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Investigation of biofertilizer and selective herbicides application on control of *Convolvulus arvensis* L. and *Hibiscus trionum* L. in maize

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Key words: *Pseudomonas* spp. weed biomass, weed density, grain yield.

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. Application and non-application of biofeltilizer (a strain of *Pseudomonas* spp.) was the main treatments. The sub-treatments were application of herbicides consisted of EPTC, atrazin, nicosulfuron, foramsulfuron, rimsulfuron and 2,4-D plus MCPA respectively at 4100, 800, 80, 450, 12.5, 108 g ha^{-1} and weedy check to control of Bindweed (*Convolvulus arvensis* L.) and Venice mallow (*Hibiscus trionum* L.). In this research, application of biofertilizer on efficiency of some herbicides was effective in decreasing weeds dry weight. Total evaluation indicated that all herbicides in situation, application and non-application of biofertilizer, significantly reduced weeds dry weight compare to weedy check. The result of this experiment show that sub-treatment had not significantly affect on biomass and number of Bindweed (*Convolvulus arvensis* L.). EPTC, atrazin and foramsulfuron compared with weedy check and other sub-treatment significantly reduced the number of Venice mallow (*Hibiscus trionum* L.). However, EPTC compared with weedy check and other sub-treatment significantly reduced the number of this weed. Application of atrazin and EPTC resulted in significant increase of maize grain yield and the number of ear. Application of atrazin caused the highest 1000-grain weight of maize, but was not significantly different from nicosulfuron and foramsulfuron.

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Introduction

Corn with the scientific name *Zea mays* L. is one of the four major grains in the world. And its production after wheat and rice is third (Mousavi, 2001). Lands under corn plantation in Iran in 2011, was approximately 252 thousand hectares and rate of production was estimated 2.2 million tone (FAO, 2013). One of the problems that related to corn production is the presence of weeds which causes interference in agricultural operations, increase labor and production costs, and reduces plant height, leaf area, dry weight and corn yield (Rashed Mohasel *et al*., 1999). If we don't control weeds, depended on density and varieties may reduced from 15 to 90% of corn yields (Zimdahl, 1993). Weeds of corn will struggle to nutrient availability with main plant and some of them till double of nitrogen and phosphorous and 3 times of potassium available in unit dry weight of corn plants (Tollenaar *et al*., 1999). Pandey & Singh (1994) in their research to compare the efficacy of Solfonilurea herbicides family to hand mowing to control weeds in wheat plantation reported, herbicide treatments did not have satisfactory efficacy to controlling *C. arvensis* L. Different behavior of these herbicides is because of different environment conditions or refered to difference in biotype sensitivity of this type. Bijanzade & Ghadiri, (2006) reported that rimsulfuron and atrazin herbicides treatment could not have a satisfactory control over *C. arvensis* L. In addition, separate application of Solfonilurea herbicides family did not have good control over some varieties of broadleave weeds like *C. arvensis* L. 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 other countries, many herbicides are registered to control weeds in corn. But in Iran, registered herbicide varieties are less. These herbicides (atrazin, alachlor, EPTC and 2,4-D) are being used in Iran for several years in corn fields. So now, weeds have good resistance toward herbicides (Zand *et al*., 2007). New Solfonilurea herbicides family like nicosulfuron, foramsulfuron and rimsulfuron are the selective herbicide in corn plantation (Lemieux *et al*., 2003) and due to some of individual characteristics like the lowest usage dose in surface area compared to other herbicides, higher biological activity, extent side effects, high herbicide leaching and so on, recently are being extended (Russell *et al*., 2002).

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). Varieties of Bacteria from *Azotobacter*, *Azospirillum*, and *Pseudomonace* strain which is known as the most important of plant growth promoting bacteria, not only facilitate the biological nitrogen fixation process and phosphate dissolution in soil. Also has a positive effect on crops growth development and crops yield by producing growth promoting hormones such as auxin, gibberellin and cytokinin (Zahir *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). Forlani *et al*., (1995), carried out a research to address the effect of Solfonilurea and imidazolinons herbicides family on 18 isolations from bacteria exists in soil just like varieties from *Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonace* and *Seratia*. It is showed that chlorsulfuron and rimsulfuron from sulfonylurea herbicides family prevent the growth of one of two strains of

Azospirillum and one of the four *Pseuodomonas* strains.

