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Evaluation of biofertilizer effect on selective soybean herbicides efficiency to control of *Xanthium spinosom* and *Datura stramonium*

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Key words: *Bradyrhizobium japonicum*, grain yield, weed biomass, weed density.

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Abstract

This experiment was conducted during 2009 growing season as split plot with randomized complete block (RCBD) design arrangement in a field of Faculty of Agriculture, Islamic Azad University, Karaj branch, Iran. Treating or not treating with biofeltilizer (*Bradyrhizobium japonicum*) was the main treatments. The sub-treatments were application of herbicides consisted of trifluralin, ethalfluralin, metribuzine, oxyflorfen, bentazon, aciflourfen+bentazon respectively at 1.2, 1.16, 0.35, 0.48, 1.2, 1.06 kg ha^{-1} and weedy check. In this research, application of biofertilizer on efficiency of some herbicides was effective in decreasing weeds dry weight. The biomass of cocklebur (*Xanthium spinosom*) in treatments trifluralin, ethalfluralin, aciflurfen+bentazon or bentazon, was significantly less than weedy check, but oxyflorfen and metribuzin had no significant effect on the biomass of this weed. However, the herbicides, compared with weedy check, had no significant effect on the density of cocklebur. All herbicides, compared with weedy check, significantly reduced the density and biomass of jimsonweed (*Datura stramonium*). Regard less of statistic analysis, the mean biomass of jimsonweed in the treatment of aciflourfen+bentazon was less than the other treatments. Application of aciflourfen+bentazon, bentazon compared with weedy check resulted in significant grain weight of soybean. Compared with weedy check, all treatment except oxyflorfen, increased soybean 1000-grain weight. Although the soybean 1000-grain weight in the treatments aciflourfen+bentazon, bentazon and ethalfluralin was higher than other treatment, but these treatments were not significantly different from metribuzin and ethalfluralin.

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Introduction

Lands under soybean plantation in Iran in 2011 were 70 thousand hectare and rate of production was estimated, 170 thousand tone (FAO, 2013). *Xanthium spinosum* L. decreased 70% yield of bean (Mclelland, 2002). Result of Friesen (1987) showed the presence 13 plants of *Sinapis arvinsis* in each meter of plant line can decrease *Helianthus annus* L. yield by ratio of 35%. Shaw *et al*., (1990) Reported American farmers spend yearly up to 6.2 billion of dollars on controlling of farm and pasturage weeds. From this amount 3.6 billion of dollars goes on buying 200 million kilograms of herbicides. The average damage of weeds in corn plantation in Iran is reported at ratio of 86% (Mousavi, 2001). Hence, herbicides consumption trend in Iran during the previous years represented that this measure is going up. Already just about half of 24 million liter or kilogram of venom consumption in the term of agriculture devote to herbicides (Zand *et al*., 2009). Hadizade *et al*., (1998) reported competition between soybean and weeds cause to morphological changes in soybean canopy just like plant height, increase in middle node, decrease diameter of original stem and decrease in length and number of sub branches. Stoller & Woolley (1985) reported the unlimited habit of weeds growth like *D. stramonium* lead to increase the length of them. In convergence of crop growth *D. stramonium* establishes its leaves on top of the canopy in order to light competition with crops which makes it dominant over the main crop (Regnier & Stoller. 1989; Pike *et al.,* 1990). Stephan & Roskamp (1998) demonstrated that thifensulfuron at 2.2 to 8.8 g/ha in combination with bentazon at 420 g/ha may be safely applied to soybean for broadleaf weed control such as *D. stramonium* and *X. spinosom*. According to Brickell & Jordan (1980) oxyfluorfen, all at 0.38-0.5 lb/acre and bifenox at 1.5 to 2 lb/acre gave good selective control of jimsonweed (Datura stramonium), Capsella bursa-pastoris and Chenopodium album. Control was less satisfactory when the treatments were incorporated before sowing the soybeans. Post-emergence treatments with acifluorfen and MC 10978 (acifluorfen-sodium) at 0.5, 0.75 and 1 lb/acre also performed well. Ovesen, (2001) showed application of aciflourfen+bentazon controlled *X. spinosom* L. by the rate of 80%. Marwat *et al*., (1995) showed interference of *Abutilon theophrasti* L. with soybean in each square meter decreases soybean yield by 35%. Also competition of 10 and 15 *A. theophrasti* L. in each square meter decreased soybean yield by 58 and 74, respectively. Also, they declared weed species which has less height than corps decreases the yield when they compete for water. Even though nutrient increase the plant growth but weeds use it more than crops.

