which they revealed bentazon has no injury to soybean. In contrast of dual solution herbicide treatments highest SOD infestation registered at imazethapyr + bentazon treatment, which respectively at 50 and 48 gr a.i ha^{-1} (equals to full rate recommended of each herbicide component) it was 6.1 i.u. this high SOD infestation could be due to presenting of two broadleaf herbicide component with high active ingredient in one solution that they have mutual site of actions (cytosol and chloroplast) with SOD. Not only imazethapyr + bentazon treatment impact broad spectrum on weeds, but also it can harm soybean too. Thus soybean resist against and detoxify this herbicide with excess SOD activity that it happened at full rate, which can promote soybean stomatal closure and ROS accumulation but at reduced rate of this herbicide treatment SOD activity reduced.

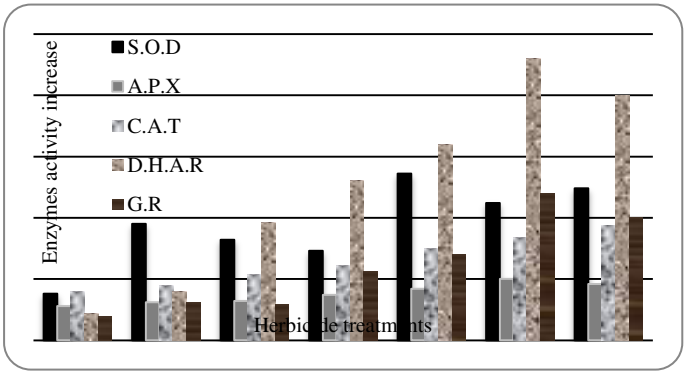

Fig.1. Mean of Soybean antioxidant enzymes increase in comparison to control treatment at different herbicide treatments.

I=imazethapyr B=betazon S= sethoxydim, IB= imazethapyr + bentazon IS= imazethapyt + sethoxydim, BS= bentazon + sethoxydim and IBS= imazethapyr + bentazon + sethoxydim.

Lowest SOD activity in doubled tank-mix herbicide demonstrated in Bentazon + Sethoxydim treatment, which it was 4.7 i.u at 480 and 187 gr a.i ha^{-1} respectively that in comparison to control treatment it raised 7.3 time. This demonstrate soybean tolerance against this herbicide treatment is less affinity to SOD activity and it fight back by other procedure like none enzyme procedure. During this research minimum SOD activity through all treatment demonstrated at tripled tank-mix herbicide treatment contain of Imazethapyr + Bentazon + Sethoxydim active ingredient, which it was 1.9 i.u at 10, 96 and 37 gr a.i ha^{-1} respectively that it equals to reduced rate of 30% of recommended dose of each herbicide component. Otherwise it show by using tank-mixed herbicide the same or lower SOD activity will obtain with reduced herbicide rates. This result registered maximum soybean yield and minimum weed interfere at this treatment. It confirm this hypothesis that reduced dose of mixed herbicide will reduce soybean harm and it can resist easily. In all herbicide treatment by reducing herbicide rate from full recommendation dose to reduced 30% of recommended dose SOD activity diminish and confirm this hypothesis gain. APX activity in control treatment was 1.2 i.u. Highest APX activity registered at bentazon treatment at 960 gr a.i ha^{-1} (full rate) where it was 7.2 i.u (table 3) .This result could be due to the same action place of both herbicide and enzyme where they both work at chloroplast. Bentazon work at chloroplast in thylakoid membrane to impact on photosynthesis, which could increase ROS activity that it promote APX activity to scavenge and oxidase H_2O_2 to water and oxygen, which former produced by SOD. Minimum APX activity noted at imazethapyr treatment in all rates specially reduced rate of 30 gr a.i ha^{-1}, which it recorded 4.2 i.u that it raised 3.5 times in compare to control. Through doubled herbicide mixture maximum APX activity occurred in bentazon + sethoxydim treatment through 480 and 187 gr a.i ha^{-1} rate respectively which it measured 5.1 i.u. This high APX activity could be in a result of same site of action of these treatment particles with APX activity place, which both herbicide interference at chloroplast and mitochondria that it was the same with APX. Furthermore both participated particle in this herbicide mixture used as high active ingredient per acre that it will promote stomatal closure which it induce APX accumulation. Aksoy and Dinler (2012) confirm high activity of APX over abiotic stress on soybean, which it demonstrated in our study. Minimum APX activity through all herbicide treatment registered at imazethapyr + bentazon + sethoxydim which induced at 10. 96 and 37 gr a.i ha^{-1} rate (equals to reduce to 30% of recommended rate of each herbicide), where it was 3.5 i.u. comparing of this herbicide treatment at full rate (33, 320 and 125 gr a.i ha^{-1} of each herbicide component) using with reduced to 30% rate demonstrate that just 0.4 i.u APX activity will change (APX