Even though biofertilizer application improved in Iran, their interaction with herbicides is very less. Therefore, the aim of this research were evaluation of herbicides efficacy in control of corn weeds and checked their interaction with biofertilizer like (*Pseuodomonas* spp.), and the effect of biofertilizer on corn 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 two main treatments and seven sub-treatments in four repetitions 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 (*Pseuodomonas* spp.), with the population of 5×10^9 bacteria per each gram. For every kilogram of corn seed (*Zea maize* SC. 704) cultivar by ratio of 50 ml of solution was added and mixed well, till all the seeds to become uniformed with the substance. Then seeds were kept on the ground for period of 10 minutes in open air, in shadow. Then corn planted and watered immediately. The sub-treatments were application of herbicides consisted of EPTC EC 82%, atrazin WP 80%, nicosulfuron SC 4%, foramsulfuron OD 22.5%, rimsulfuron DF 25% and 2,4-D plus MCPA SL 67.5% respectively at 4100, 800, 80, 450, 12.5, 108 g ha^{-1} (Vencill, 2002; Tomlin, 2003) and weedy check. EPTC as pre-plant and soil incorporation, atrazin as pre-emergence, 2,4-D+MCPA at 10-15 cm of corn height and other herbicides applied as post-emergence in 3-4 leaves stage of corn. 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 3 m wide and 6 m length. Plots length consisted of four rows of plant with 6 m length. Distance of plots in every block was 75 cm. and distance of blocks from each other was 2 m. Also distance of plant lines were 75 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 accidently 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 accidently 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 corn yield, harvest was done from 2 middle lines of 6 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, 3 weeks after applying herbicide treatments, there is no evidence of plant phytotoxicity for using herbicides in advised time and recommended dose application. Among native weeds, analysis in herbicides applications were achieve on dominant weeds of area and are explained.

Weed Control

Convolvulus arvensis L.

Variance dissolve results showed (Table 1) that in none applications of biofertilizer none of herbicides had not significant difference in dry weight of *C. arvensis* L. compare to weedy check, although in biofertilizer application with atrazin and nicosulfuron the dried weight of these weed was significantly higher than EPTC, foramsulfuron and weedy check (Fig. 1). Also there was no significant difference among herbicide treatments in decrease of number and dry weight of C. *arvensis* L. (Figs. 2 & 3).

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

			Mean Square			
S.O.V	**DF**	**Grain yield (kg/ha)**	**Density (p/m²)**		**Biomass (g/m²)**	
			CONAR	**HIBTR**	**CONAR**	**HIBTR**
Rep	3	27891	0.0585	0.1471	0.2683	0.7042
A (Biofertilizer)	1	$5540^{n.s}$	$0.0672^{n.s}$	$0.0144^{n.s}$	$1.8624^{n.s}$	$0.1304^{n.s}$
E(a)	3	7977	0.1461	0.0299	0.6240	0.8002
B (Herbicide)	6	53105^{**}	$0.1952^{n.s}$	0.0995^{*}	$0.3071^{n.s}$	1.0265^{*}
A*B (Biofertilizer*Herbicide)	6	$8596^{n.s}$	$0.1672^{n.s}$	$0.0321^{n.s}$	0.8205^{*}	$0.4181^{n.s}$
E(b)	36	9669	0.1548	0.0390	0.3020	0.3963
C.V. %		17.34266	13.16001	7.031830	18.23120	20.04141

			A*B Effect Sliced by A			
Biofertilizer	**DF**	**Grain yield (kg/ha)**	**Density (p/m²)**		**Biomass (g/m²)**	
			CONAR	**HIBTR**	**CONAR**	**HIBTR**
Application	6	$19226.87^{n.s}$	$0.321022^{n.s}$	$0.077635^{n.s}$	0.975347^{*}	$0.403231^{n.s}$
Non-Application	6	42475.06^{**}	$0.041530^{n.s}$	$0.054122^{n.s}$	$0.152335^{n.s}$	1.041435^{*}

