



RESEARCH PAPER

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Investigation of bio-fertilizer and selective herbicides application on control of *Chenopodium album* and *Amaranthus retroflexus* in soybean

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Abstract

This experiment was conducted during 2009 growing season as split plot with randomized complete block design arrangement in a field of Faculty of Agriculture, Islamic Azad University, Karaj branch, Iran. Application and non-application of bio-fertilizer (*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 to control of redroot pigweed (*Amaranthus retroflexus*) and lamb's quarters (*Chenopodium album*). In this research, application of bio-fertilizer on efficiency of some herbicides was effective in decreasing weeds dry weight. The biomass of redroot pigweed (*Amaranthus retroflexus*) in treatment oxyflorfen was significantly less than weedy check and other sub-treatment. However, the herbicides compared with weedy check, had significant effect on the number of redroot pigweed plants. The biomass of lamb's quarters (*Chenopodium album*) in treatments metribuzine, ethalfluralin was significantly less than weedy check and other sub-treatment. Application of metribuzine compared with weedy check and other sub-treatment, significantly reduced the density of lamb's quarters (*Chenopodium album*). Application of aciflourfen+bentazon, bentazon compared with weedy check, resulted in significant grain weight of soybean.

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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 report, 2013). *Xanthium spinosum* L. decreased 70% yield of bean (McLelland, 2002). *A. retroflexus* L. in 70 countries and in 60 farm plants are reported, as one of the current weeds in Canada soybean fields (Holm *et al.*, 1997). 50 percent decrease in soybean yield is because of competition with *A. retroflexus* L. (Cowan, 1998). 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. 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. (Jannink *et al.*, 2000) reported that soybean are not strong competitors in the early part of the season, therefore weeds out grow them. If the crop is not kept weed free, light competition takes place after 4 weeks when the weed grow taller than soybeans and intercept photosynthetically active radiation (Jannink *et al.*, 2000). Where weed density of *Xanthium pensylvanicum*, *C. album*, *A. retroflexus* and *D. stramonium* exceeded 1-2 plants/m², a full canopy over soybean formed which intercepted 44-56% of the photosynthetic active radiation, and yield reductions varied from 18-80%. 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).

In accordance with sustainable agriculture, some of soil micro organisms which have symbiosis with plants and use as bio-fertilizers to supply food elements are in extension (Sharma, 2003). This 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 *rhizobioumes* 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 bio-fertilizer 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 bio-fertilizer like (*Bradyrhizobium japonicum*), and the effect of bio-fertilizer on soybean yield.

Materials and methods

This experiment was conducted during 2013 growing season as split plot with randomized complete block 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 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 bio-fertilizer (*Bradyrhizobium japonicum*), with the population of 2.3-10⁸ bacteria per each gram. For every kilogram of soybean seed *Glycine max* L. Var 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. 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 m² 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 m² 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. 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 bio-fertilizer 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

Amaranthus retroflexus L.

Statistical analysis showed (table 1) no significant differences between biomass and density of *A. retroflexus L.* within application and none application bio-fertilizer but there was scientifically differences between herbicides treatments on the level of 5%. There was no significant differences interaction between bio-fertilizer and herbicides. In this valuation, oxyflorfen application in compare to weedy check and other herbicide treatments significantly decreased the dry weight of this weed by ratio of 83.60%. All herbicides scientifically decreased the biomass of *A. retroflexus L.* in confine of 61-86% in compare to weedy check (fig. 1 & 2).