In accordance with sustainable agriculture, some of soil micro organisms which have symbiosis with plants and use as biofertilizers to supply food elements are in extension (Sharma, 2003). These micro organisms usually are from bacteria, and equipped with an enzymatic system that enables them to break the triple bond between two nitrogen atmospheric atoms and produces ammoniac, that similar to industrial processes but has no expense for unrenewable energy resources (Dalla Santa *et al*., 2004). In agriculture, herbicides have been used in large scale. But often, there is no research on their sub effects. This is very important in crops. Because, herbicides not only will have adverse effect on plant growth, also influence on the interaction relation between symbiosis bacteria just like *rhizobioum*es and plant growth promoting bacteria (Brock, 1975). Studies showed some of the herbicides keep adverse effect on plants nodulation. These effects on main roots nodules were more than sub roots. Trifluralin decreases the growth of sub roots and makes nodules in soybean (Kust *et al*., 1971; Eberbachk *et al*., 1989). Even though biofertilizer application improved in Iran their interaction with herbicides are very less Therefore the aim of this research were efficacy evaluation of herbicides in control of soybean weeds and checked their interaction with biofertilizer like (*Bradyrhizobium japonicum*), and the effect of biofertilizer on soybean yield.

Materials and methods

Site description and experimental design

This experiment was conducted during 2013 growing

season as split plot with randomized complete block (RCBD) design arrangement with four replications in a field of faculty of agriculture Islamic Azad University Karaj, Iran. In position of (35° 45′N, 51° 56′E; 1313 m above the sea level). This area has average temperature with relative humidity of 36 to 73 percent and less rain per year (Anonymous, 2009). Main factors were application and non-application of biofertilizer (*Bradyrhizobium japonicum*), with the population of $2.3 \cdot 10^8$ bacteria per each gram. For every kilogram of soybean seed *Glycine max* L. ver. of Williams cultivar an amount of 20 ml of solution was added and mixed well till all seeds were mixed with this substance well then seeds were kept in an open air for a period of half an hour in the shadow then soybean planted after planting soybeans seeds watering started immediately. The sub-treatments were application of herbicides consisted of trifluralin EC 48%, ethalfluralin EC 33.3%, metribuzine WP 70%, oxyflorfen EC 24%, bentazon SL 48%, aciflourfen+bentazon SL 6.42% respectively at 1.2, 1.16, 0.35, 0.48, 1.2, 1.06 kg ai ha^{-1} (Vencill, 2002; Tomlin, 2003) and weedy check. Trifluralin and ethalfluralin as pre-plant and soil incorporation, oxyflorfen and metribuzine pre-emergence and other herbicides applied as post-emergence in 4-6 leaves stage of soybean. All herbicides were sprayed with hand lever knapsack sprayer equipped with standard flat fan T-jet nuzzle and calibrated to deliver 375 L ha^{-1} of spray solution at a pressure of 2.5 bar. Immediately after using pre-plant and soil incorporation herbicide helped the rake to mix them with soil, Mixed done well, up to depth of 10 cm. Size of each plot was 2.5 m wide and 6 m length. Plots length consisted of five rows of plant with 6 m length. Distance of plots in every block was 50 cm and distance of blocks from each other was 2 m. Also distance of plant lines were 50 cm. Sub plots were managed in such a way that while irrigation, water should not enter the other plot. All operations like fertilizing, irrigation, pest control were done according to the technical advises.

Weed and crop measurements

Evaluation including weeds population was measured separately for each weed species by counting the number of weeds 21 days after last treatment (DAT) within two fixed 0.5 m^2 quadrates that were dropped in to the treated of each plot accidentally which showed total weeds of that plot. In kernel filling stage in ear by keeping quadrates 0.5 m^2 in two points from every plot accidentally which declares total weeds of that plot. All weeds were mow at the ground level, separated by species and oven dried at 75°C for 48 hour. Then the biomass of all weed species was weighted. After seed maturity to value the soybean yield, harvest was done from 3 middle lines of 4 m and then weighted. After harvest, sampling from seeds was done by each plot. 1000 grain weight in separate plot was determined.