activity change from 3.5 i.u at reduced to 30% rate to 3.9 i.u at full rate of herbicide treatment). This result shows in this herbicide treatment by reducing rate from full recommended rate to 30% of recommended rate just a little APX activity will raise and regarding to reduce rate benefits can use reduced rate with proper soybean yield and weed control. Moreover by reducing herbicide rate from full rate of label recommended to reduce to 30% of label recommended herbicide rate, APX activity was poor through all herbicide treatment. Otherwise it demonstrate that reduced rate of tank-mixed herbicide has minimum side effect on soybeans and its antioxidant enzyme activity thus we could lower herbicide rate by using herbicide tank-mixing. Catalase (CAT) activity through control treatment registered 0.5 i.u. in contrast of SOD and APX, CAT main.

Table 3. Mean of both locations by induction treatment on superoxide dismutse (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and dehidroxyascorbate reductase (DHAR) activity

		Treatments (Herbicide and Herbicide Rates)										
		IBS100	**IBS60**	**IBS30**	**IB100**	**IB60**	**IB30**	**IS100**	**IS60**	**IS30**	**BS100**	**BS60**
Enzymes activity (i.u)	**SOD**	3.9	3.5	1.9	6.1	5.1	3.1	5.2	4.5	2.7	4.7	4.1
	APX	3.9	3.7	3.5	4..1	3.8	3.6	4.7	4.1	3.7	5.1	4.5
	CAT	2.4	2	1.7	2.7	2.2	1.9	3.1	2.6	2.4	3.5	3
	GR	0.4	0.3	0.2	0.6	0.5	0.3	0.8	0.6	0.4	1.1	0.9
	DHAR	0.2	0.1	0.07	0.3	0.2	0.1	0.4	0.3	0.2	0.5	0.4

I=imazethapyr B=betazon S= sethoxydim, IB= imazethapyr+bentazon IS= imazethapyt+sethoxydim, BS= bentazon+sethoxydim and IBS=imazethapyr+bentazon+sethoxydim. 100= full recommended rate of herbicide 60= reduced to 60 percent of label recommended rate 30= reduced to 30 percent of label recommended rate.

Continue table 3.

		BS30	**I100**	**I60**	**I30**	**B100**	**B60**	**B30**	**S100**	**S60**	**S30**	**control**
Enzymes activity (i.u)	**SOD**	2.2	8.8	6.9	4.7	7.5	5.7	3.7	8.1	6.4	4.1	0.5
	APX	3.9	5.9	5.1	4.2	7.2	6	4.9	6.5	5.7	4.6	1.2
	CAT	2.7	4.2	3.9	3.2	4.7	4.3	3.7	5.2	4.8	4.1	0.5
	GR	0.6	1.4	1.1	0.8	2.1	1.8	1.5	1.8	1.5	1.2	0.15
	DHAR	0.3	0.6	0.5	0.4	0.8	0.7	0.6	0.7	0.6	0.5	0.029

I=imazethapyr B=betazon S= sethoxydim, IB= imazethapyr+bentazon IS= imazethapyt+sethoxydim, BS= bentazon+sethoxydim and IBS=imazethapyr+bentazon+sethoxydim. 100= full recommended rate of herbicide 60= reduced to 60 percent of label recommended rate 30= reduced to 30 percent of label recommended rate.