Table 1. Analysis of variance for different bio-fertilizer, 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/m ²)		Biomass (g/m ²)	
			AMARE	CHEAL	AMARE	CHEAL
Rep	3	107981.190	0.3242	0.0174	0.8357	0.1589
A (Bio-fertilizer)	1	0.714 ^{ns}	0.1244 ^{ns}	0.0091 ^{ns}	0.0017 ^{ns}	0.2261 ^{ns}
E(a)	3	31756.428	0.0444	0.0116	0.2545	0.6639
B (Herbicide)	6	1508957.857**	0.3341*	0.1813**	0.9503*	3.1284**
A*B (Bio-fertilizer*Herbicide)	6	70673.214 ^{ns}	0.0204 ^{ns}	0.0801 ^{ns}	0.4433 ^{ns}	2.4103*
E(b)	36	39874.8948	0.1186	0.0461	0.3229	0.7661
C.V. %		23.67863	11.93617	7.451036	17.11089	25.05135
A*B Effect Sliced by A						
Bio-fertilizer	DF	Grain yield (kg/ha)	Density (p/m ²)		Biomass (g/m ²)	
			AMARE	CHEAL	AMARE	CHEAL
Application	6	762700 ^{ns}	0.223633 ^{ns}	0.073062 ^{ns}	0.691740 ^{ns}	2.236579*
Non-Application	6	816930 ^{ns}	0.130939 ^{ns}	0.188448**	0.701939 ^{ns}	3.302161**
A*B Effect Sliced by B						
Herbicide	DF	Grain yield (kg/ha)	Density (p/m ²)		Biomass (g/m ²)	
			AMARE	CHEAL	AMARE	CHEAL
Trifluralin	1	10351.25000 ^{ns}	0.016685 ^{ns}	0.126184 ^{ns}	0.050574 ^{ns}	0.000474 ^{ns}
Ethalfuralin	1	37845.00000 ^{ns}	0.012282 ^{ns}	0.036036 ^{ns}	0.927360 ^{ns}	7.324868**
Metribuzine	1	16290 ^{ns}	1.061669 ^{ns}	0.004171 ^{ns}	0.088145 ^{ns}	0.080488 ^{ns}
Oxyflorfen	1	46080.00000 ^{ns}	0.003292 ^{ns}	6.162976 ^{ns}	0.207188 ^{ns}	0.009718 ^{ns}
Bentazon	1	12500.00000 ^{ns}	0.013625 ^{ns}	0.230851*	0.030972 ^{ns}	5.157479*
Aciflourfen + Bentazon	1	361.25000 ^{ns}	0.097214 ^{ns}	0.084470 ^{ns}	0.856531 ^{ns}	0.949707 ^{ns}
Weedy check	1	154000 ^{ns}	0.103783 ^{ns}	0.008277 ^{ns}	0.500831 ^{ns}	1.165283 ^{ns}

(AMARE): *Amaranthus retroflexus* L., (CHEAL): *Chenopodium album* 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.

Chenopodium album L.

Statistical analysis showed (table 1) except two case there was no significant difference between interaction of bio-fertilizer and herbicides in biomass of *C. album* L. Treatment of ethalfuralin and bio-fertilizer showed no significant difference in dry weight of *C. album* compared to weedy check, whereas none application of bio-fertilize scientifically decreased the biomass of this weed compare to weedy check in level 1% (fig. 3). Despite of ethalfuralin, the application of Bentazon with bio-fertilize decreased the biomass of this weed in level 5%. However there was no significant difference in biomass compare to weedy check when bio-fertilizer applied (fig. 3). The effect of application and none application of bio-fertilizer in herbicides efficiency to decrease the biomass of this weed was the same, in this case there was no significant difference between application and

none application of bio-fertilizer with herbicide in number and dry weight of this weed (fig. 3). Variance dissolve results (table 1) showed there was significant difference by herbicide application in level 1%. So that metribuzine application scientifically decreased the density of *C. album* L. by ratio of 93% in compare to weedy check and other herbicide treatments (fig. 1). Biomass of *C. album* L. decreased by metribuzine and trifluralin in ratio of 85.40% and 72.40% respectively in compare to other treatments and weedy check (fig. 4&5).

Grain Yield

Variance dissolve results (table1) showed although interaction between bio-fertilizer 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 (fig. 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 (fig. 7).