Data analysis

All data were analyzed statistically using program procedure in SAS statistical software (SAS institute, 2000). Duncan multiple rang test (DMRT) set at 0.05 was used to determined the significance of the difference between treatment means and by using excel software graphics were drawn.

Results

Plant Phytotoxicity

In this research, after 3 weeks among herbicides application bentazon caused chlorosis and leaf necrosis. These marks appeared as brown stains in old leaves but had not bad effect on soybean yield. Appearance of necrotic effects in both original treatments of biofertilizer application and none application were same. Other herbicides did not make any remark on soybean plants. Among native weeds, analysis in herbicides applications were achieve on dominant weeds of area and are explained.

Weed Control

Datura stramonium L.

Variance dissolve results (Table 1) showed, despite none application biofertilizer treatment, the application of that with bentazon showed no significant difference in biomass of *D.stramonium* L. Also trifluralin with biofertilizer application compare to none application significantly achieved less

biomass of this weed (Figure 1). There was no significant difference between application and none application of biofertilizer with herbicide in number and dry weight of this weed (Figure 1). There was significant difference by herbicide application in number and dry weight of this weed in level 1%. In done evaluations, all herbicides treatments decreased the number of this weed in ratio of 93-64% and its biomass in ratio of 99-85% (Figures 2 & 3).

Table 1. Analysis of variance for different biofertilizer, herbicide and their interaction treatments on density and biomass of weeds and soybean grain yield.

Mean Square						
S.O.V	DF	Grain yield (kg/ha)	Density (p/m²)		Biomass (g/m²)	
			DATS	XANST	DATST	XANST
Rep	3	107981.190	0.0527	0.0869	0.4465	0.1141
A (Biofertilizer)	1	$0.714^{n.s}$	$0.0006^{n.s}$	$0.0077^{n.s}$	$1.2170^{n.s}$	$0.0053^{n.s}$
E(a)	3	31756.428	0.0142	0.0422	0.4275	0.2152
B (Herbicide)	6	1508957.857^{**}	0.0747^{**}	$0.0523^{n.s}$	6.0793^{**}	0.4444^{*}
A*B (Biofertilizer* Herbicide)	6	$70673.214^{n.s}$	$0.0306^{n.s}$	$0.0199^{n.s}$	1.9702^{**}	$0.0447^{n.s}$
E(b)	36	39874.8948	0.0173	0.0488	0.2799	0.1754
C.V. %		23.67863	4.800565	8.040375	16.43836	14.33782
A*B Effect Sliced by A						
Biofertilizer	DF	Grain yield (kg/ha)	Density (p/m²)		Biomass (g/m²)	
			DATST	XANST	DATST	XANST
Application	6	$762700^{n.s}$	0.043837^{*}	$0.040145^{n.s}$	1.290095^{**}	$0.301471^{n.s}$
Non-Application	6	$816930^{n.s}$	0.061545^{**}	$0.032199^{n.s}$	6.759509^{**}	$0.187772^{n.s}$
A*B Effect Sliced by B						
Herbicide	DF	Grain yield (kg/ha)	Density (p/m²)		Biomass (g/m²)	
			DATST	XANST	DATST	XANST
Trifluralin	1	$10351.25000^{n.s}$	$0.036036^{n.s}$	$0.013932^{n.s}$	2.266581^{**}	$0.006781^{n.s}$
Ethalfluralin	1	$37845.00000^{n.s}$	0.097214^{*}	$0.033350^{n.s}$	$0.324034^{n.s}$	$0.121537^{n.s}$
Metribuzine	1	$162900^{n.s}$	$0.015687^{n.s}$	$0.004171^{n.s}$	$0.049599^{n.s}$	$0.004533^{n.s}$
Oxyflorfen	1	$46080.00000^{n.s}$	$0.015687^{n.s}$	$0.034561^{n.s}$	$0.335145^{n.s}$	$0.046919^{n.s}$
Bentazon	1	$12500.00000^{n.s}$	$0.004171^{n.s}$	$0.033350^{n.s}$	1.597596^{*}	$0.033759^{n.s}$
Aciflourfen + Bentazon	1	$361.25000^{n.s}$	$0.015687^{n.s}$	$0.004171^{n.s}$	$1.531838^{n.s}$	$0.059584^{n.s}$
Weedy check	1	$154000^{n.s}$	$0.000052011^{n.s}$	$0.004171^{n.s}$	8.465884^{**}	$0.000758^{n.s}$

(DATST): *Datura stramonium* L., (XANST): *Xanthium spinosom* L

ns: not-significant

**,* means within each column followed by same letter are not significantly different according to Duncan's multiple range test at the 1 % and 5 % probability level.

