Journal of Crop Nutrition Science
ISSN: 2423-7353 (Print) 2322-3227 (Online)
Vol. 1, No. 2, 2015
http://JCNS.iauahvaz.ac.ir OPEN ACCESS

Effects of Rhizobium– Mycorrhizae Symbiosis and Foliar Application of Urea on Some Traits of Soybean

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RESEARCH ARTICLE

ARTICLE INFO.
Received Date: *15 Mar. 2015*
Received in revised form: *20 Apr. 2015*
Accepted Date: *15 May. 2015*
Available online: *1 Jul. 2015*

To Cite This Article:
Alireza Safahani, Azin Irani, Farshad Ghooshchi. Effects of Rhizobium– Mycorrhizae Symbiosis and Foliar Application of Urea on Some Traits of Soybean. ***J. Crop. Nut. Sci., 1(2)****, 1-11, 2015.*

ABSTRACT

In order to investigate the effect of rhizobium bacteria, mycorrhiza fungi and foliar application of urea on qualitative and quantitative traits of soybean an experiment was conducted based on randomized complete block design with four replications. The experiment included six treatments as: Control (C), Rhizobium bacteria (R), Rhizobium bacteria + Mycorrhiza fungi (R+M), Rhizobium bacteria + Foliar application of Urea (R+U), Rhizobium bacteria+ Mycorrhiza fungi + Foliar application of Urea (R+M+U), Foliar application of Urea (U). Results showed that the effect of rhizobium and mycorrhiza and foliar application of urea on grain yield and yield components of soybean was not significant, but their effect on grain protein, grain oil, and the number of grains per lateral pod was significant. Rhizobium- mycorrhiza symbiosis and foliar application of urea had significant effect on the rate of grain oil, protein, and yield increased number of grains per pod. Combined use of rhizobium, mycorrhiza, and foliar application of urea increased leaf area and the highest leaf area index belonged to rhizobium treatment (R) 80 days after planting and after that (R+U) treatment 90 days after planting. The highest rate of dry matter belonged to rhizobium treatment (R). Stem height, number of nodules, nodule dry weight, and root dry weight during the growth season increased similarly in all treatments. The highest stem height belonged to (R+M) treatment, the lowest stem height belonged to (C) treatment, the highest nodule dry weight and root dry weight belonged to (R) and (R+M+U) treatments, respectively and the lowest one belonged to (C) treatment.

Keywords***:*** *Agronomic Characteristics, Glycine max, Yield.*

INTRODUCTION

Soybean is one of the most important crops in Iran and around the world which have a special place among the crops due to the nutritional value of its grain (20% oil, 40% protein) (Ahmad, 2013). Soybean crop is getting more and more important because of many medicinal and industrial uses and more