			A*B Effect Sliced by B			
Herbicide	**DF**	**Grain yield (kg/ha)**	**Density (p/m²)**		**Biomass (g/m²)**	
			CONAR	**HIBTR**	**CONAR**	**HIBTR**
EPTC	1	$820.13^{n.s}$	$0.059185^{n.s}$	$1.577722^{n.s}$	$0.173993^{n.s}$	$0.097709^{n.s}$
Atrazin	1	$4465.13^{n.s}$	$0.596288^{n.s}$	$0.015687^{n.s}$	2.050037^{*}	$0.057529^{n.s}$
Nicosulfuron	1	$2964.50^{n.s}$	$0.000750^{n.s}$	$0.002516^{n.s}$	1.820147^{*}	$0.031074^{n.s}$
Foramsulfuron	1	$10224.50^{n.s}$	$0.272442^{n.s}$	$0.138720^{n.s}$	$0.065736^{n.s}$	$0.661418^{n.s}$
Rimsulfuron	1	$242^{n.s}$	$0.038886^{n.s}$	$0.014874^{n.s}$	$0.855684^{n.s}$	$0.784224^{n.s}$
2,4-D + MCPA	1	$36.13^{n.s}$	$0.047203^{n.s}$	$0.031466^{n.s}$	1.435818^{*}	$0.019044^{n.s}$
Weedy check	1	$38364.50^{n.s}$	$0.056259^{n.s}$	$0.004317^{n.s}$	$0.384418^{n.s}$	$0.988325^{n.s}$

(CONAR): *Convolvulus arvensis* L., (HIBTR): *Hibiscus trionum* 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.

Maize Grain Yield

Variance dissolve results showed (Table 1) that in situation of biofertilizer application, atrazine was the sole treatment which made significant increase in corn grain yield. But other herbicides treatment had no significant difference compare to weedy check (Fig. 7). In none-application of biofertilizer, all herbicides treatment had significant increase in grain yield compare to weedy check. And also in this case herbicides had no significant differences with each other. The herbicides efficiency in grain yield in both situation application and none application of biofertilizer statistically was the same (Fig. 7). The average comparison in Duncan test (Fig. 8) showed that in herbicide treatments in level of 5%, there was significant difference. All herbicides treatments made increase in corn grain yield. In such, grain yield in atrazin and nicosulfuron treatments by the ratio of 56.80% and 51% significantly was more than weedy check. In this view, atrazin, nicosulfuron, EPTC, foramsulfuron and rimsulfuron were kept in a same statistics group. Also 2, 4-D+MCPA achieved the least increase in grain yield by the rate of 22% compare to weedy check.

Hibiscus trionum L.

Variance dissolve results (Table 1) showed in none application of biofertilizer, treatments of atrazine, EPTC & foramsulfuron had significant reduce in dry weight of *H. trionum* L. compared to weedy check. But in this case other herbicides had no significant difference with weedy check (Fig. 4). Whereas in biofertilizer application none of herbicides had significant difference compare to weedy check (Fig. 4). Herbicide treatments on the level of 5% showed significant differences. So that, herbicides like atrazin, EPTC and foramsulfuron compare to weedy check and other treatments, significantly decreased the dry weight of this weed by ratio of 93%, 91% and 65.73% respectively. Only EPTC and atrazin significantly decreased the number of *H. trionum* L. by ratio of 97.53% and 84.61% in compare to weedy check and other herbicide treatments (Fig. 5 & 6).

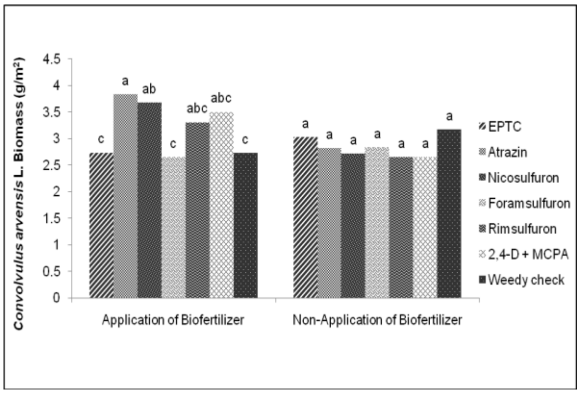

Fig. 1. Comparison of *Convolvulus arvensis* dry weight in treatment's experiment. Mean within each column followed by same letter are not significantly different (Duncan 5%).