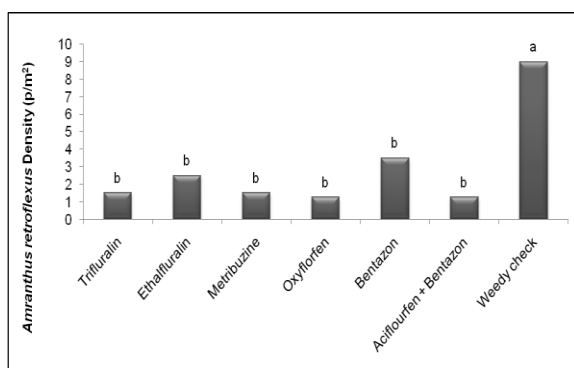


Fig. 1. Effect of different herbicide treatments on redroot pigweed (*Amaranthus retroflexus*) populations reductions. Means within each column followed by same letter are not significantly different (Duncan 5%).

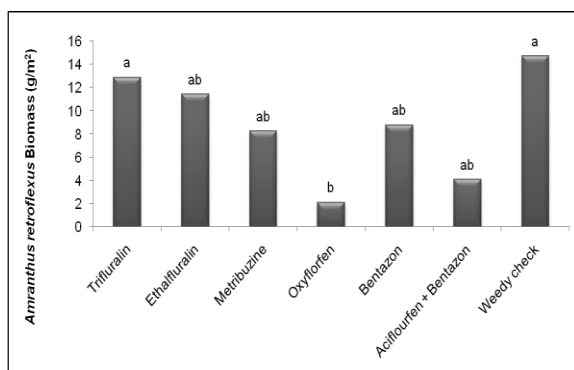


Fig. 2. Effect of different herbicide treatments on redroot pigweed (*Amaranthus retroflexus*) biomass reductions. Means within each column followed by same letter are not significantly different (Duncan 5%).

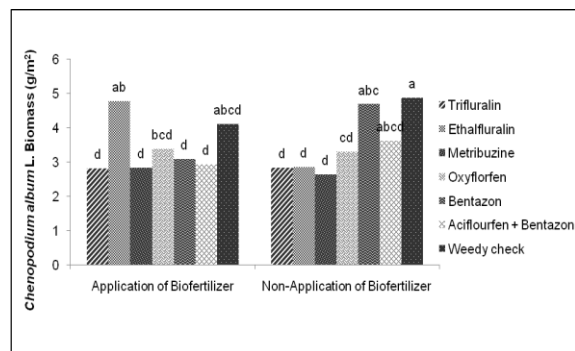


Fig. 3. Comparison of *Chenopodium album* dry weight in treatment's experiment.

Mean within each column followed by same letter are not significantly different (Duncan 5%).

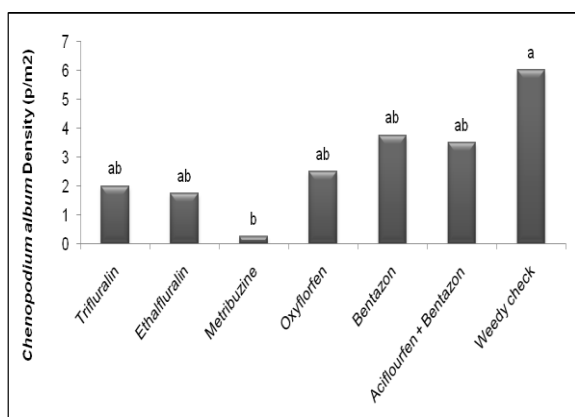


Fig. 4. Effect of different herbicide treatments on common lambsquarters (*Chenopodium album*) population reductions. Means within each column followed by same letter are not significantly different (Duncan 1%).

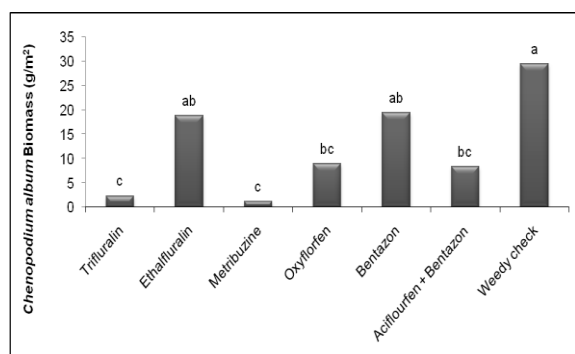


Fig. 5. Effect of different herbicide treatments on common lambsquarters (*Chenopodium album*) biomass reductions. Means within each column followed by same letter are not significantly different (Duncan 1%).