Xanthium strumarium L.

Variance dissolve results (Table 1) showed there is no significant difference between application and none application of biofertilizer in density and biomass of *X. strumarium* L. Also no significant difference was observed in interaction of biofertilizer with herbicide. Evaluations showed neither of herbicides treatment could affect on decreasing density of *X. strumarium* L. compared to weedy check (Figure 4). Whereas bentazon, aciflourfen+bentazon, ethalfluralin and

trifluralin application respectively decreased weed biomass by rate of 78, 70, 55 and 38.59% compare to weedy check. Oxyflorfen had no efficacy on this weed biomass (Figure 5).

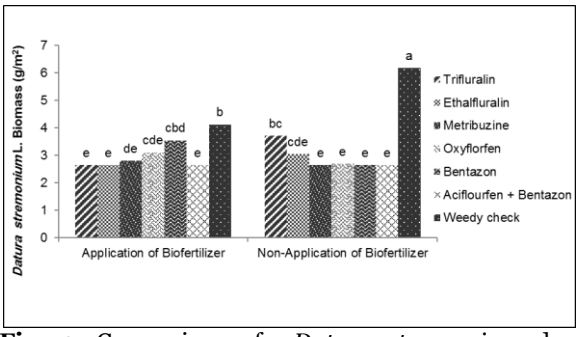

Fig. 1. Comparison of *Datura stramonium* dry weight in treatment's experiment. Mean within each column followed by same letter are not significantly different (Duncan 5%).

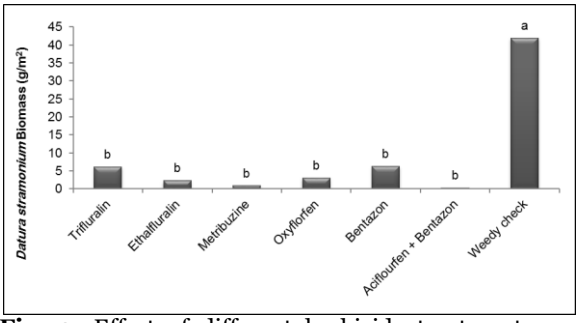

Fig. 2. Effect of different herbicide treatments on jimsonweed (*Datura stramonium*) populations reductions. Means within each column followed by same letter are not significantly different (Duncan 5%).

Soybean Grain Yield

Variance dissolve results (Table 1) showed although interaction between biofertilizer with herbicide had no significant efficacy on grain yield, there were significant differences in categorizing treatments in variance dissolve results between those. As in both fertilizer conditions just aciflourfen+bentazon had maximum grain yield compare to weedy check. But in this province other herbicides did not have any significant differences compare to weedy check (Figure 6). There were significant differences between herbicides treatment in level of 1%. Among herbicides aciflourfen+bentazon had maximum grain yield which was statistically equaled with bentazon. The least grain yield rate was belonged to weedy check and all of the herbicides treatments increased grain yield. Grain yield weight in aciflourfen+bentazon by rate of 40% was more than weedy check and other treatments (Figure7).

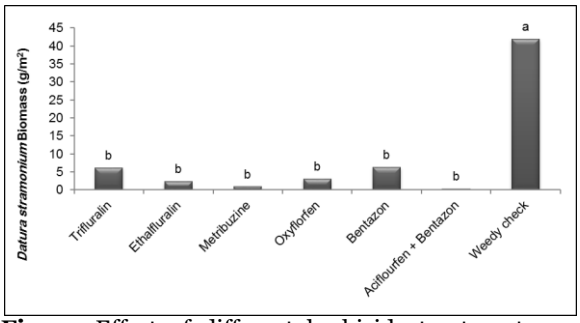

Fig. 3. Effect of different herbicide treatments on jimsonweed (*Datura stramonium*) biomass reductions.Means within each column followed by same letter are not significantly different (Duncan 5%).