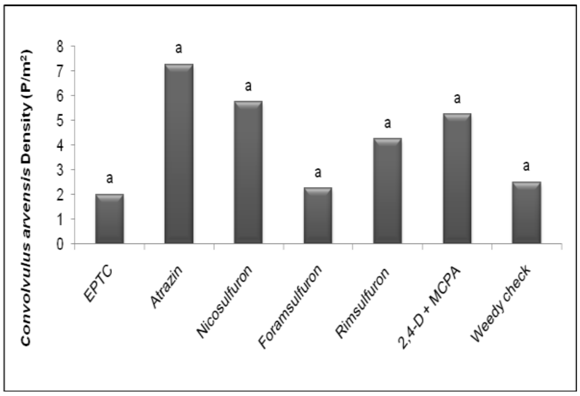

Fig. 2. Effect of different herbicide treatments on Bindweed (*Convolvulus arvensis* L.) populations reductions. Means within each column followed by same letter are not significantly different (Duncan 5%).

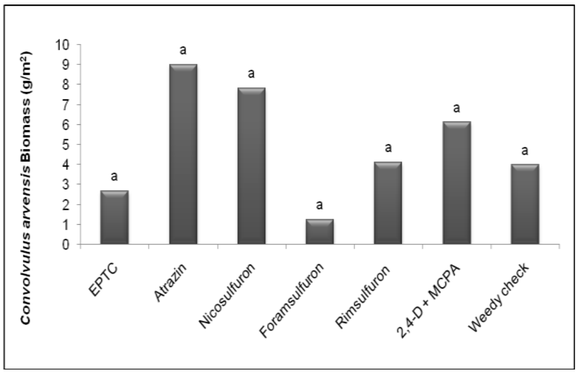

Fig. 3. Effect of different herbicide treatments on Bindweed (*Convolvulus arvensis* L.) biomass reductions. Means within each column followed by same letter are not significantly different (Duncan 5%).

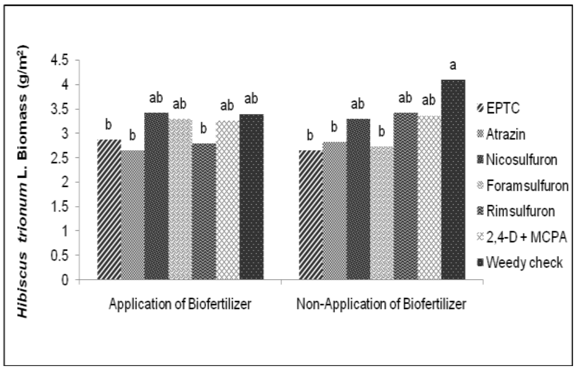

Fig. 4. Comparison of *Hibiscus trionum* dry weight in treatment's experiment. Mean within each column followed by same letter are not significantly different (Duncan 5%).

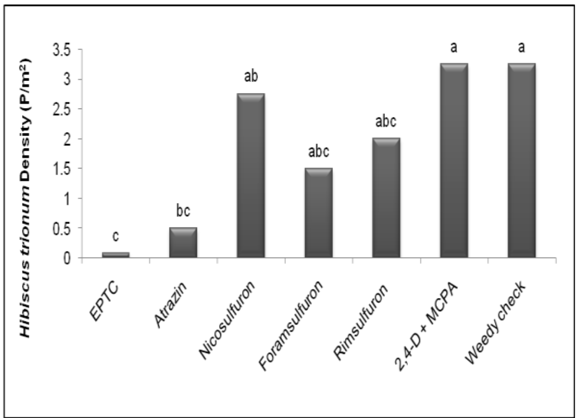

Fig. 5. Effect of different herbicide treatments on Venice mallow (*Hibiscus trionum* L.) population reductions. Means within each column followed by same letter are not significantly different (Duncan 5%).

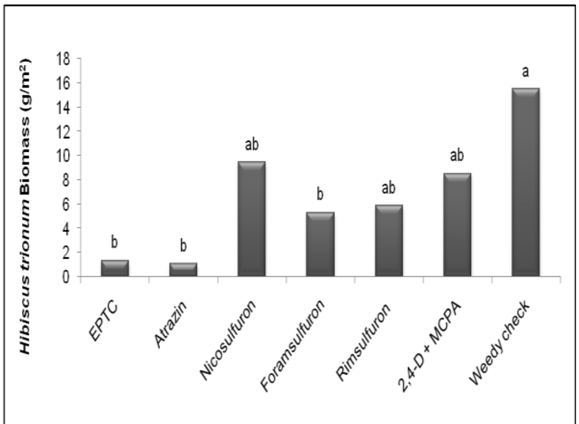

Fig. 6. Effect of different herbicide treatments on Venice mallow (*Hibiscus trionum* L.) biomass reductions. Means within each column followed by same letter are not significantly different (Duncan 5%).