Activity takes apart at setoxidym within all treated rates but maximum activity registered at full rate (375 gr a.i ha^{-1}). This same activity of setoxydim and CAT could be due to the same working place of them, which catalase work within peroxysomes and mitochondria to detoxify hydrogen peroxide, whereas setoxidym has maximum site of action to prohibit of lipid synthesis. This work sight can connect their activity and increase them parallel. According to forecasting minimum CAT activity through singled herbicide occured at imazethapyr in 30 gr a.i ha^{-1} , where it registered 3.2 i.u, which it was the lowest CAT activity through single herbicides (table3). This can explain by different

working place of both herbicide and CAT enzyme, which imazethapyr site of action is within chloroplast when CAT doesn't exist there. Throughout doubled component tank-mixed herbicide minimum CAT activity (1.9 i.u) sighted at imazethapyer + bentazon when they used by reduced rate of 15 and 144 gr a.i ha^{-1} (reduced to 30% of recommended rate of each solution) respectively. This minimum activity spectrum throughout doubled solution herbicide is due to different site of action of each compartment herbicide in comparison to CAT activity. Lowest CAT activity through all herbicide treatment registered at imazethapyr + bentazon + sethoxydim using at 10, 96 and 37 gr a.i ha^{-1} rate respectively (equals to reduce to 30% of recommended dos), which it obtained 1.7 i.u. This result demonstrate that by manipulating herbicide site of actions, which it present by tank-mixed herbicide can lower herbicide rate to minimum impact on soybean and yield without any yield loss or diminish loss. Glutathione Reductase (GR) activity changed through herbicide treatment, where its maximum activity initiated at bentazon during full rate application (960 gr a.i ha^{-1}). It also confirmed by Aksoy and Dinler (2012), which noted that high GR activity occurred at maximum abiotic stress. Not only bentazon activity is just through chloroplast specially on thylakoid electron transfer, but also GR main activity is the same at chloroplast, which it promote their activity induction parallel. Nevertheless according to visual injury index bentazon had no harm to soybean that it could be as a result of soybean power to closuring stomatal immediately against bentazon that it can accelerate accumulation of ROS that led to initiate scavenger enzyme activity specially GR. According to former forecast minimum GR activity through single solution herbicide registered at imazethapyr treatment when it induced to soybean within reduced rate of 30 gr a.i $ha^{-1,}$ its activity was 0.8 i.u. as a result of different activity place between GR and imazethapyr, which GR main activity is through chloroplast but imazethapyr main site of action is through cytosol this hypothesis corroborate this results. Among dual solution herbicides treatment highest GR activity recorded at bentazon + sethoxydim in full rate use, which it was 480 and 187 gr a.i ha^{-1} that induced 1.1 i.u activity of GR. Presenting two herbicide in this dual solution herbicide that both them target focused on chloroplast, can be the main cause of increasing GR activity in this treatment. Imazethapyr + bentazon + sethoxydim registered minimum activity of GR, equals to 0.2 i.u (in comparison to control which it was 0.15 i.u) at reduced dose of 10, 96 and 37 component respectively (equals to reduced rate of 30%). This demonstrates by tank mixing this component as a one solution, which led to increase site of action can lower herbicide rate to reduce herbicide side effects on soybean. This can be due to incessant stomatal opening through herbicide usage because of the less side effect of reduced rate and different site of action that induced less ROS production that it is the main reason for lowering GR activity. According to data shown in table 3 by reducing herbicides

rate in all treatment GR activity decrease due to lower ROS production. Dehydroxy Ascorbate Reductase (DHAR) activity at control treatment was 0.029 i.u, albeit maximum activity of this enzyme registered at bentazon treatment during 960 gr a.i ha^{-1} full rate induction to soybean. This confirmed by Aksoy and Dinler (2012). DHAR main activity is through chloroplast where it produce ascorbate by reducing dehydro ascorbate to proceed scavenging ROS, thus it simultaneously work at the same place of bentazon site of action that take apart at chloroplast, these all led to increase DHAR by using bentazon at full rate. Minimum DHAR activity among single solution treatment registered at imzethapyr at 30 gr a.i ha^{-1} rate where it was 0.4 i.u. This was due to different placement of both activities. According to Figure1 that it reveals how many times enzymes activity raised, maximum enzyme level accordance to control treatment registered at Bentazon and DHAR enzyme which DHAR raised 23 times in contrast to control. It shows that bentazon caused maximum ascorbate consumption to scavenge ROS that this ascorbate produces by DHAR enzyme. Similarly maximum GR increase registered at bentazon. Generally minimum enzyme increase registered at imazethapyr + bentazon + sethoxydim treatment as presented former. Moreover at tank-mixed treatment the lowest enzyme increasing registered, which in contrast maximum enzyme increasing registered through singlet herbicide application. Imazethapyr + bentazon + sethoxydim and imazethapyr + bentazon treatment demonstrated more SOD rising in comparison other enzyes, it would be due to lower toxicity of this treatment herbicide that it just scavenge by SOD and CAT simontaneosly, which APX and GR had less activity to scavenge ROS.