Discussion

At present in Iran there is no report in relation with herbicides interaction on biofertilizers. Herbicides are used in broad level in agriculture. But there is no research on their sub effects. This matter has special importance about cereals. Because herbicides not only will have adverse effect on plant growth, also influence on the interaction relation between symbiosis bacteria just like *rhizobioum*es and plant growth promoting bacteria (Brock, 1975). Studies showed some of the herbicides keep adverse effect on plants nodulation. These effects on main roots nodules were more than sub roots. Trifluralin decreases the growth of sub roots and makes nodules in soybean (Kust *et al*., 1971; Eberbachk *et al*., 1989). Bollich *et al*., (1988) reported that pendimetalin and trifluralin made disturbance in soybean germination and decreased number of nodes, dry weight and nitrogen fixed, but had no inhibition effect on nodulation and nitrogen fixation in grain yield. Upon experiments, pre-emergence application of alachlore and metribuzine decreased nodulation in soybean root but had no negative effect on grain yield (Mallik *et al*., 1985). Moorman (1986) reported a reduction in soybean-node-weight by treating alachlor, linuron and trifluralin herbicides without any diminishing in crop yield. Eberbachk *et al*., (1989) reported the phenoxy group pesticides had harmful effects in root

nodulation. Dunigan *et al*., (1972) reported 2,4-D and MCPA herbicides stopped different verities of rhizobioums activity. Brock, (1975) reported dalapon treatment had no effect on nodes number or total weight in bird foot clover, also 2,4-DB decreases the both of mentioned subjects. Rennie *et al*., (1984) Reported nitralin and prometryn treatments had harmful effects on soybean nodulation and also linuron and trifluralin treatments respectively decreased nodules in soybean by ratio of 1.12 and 0.84 kg ha^{-1}. Mallik *et al*., (1985) reported pre-emergence applications of alachlor and metribuzine at 1.7 and 0.34 kg ha^{-1} in soybean significantly decreased nodulation; nitrogenize activity and total amount of nitrogen. Also trifluralin at 0.56 kg ha^{-1} decreased nodulation and nitrogenizes activity.

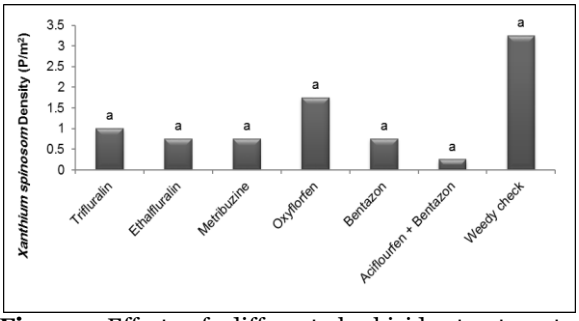

Fig. 4. Effect of different herbicide treatments cocklebur (*Xanthium spinosom*) population reductions. Means within each column followed by same letter are not significantly different (Duncan 5%).

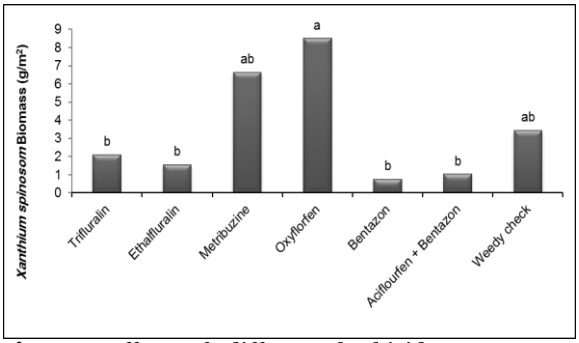

Fig. 5. Effect of different herbicide treatments cocklebur (*Xanthium spinosom*) biomass reductions.Means within each column followed by same letter are not significantly different (Duncan 5%).

Therefore, with regards to above statements, nodulation and azote biological fixation by plant growth promoting bacteria such as *Bradyrhizobium japonicum* in crops like soybean can be under impression of environmental circumstances and various herbicides. In such, these herbicides cause to reduction or inefficacy of plant growth promoting and azote biological fixation current in crop plants, in this research nodulation was not occur which needs more researches.