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Soybean is one of the plants need large amounts of nitrogen for production as for the production of one tone of soybean about 100 $kg.ha^{-1}$ nitrogen is estimated to be needed (Beiranvand *et al*., 2003). Supplying nitrogen through chemical fertilizers, in addition to its relatively high costs, has negative irreparable effects on the environment. Soybean is one of the legumes that do not respond positively to nitrogen and much of the required nitrogen will be supplied by the symbiosis through the nitrogen fixation (Bhat *et al*., 2011). Mycorrhizal fungi form a symbiotic relationship with plant roots. Arbuscular mycorrhizae are the most common type of mycorrhiza, where the fungi colonize the interior of the root and form specialized structures, known as arbuscules, for nutrient exchange with the host. Fungal hyphae extend from the root and explore the soil more efficiently than would fine plant roots. Arbuscular mycorrhizae can provide the plant with supplemental phosphorus (P), nitrogen (N), and micronutrients since the plant roots alone are not able to maximize the interception of nutrients (Allen *et al*., 2003). Kumutha *et al*. (2004) stated that although there is too much nitrogen in atmosphere and soil organic matters, plants cannot directly use atmospheric and organic matter nitrogen. Therefore, inoculation of leguminous seeds with some useful soil microorganisms (such as rhizobium bacteria) not only supplies the nitrogen needed for plants but also reduces environmental pollution caused by the extreme use of chemical fertilizers. Rhizobium is an active micronutrient in soil which is able to stabilize nitrogen after the establishment within the roots nodules of the host plant. For optimal use, soybean can supply about 80% of its need through symbiosis with brady rhizobium japonicum. This bacterium is not found naturally in soil in Iran and thus the biological fertilizers available in the market that contain various races of this bacterium need to be used (Coskan *et al*., 2009). Beneficial soil microorganisms provide soil minerals through biological fixation of nitrogen, solubilize phosphorus and potassium, control pathogens, produce various plant growth promoting and regulating hormones, and affect plants yield as well (Purcell *et al*., 2002). Osborne and Riedell (2006) stated that biological nitrogen fixation which is done by diazotroph bacteria and with the help of nitrogenase enzyme is one of the most important biological processes after photosynthesis. Krikun *et al*. (2010) stated that biological nitrogen fixation provides the nitrogen required for rhizobium symbiotic plants, and the subsequent crops will also take advantage of residual nitrogen via fixation process. Abdel-Fattah *et al*. (2002) studied broad bean and concluded that mycorrhizal inoculation significantly increased yield, dry matter production, and photosynthetic pigments of broad bean compared with non-mychorrhizal plants. Ogoko *et al*. (2006) stated that the number of nodules depended on the rate of assimilation and had a positive effect on nitrogen nutrition. In the past few decades, conventional farming systems which depend on external inputs such as chemicals have had a significant role in the production of crops (Smith *et al*., 2004). The efficiency of such systems is questionable for several reasons such as the rising cost of acquiring energy and chemicals used in farms, contamination of surface and ground water due to consumption of chemicals, etc (Son *et al*., 2006). With regard to the cultivation area of legumes around the world (250 million hectares) the importance of symbiosis between legumes and rhizobium bacteria becomes more evident (Rezvani *et al*., 2011). One of the most leading microorganisms in root is mycorrhiza. These fungi make a plenty of benefits for their hosts through a symbiotic relationship with crops. The increase of water and nutrients intake by the host plant and consequently the increase of growth and resistance to drought stress and pathogens, plant hormone production, soil structure improvement through facilitating the creation of aggregates are some of the advantages that the host plant will benefit from in such a symbiotic relationship (Hause *et al*., 2007).

Coskan *et al*. (2003) stated that foliar application of soybean during the grain filing stage not only improves gradation but also enhances the qualitative yield of soybean. This research aims to investigate the separate and combined application of rhizobium, mycorrhiza, and foliar application of urea in order to achieve the optimal yield and to reduce the consumption of chemical fertilizers in order to achieve sustainable agriculture.

MATERIALS AND METHODS

Field and Treatment Information

The experiment was carried out as a randomized complete block design with 6 treatments and four replications in Farakhil region (District of Ghaemshahr) (36° 11' N, 52° 37' 24 E and altitude 689 m). Experimental treatments included: Control (C), Rhizobium bacteria (R), Rhizobium bacteria + Mycorrhiza fungi (R+M), Rhizobium bacteria + Foliar application of Urea (R+U), Rhizobium bacteria + Mycorrhiza fungi + Foliar application of Urea (R+M+U), Foliar application of Urea (U). Each experimental plot included four planting rows with 50 cm width. Fifty $kg.ha^{-1}$ nitrogen was used as the starter for all the treatments during the planting. Soil properties of the experimental site are displayed in table (1).