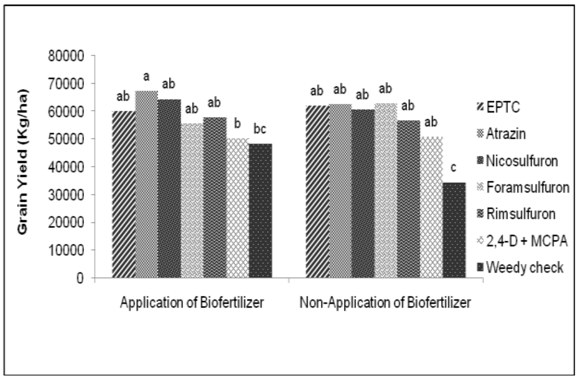

Fig. 7. Comparison of corn grain yield in treatment's experiment. Mean within each column followed by same letter are not significantly different (Duncan 5%).

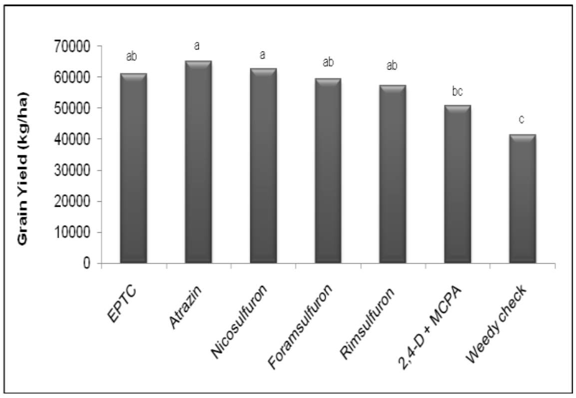

Fig. 8. Effect of different herbicide treatments on corn grain yield. Means within each column followed by same letter are not significantly different (Duncan 5%).

Discussion

In Iran, there is no report in relation with herbicides interaction on biological nitrogen fixation in crops for example, corn with plant growth promoting bacteria such as *Azotobacter*, *Azospirillum* and *Pseuodomonas*. 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). Forlani *et al.*, (1995), carried out a research to address the effect of Solfonilurea and imidazolinons herbicides family on 18 isolations from bacteria exists in soil just like varieties from *Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonace* and *Seratia*.

It is showed that chlorsulfuron and rimsulfuron from sulfonylurea herbicides family prevent the growth of one of two strains of *Azospirillum* and one of the four *Pseudomonas* strains. Also imazapyr and imazethapyr from imidazolinones herbicides family had negative effect on two of five *Bacillus* isolations. Green house reaserches were done to evaluation of herbicides inhibition effect on bacteria growth on farm condition. Thus corn seeds with two strains of sensitive bacteria to Solfonilurea herbicides family inoculationed and rimsulfuron with ratios of 0, 0.2, 0.5 mol a.i.kg^{-1} applied. Significant difference was seen in concentration value of bacteria in root by using two strains of resistance and sensitive bacteria to herbicide. Rimsulfuron application significantly was the cause of increase resistance bacteria concentration on the root. Changing the structure of the bacteria in rhizospher was the result of consecutive usage of AHAS inhibitor herbicides in order to control weeds. Martenson and Nilson reported (1989) that when chlorsulfuron was used at rate of 4 and 8 g ha^{-1} for white clover and bird's foot terifoil, there was no node in plant. Eberbachk and douglas (1989) reported when amitrole at advised dose was used, then plant growth and nitrogenize activity and nodulation in clover decreased. They also declared 2, 4-D and diquat herbicides remains effected in nodulation and nitrogenize activity in clover. They declared diquat was initially effect on plant growth rather than appearance of nodes (Eberbachk *et al.*, 1989). Farm experiments showed, when simazine was used at the rate of 3 kg ha^{-1} for 3 consecutive years in corn, and then at fourth year it had reductive effect on soybean nodulation (Dunigan *et al.*, 1972). Moorman (1986) reported a reduction in soybean-node-weight by treating alachlor, linuron and trifluralin herbicides without any diminishing in crop yield. Mallik and Tesfa (1985) reported pre-emergence application of alachlor at 1.7 kg ha^{-1} and metribuzin at 0.34 kg ha^{-1} in soybean, significantly decrease the nodulation, nitrogenization activity and total rate of azote. It is observed that application of 2, 4-D & MCPA lead to decrease the activity of rhizobioum varieties (Dunigan *et al.*, 1972).