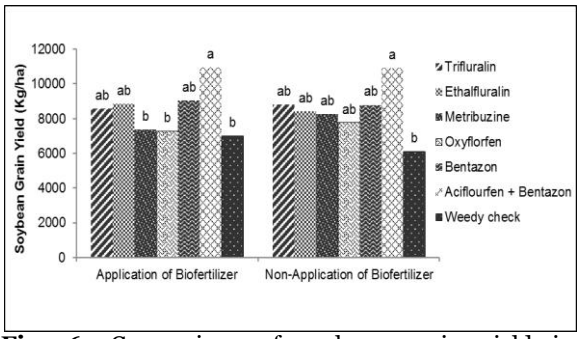

Fig. 6. Comparison of soybean grain yield in treatment's experiment. Mean within each column followed by same letter are not significantly different (Duncan 5%).

Stephan & Roskamp (1998) demonstrated that thifensulfuron at 2.2 to 8.8 g/ha in combination with bentazon at 420 g/ha may be safely applied to soybean for broadleaf weed control such as *D. stramonium* and *X. spinosom*. Veldon *et al*., (1987) showed in greenhouse condition bentazon and aciflourfen applications are effective on decrease rate of moisture percentage in *D. Stramonium* L., even though bentazon was more effective than aciflourfen to decrease density and biomass of this weed. Karaminejad *et al*., (2010) reported among the herbicides treatment, application of metribuzine effectively controlled *D. stramonium* L. in range of 64-77%. According to Brickell & Jordan (1980) oxyfluorfen, all at 0.38-0.5 lb/acre and bifenox at 1.5 to 2 lb/acre gave good selective control of *D.stramonium* and *C. album*. Control was less satisfactory when the treatments were incorporated before sowing the soybeans. Post emergence treatments with acifluorfen and MC 10978 (acifluorfen-sodium) at 0.5, 0.75 and 1 lb/acre also performed well. Weaver, (1991) showed metribuzine application had major role in decrease of *X. spinosom* L., *D. stramonium* L. and *A. theophrasti* L. density.

Suitable control of this weed by the herbicides were related to low density of it compare to *X. spinosom* L. McWhorter and Anderson (1976 a,b); Weaver, (1980) reported post-emergence application of bentazon at 1.1-0.6 kg ha^{-1} and aciflourfen at 1.1 kg ha^{-1} had satisfactory control on *X. stramonium* L. in soyabean. The suitable control of this weed by applied herbicides is because of *D. stramonium* photosynthesis process and also lower profusion compare to *X. spinosom*. Ovesen, (2001) showed application of aciflourfen+bentazon controlled *X. spinosom* L. by the rate of 80%. Suitable control of this weed by mentioned herbicide is related to better absorption of herbicides by this weed. Because of trifluralin and ethalfluralin are pre-plant and soil incorporation herbicides and aciflourfen+bentazon are post-emergence herbicides. Kapusta, *et al* (1986) reported bentazon treatment application at 0.8 and 1.1 kg ha^{-1} and aciflourfen at 0.4 and 0.6 kg ha^{-1} did not decrease soybean yield. Fronning & Kegode, (2004) in their research to control of *Artemisia biennis* L. in soybean in Wyndmere showed, bentazon+aciflourfen at 240+120 g ha^{-1} had suitably controlled as good as weed free treatment and increase soybean yield. Also bentazon at 560 g ha^{-1} ranked in next step. Research at almond to control *A. retroflexus* L. with aciflourfen+bentazon at advised doses marvelous increase yield achieved Grichar, (1997).

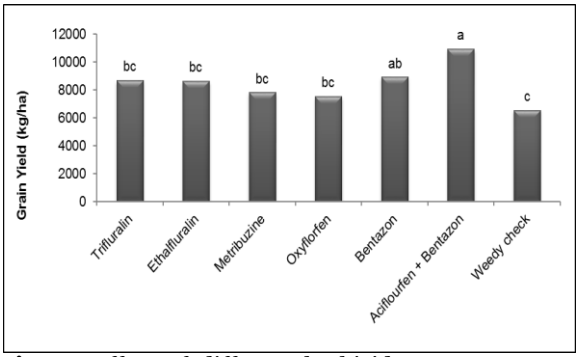

Fig. 7. Effect of different herbicide treatments on soybean grain yield. Means within each column followed by same letter are not significantly different (Duncan 5%).

Since in this experiment all herbicides decreased the dry weight of weeds, then relative increase by oxyflorfen+bentazon treatment can be related to decrease of weeds biomass in these treatments or can be attributed to better absorption of herbicides via weed, because trifluralin and ethalfluralin are pre-plant soil incorporation herbicide and mixture of aciflurfen plus bentazon is pre-emergence.

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