Table 1. Soil properties of the experimental site (Soil depth: 0-30 cm)

EC ($ds.m^{-1}$)	pH	OC (%)	TNV (%)	P (ppm)	K (ppm)	Fe (ppm)	Mn (ppm)	N (ppm)	Zn (ppm)
0.56	7.3	2.56	45	15	180	11	2.1	0.33	1.2
Sand	Silt (%)	Clay (%)	Class	-	-	-	-	-	-
16	56	28	Si-C-L	-	-	-	-	-	-

Crop Management

Seed inoculation was done according to Research Institute of Soil and Water. Seeds of Jk cultivar were mixed and stirred with 5 ml sticky gum Arabic and packages containing open bacteria (10^8 bacteria per ml) or packages containing open mycorrhizal fungi strains (*Glomus intraradices*) (10^6 fungi per ml) and after stirring for 10 minutes in shadow they were dried and cultivated. Foliar application of urea as the rate of 5 per 1000 was sprayed in desired treatments at during flowering stage.

Traits measure

To measure plant growth parameters and to investigate factors influencing soybean yield, sampling was done about 3 weeks after plant emergence once every 7 to 10 days in 6 steps. In each step, 5 plants from each experimental plot were harvested and after biomass were dried in oven at 75°C for 48 hours. Leaf area index was measured by leaf area meter instrument and then it was calculated for an area of 1 m^2 of land. In order to determine the root nodes in 6 steps during the growth season, five plants in the rows were randomly removed from the soil then the roots were washed by water to get completely clean. The nodes were separated from the roots and after the counting were dried for 24 hours in the oven at 70° C and measured. In order to determine the yield of grain and crop straw, at the harvest time plants were harvested manually from the two middle lines in an area of about 3 m^2.

Then, the grains were separated from the pods by combine machine and the grains and straw of each treatment were separately measured and dried for 48 hours in the oven at 75℃. After drying, dry weight of grains in each plot was measured and the crop yield was calculated with 13% humidity.

Oil was measured by Soxhlet apparatus via AOCS (1997) .Total nitrogen of grain was measured by Kjeltec (Tector Auto Analyzer) through AOCS (1997) method and was multiplied by conversion coefficient of 5.71 (FAO, 2003) to calculate grain protein. Nitrogen harvest index was calculated as follows (Smith and Read, 2008):

Formula. 1. Nitrogen Harvest Index= (Grain nitrogen/ Grain nitrogen + straw nitrogen). 100.

Statistical analysis

Analysis of variance of experimental data and comparison of the means were done test using SAS software (Ver. 8) and least significant difference (LSD). Figures were drawn using Excel (Ver. 2007) software.

RESULTS AND DISCUSSION

The ANOVA results showed treatments had positive but insignificant effects on grain yield, biological yield, harvest index, number of grain per pod, and 1000-grain weight (Table 2). Effect of treatments on number of grain per lateral pod was insignificant at 5% level (Table 2). Mean comparison of number of grain per lateral pod showed highest number of grain per lateral pod belonged to rhizobium + mycorrhiza + foliar application of urea (R+M+U) and lowest one belonged to rhizobium + mycorrhiza (R+M), rhizobium + foliar application of urea (R+U), and foliar application of urea (U) (Fig. 1).

Table 2. The ANOVA results of the effect of rhizobium, mycorrhiza, and foliar application of urea on soybean yield

S.O.V	df.	Grain yield	Biological yield	Harvest index	1000-grain weight	Number of Grain per main pod	Number of grain per sub pod
Block	3	339616.66^{ns}	3212105.556*	13.231^{ns}	77.410^{ns}	0.014^{ns}	0.003*
Treatment	5	65630^{ns}	756256.66ns	7.206^{ns}	28.179^{ns}	0.005^{ns}	0.004*
Error	15	126216.67	765785.556	11.583	52.587	0.017	0.001
CV (%)	-	9.28	8.75	7.31	4.23	5.47	1.95

ns: non-significant; *: significant at 5% probability level; **: significant at 1% probability level.

Tufenkci *et al*. (2005) stated that even though environmental conditions significantly affect 1000-grain weight after grain formation, 1000-grain weight is mainly influenced by genotype and is considered as one of the most important components determining ultimate yield along with the number of grains per pod. Grain size specifies grain saving capability. Coskan *et al*. (2009) reported that inoculation with rhizobium and mycorrhiza significantly increased grain yield through the increase of vegetative growth of plant and yield components.