Table 4. Mean comparison of main effect of herbicide treatment on superoxide dismutse (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and dehidroxyascorbate reductase (DHAR) activity on soybean.

Herbicide treatment	Soybean antioxidant enzymes				
	SOD	APX	CAT	GR	DHAR
Imazethapyr+bentazon+sethoxydim	3.1 G	3.7 G	2.1 G	0.35 G	0.15 G
Imazethapyr+ bentazon	4.8 D	3.8 F	2.3 F	0.51 F	0.22 F
Imazethapyr+sethoxydim	4.1 E	4.2 E	2.7 E	0.65 E	0.32 E
Bentazon+sethoxydim	3.7 F	4.5 D	3.1 D	0.91 D	0.43 D
imazethapyr	6.8 A	5.1 C	3.8 C	1.1 C	0.53 C
Bentazon	5.6 C	6.1 A	4.2 B	1.8 A	0.72 A
sethoxydim	6.2 B	5.6 B	4.7 A	1.5 B	0.63 B

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).

Table 5. Mean comparison of main effect of herbicide doses on SOD, APX, CAT, GR, DHAR.

Herbicide dose	Soybean antioxidant enzymes				
	SOD	APX	CAT	GR	DHAR
100%	6.3 A	5.3 A	3.7 A	1.2 A	0.52 A
60%	5.2 B	4.7 B	3.3 B	1 B	0.43 B
30%	3.2 C	4.1 C	2.8 C	0.76 C	0.33 C

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).

According table 4 which demonstrate mean comparison of main effect of herbicide treatment on enzymes activity each herbicide effect on enzyme grouped at different bunch, which demonstrate broad spectrum of herbicides and their interactions on antioxidant enzyme, causing different herbicide site of actions on soybean that it promote different enzyme to scavenge herbicide side effect on cells. Although at end of table, which minimum enzymes activity revealed, tank-mixed herbicide presented. This confirm it by manipulating herbicide site of actions and reduced rate of active ingredient per acre when herbicide mixed each other, ROS activity will reduce minimum level that it will led to lower antioxidant enzyme activity to scavenge ROS harm to soybean. According to this table at front line of enzyme activity single herbicide placed which have more active ingredient rate per acre which can induce stomatal closure and ROS concentration, hence it will led to increase enzyme activity. According table 5 which demonstrate mean effect of herbicide rates on enzyme activity, by reducing herbicide dose all five enzymes activity reduced and each dose led to different enzyme response, which it confirmed by their different grouping. So lowest enzymes activity registered at reduced to 30% of recommended dose, throughout all treatments. This can be due to minimizing stomatal closure and absorb CO_2 through leaf cells without any constraint it prevent ROS production and reduce antioxidant enzymes.

5. CONCLUSION

Our result revealed that by reducing herbicide rate ROS production will be lower, hence antioxidant enzymes activity will reduce. Furthermore by tank-mixing herbicide it will be possible to reduce herbicide rate without yield loss and weed control, this positively led to lower enzyme activity. In contrast minimum enzyme activity registered at imazethapyr + bentazon + sethoxydim at reduced rate of 30% of label recommended dose, throughout all treatments. Even though maximum enzymes activity registered when single herbicides used at full recommended dose. This information will help managing soybean fields against weeds by using reduced dose of tank mixed herbicide.

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FOOTNOTES

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