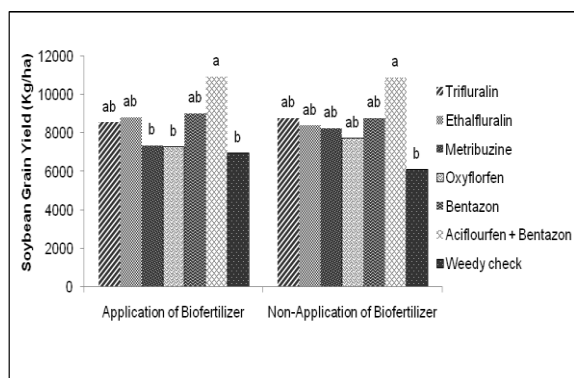


Fig. 6. Comparison of soybean grain yield in treatment's experiment. Mean within each column followed by same letter are not significantly different (Duncan 5%).

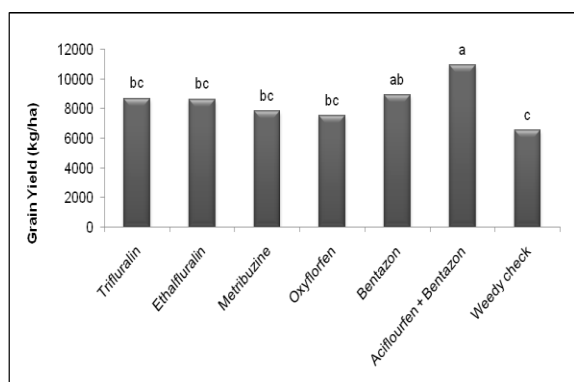


Fig. 7. Effect of different herbicide treatments on soybean grain yield. Means within each column followed by same letter are not significantly different (Duncan 5%).

Discussion

At present in Iran there is no report in relation with herbicides interaction on bio-fertilizers. 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 *rhizobioumes* 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 nitratin 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.

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.

Qasem (2007) in his researches showed pre-plant and after-plant application of oxyflorfen at 2.5 L ha⁻¹ decreased weed biomass and increased cauliflower yield.

Pannacci *et al.*, (2007) showed sunflower had least weed canopy by pre-emergence application of oxyflorfen at 240 g ai ha⁻¹ and metolachlor+oxyflorfen at 168+720 g ai ha⁻¹ as pre-emergence. Whereas, in this research between both mentioned herbicide there was no significant difference in *A. retroflexus* biomass reduction. Karen *et al.*, (1998) reported post-emergence application of metribuzine at 140 g ha⁻¹ had satisfactory control on *C. album* L. and *A. retroflexus* L. Ackley *et al.* (1996a) and Robinson *et al.* (1996) reported Pre and post-emergence application of metribuzine because of effective and suitable control of most broadleaf weeds and grasses assumed as one of the main parts in weeds management in potatoes crop. Suitable control of this weed by mentioned herbicides is because of better absorption of this herbicide by weed. Because trifluralin is pre-plant and soil incorporation and metribuzine is pre-emergence herbicide. The suitable control of this weed by mentioned herbicides can be attributed to better absorption of this herbicide by the weed compared to trifluralin as a pre-plant herbicide, because metribuzin acted as a pre-emergence herbicide. 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. Suitable control of this weed by mentioned herbicides 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. 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). Kapusta *et al.*, (1986) reported application of bentazon at 0.8 and 1.1 Kg ha⁻¹ and aciflurfen at 0.4 and 0.6 Kg ha⁻¹ advised dose did not reduce soybean yield. Since 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.

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