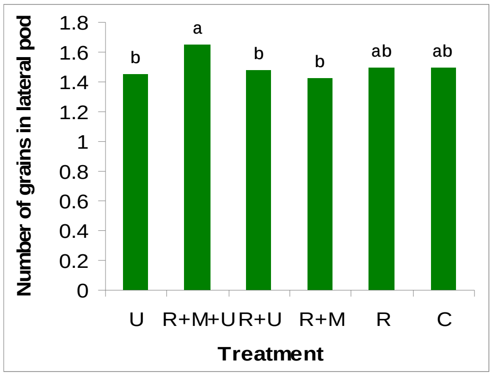

Fig. 1. Mean comparison of the number of grains in lateral pod of soybean: Control (C), Rhizobium (R), Rhizobium + Mycorrhiza (R+M), Rhizobium + foliar application of urea (S + U), Rhizobium + Mycorrhiza + foliar application of urea (R+M+U), foliar application of urea (U), Via LSD method ($p<0.05$).

The increase of crop yield through the seed inoculation mainly resulted from the increase of average weight of grain and the increase of number of pods and number of grain per pod. TaherKhanchi *et al.* (2013) stated that the combined use of soybean seed inoculation and foliar application would increase soybean grain yield when sufficient water was supplied for the plant during the growth season. Ahmad (2013) stated that *Rhizobium Japonicum* strain TAL 110 increased soybean yield by 83%. He also mentioned that the excessive use of nitrogen fertilizers will lead to the suppression of efficiency of inoculums on seeds. Mahmoud Taher khanchi *et al.* (2009) confirmed the positive effects of seed inoculation and the rhizobium bacteria and application of phosphorus and nitrogen fertilizers on the soybean performance and referred to the increase of yield per area unit to 23%. The ANOVA results showed that the effect of treatments on grain yield per hectare, harvest index, and nitrogen harvest index was not significant, but their effect on the grain protein and grain oil was significant at 5% and 1% probability level, respectively (Table 3).

Table 3. The ANOVA results of the effect of Rhizobium, mycorrhiza, and foliar application of urea on soybean yield

S.O.V	df	Grain Protein	Grain Oil	Nitrogen Harvest index	Plant height	Root dry weight	Nodule dry weight	Number of nodules
Block	3	2.343**	7.819*	5.164^{ns}	1.22*	5.45^{ns}	7.17^{ns}	6.45^{ns}
Treatment	5	1.436**	2.442*	5.796^{ns}	3.88*	11.46*	5.5*	9.76*
Error	15	0.218	1.935	11.66	4.67	6.75	4.14	3.29
CV (%)	-	1.30	5	3.87	1.1	7.77	2.5	3.6

ns: non-significant; *: significant at 5% probability level; **: significant at 1% probability level.

Mean comparison results of soybean grain protein showed that highest rate of protein belonged to Rhizobium + Mycorrhiza (R+M) and the lowest one belonged to treatments with Rhizobium + foliar application of urea (R+U), and rhizobium+ mycorrhiza + foliar application of urea (R+M+U) (Fig. 2). It is implied from results that foliar application of urea had a positive effect on rate of oil in seeds so that there was a negative correlation between percentage of oil and protein. There was an inverse relationship between protein and oil percentage. Treatment with highest rate of protein had lowest rate of oil and vice versa (Fig. 3). AL-Karaki and Clark (1998) have stated root colonization with mycorrhiza can change structure of grain oil and protein by changing phosphorus nutrition or removing metabolic substances from host plant in response to fungi. Similar results reported by Kumutha *et al.* (2004) on increase of nitrogen uptake in inoculation with rhizobium and mycorrhiza in soybean.