Therefore, with regards to above statements, azote biological fixation by plant growth promoting bacteria such as *Azotobacter*, *Azospirilium* and *Pseudomonas* in crops like corn 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, which needs more researches.

The result of this experiment showed that there were no suitable decrease in density and biomass of *C. arvensis*. Bijanzadeh & Ghadiri (2006) reported, rimsulfuron and atrazin application could not have satisfactory control over *C. arvensis* L. In addition, separate application of Solfonilurea herbisides family had unsatisfactory control over some of the broadleave weeds like *C. arvensis* L. whereas, rimsolforon at 0.02 kg ha^{-1} application dose only decreased biomass of this weed by ratio of 17%. Pandey & Singh (1994) in their research to compare the efficacy of Solfonilurea herbicides family with hand mowing to control weeds in wheat plantation reported, herbicide treatments did not have satisfactory efficacy to controlling *C. arvensis* L. Different behavior of these herbicides is because of different environment conditions or refered to difference in biotype sensitivity of this type. Unsatisfactory efficiency of herbicides in controlling *C. arvensis* L. is referred to physiological characteristics (rhizome, broadleave and perennial) of this weed. So that, its shoot is not improved enough to absorb herbicide at the time of spraying on plants. It is advised, when corn farm has contaminated by *C. arvensis* L., spraying on this weed should be delayed.

Zand (2006) and Baghestani (2007), in their research to evaluation the efficacy of new herbicides in controlling corn weeds in Iran reported, application of atrazin+alachlor, foramsulfuron, nicosulfuron and rimsulfuron controlled *H. trionum* L. completely. However rimsolforon at 12.5 g ai ha^{-1} subsequently were decreased population and biomass of this weed by 87.46% and 89.16%. Also EPTC at 4920 g ai ha^{-1}

were respectively decreased population and biomass of this weed by 84.09% and 66.67%. Atrazine and EPTC are standard herbicides in this experiment and because of that they had acceptable effect in biomass decrease of this weed compare to weedy check and other herbicide treatments. Osullivan & Bouw (1993) reported that atrazin, nicosulfuron and rimsulfuron treatments, even though acted satisfactory in controlling *A. retroflexus* L. and *C. album* L. in advised doses for 3 years continuously application but had not significant differences effects in corn grain yield by treatments. Burnside *et al.*, (1969) reported Using of atrazin at 2.2 kg ai ha^{-1} has effective control on weeds as well as increase of 3.1% corn grain yield in compare to weedy check. Nurse (2007), in his research in Ontario reported corn had excellent resistance to foramsulfuron in all doses application. Lume *et al.* (2005) during 2000 and 2002 made some experiments about nicosulfuron at 50, 100, 150 and 200 g ai ha^{-1} and applied times of 1, 2, 3 and 4 weeks after plant in corn and reported nicosulfuron increases corn yield by the ratio of 96% in 2000, 100% in 2001 and 34 to 54% in 2002. Zand (2006), in his research to evaluation the efficacy of new herbicides on controlling weeds in corn plantation in Iran reported corn grain yield was most effectiveness with nicosulfuron and rimsulfuron at 60 and 12.5 g ai ha^{-1} application doses by the increase ratio of 5432.50 and 5485 kg ha^{-1} in Ahvaz, foramsulfuron at 450 g ai ha^{-1} by the ratio of 16643 kg ha^{-1} in Karaj, nicosulfuron and EPTC at 60 and 4920 g ai ha^{-1} respectively by the ratio of 9300 and 9177.80 kg ha^{-1} in Kermanshah; nicosulfuron, foramsulfuron and EPTC at 60, 450 and 4920 g ai ha^{-1} respectively by the ratio of 14013, 14325 and 13917 kg ha^{-1} in Varamin achieved. Baghestani (2007) reported nicosulfuron application at 80 g ai ha^{-1} significantly increased corn grain yield, which ranked after weed-free treatment. However, 2,4-D+MCPA showed the lowest grain yield among other herbicide treatments. In most of done studies, just like this research, satisfactory efficacy of Solfonilurea herbicides family leads a suitable control of weeds and significant increase in corn yield.

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