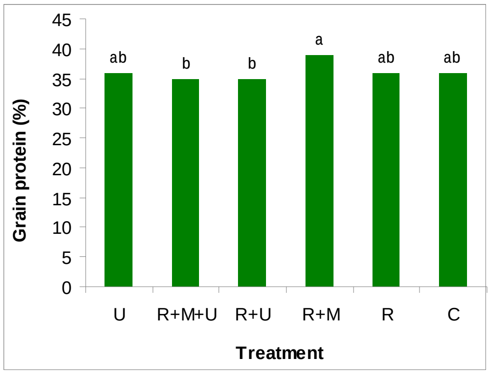

Fig. 2. Mean comparison of soybean grain protein: Control (C), Rhizobium (R), Rhizobium + Mycorrhiza (R+M), Rhizobium + foliar application of urea (R+U), Rhizobium + Mycorrhiza + foliar application of urea (R+M+U), foliar application of urea (U), Via LSD method ($p<0.05$).

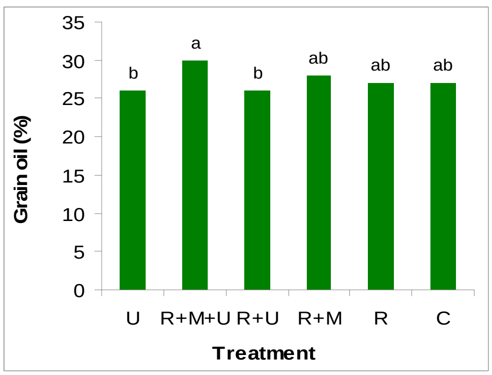

Fig. 3. Mean comparison of soybean grain oil: Control (C), Rhizobium (R), Rhizobium + Mycorrhiza (R+M), Rhizobium + foliar application of urea (R+U), Rhizobium + Mycorrhiza + foliar application of urea (R+M+U), foliar application of urea (U), Via LSD method ($p<0.05$).

Mahmoud Taher *et al*. (2009) reported the positive effects of seed inoculation with rhizobium and application of phosphorus and nitrogen fertilizers on the rate of protein (6 to 22%) and oil (12 to 35%) in soybean grain. They stated that the use of nitrogen improved the efficiency of rhizobium and phosphorus in plant. Leaf area index during the growing season showed that simultaneous consumption of rhizobium, mycorrhiza, and foliar application of urea increased leaf area index. Highest leaf area index belonged to Rhizobium (R) in 80 days after planting and after that Rhizobium + foliar application of urea (R+U) in 90 days after planting (Fig. 4). About 65 days after planting leaf area index in different treatments were close to each other and since then superiority of Rhizobium treatment (R) and rhizobium + foliar application of urea (R+U) were observed that coincided with maximum need to assimilates for production and grains filling and perhaps that is why they produced more dry matter (Fig. 4). Highest total dry matter belonged to Rhizobium (R) in 80 days after planting and then treatment with rhizobium + foliar application of urea (S+U) in 90 days after planting (Fig. 5). After grain filling stage growth of leaves decreases and lower leaves gradually begin to get yellow and consequently leaf area index decreases (Dobbelaere *et al*., 2003). Dogan *et al*. (2007) revealed that rate of dry matter accumulation continued since full flowering stage to end of grain formation stage linearly and so decreases at beginning of grain maturity and leaves falling. Rhizobium has a significant effect on increase of leaf area and dry matter.

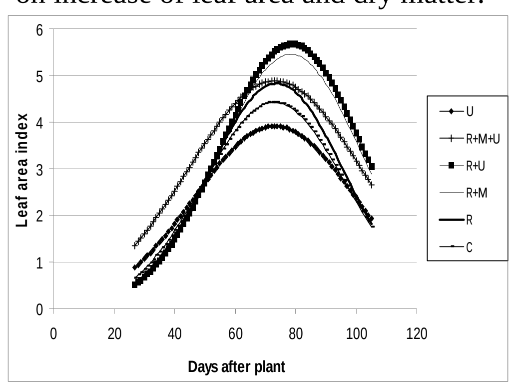

Fig. 4. Leaf area index during the growth season: Control (C), Rhizobium (R), Rhizobium + Mycorrhiza (R+M), Rhizobium + foliar application of urea (R+U), Rhizobium + Mycorrhiza + foliar application of urea (R+M+U), foliar application of urea (U), Via LSD method ($p<0.05$).

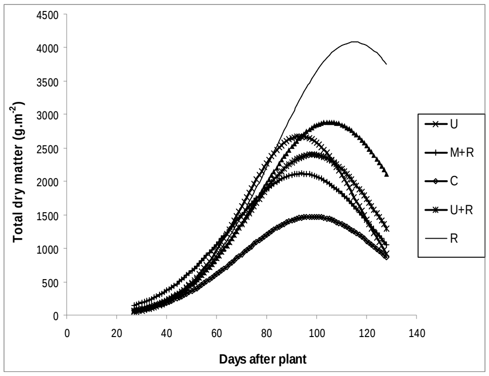

Fig. 5. Total Dry matter in soybean: Control (C), Rhizobium (R), Rhizobium + Mycorrhiza (R+M), Rhizobium + foliar application of urea (R+U), Rhizobium + Mycorrhiza + foliar application of urea (R+M+U), foliar application of urea (U), Via LSD method ($p<0.05$).

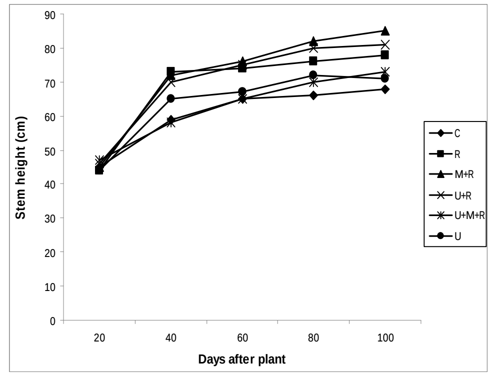

Fig. 6. Soybean stem height (cm) during growth season: Control (C), Rhizobium (R), Rhizobium + Mycorrhiza (R+M), Rhizobium + foliar application urea (R+U), Rhizobium + Mycorrhiza + foliar application of urea (R+M+U), foliar application of urea (U), Via LSD method ($p<0.05$).

Nadian (2005) stated that the mycorrhiza had a positive effect on the increase of dry weight of berseem clover compared with control treatment. The presence of rhizobium and mycorrhiza led to the increase of plant growth so that the dry weight of plant shoot and the leaf area index increased. Kaushal *et al*. (2006) and Caliskan *et al*. (2008) believed application use of the nitrogen fertilizer with the slow release capability was effective in stimulating the growth of soybean shoots would be resulted to increase the leaf area during reproductive stages particularly during the grain filling stage and ultimately would increase the grain yield. The results showed that the plant height, root dry weight, nodule dry weight, and the number of nodules during the growth season had an increasing the trend (Table 3). The highest soybean stem height belonged to the treatments with the Rhizobium + Mycorrhiza (R+M), and rhizobium + foliar application of the urea (R+U) and the lowest one belonged to the control treatment (C). The maximum soybean stem height was achieved to the 110 days after planting date (Fig. 6).

Plants show different reactions to nitrogen such as the increase of chlorophyll growth, balanced growth and development of plant, increase of leaves chlorophyll, increase of plant protein rate, increase of grain and fruit production (Coskan *et al*., 2009, Taher Khanchi *et al*., 2013). The highest root dry weight belonged to rhizobium + mycorrhiza (S+M) and foliar application of urea (U). Maximum root dry weight (6.30 g) was obtained 90 days after the growth and then it had a descending trend and reached to 130 days after planting (Fig. 7). To highest nodule dry weight in soybean belonged to Rhizobium (R) and rhizobium + Mycorrhiza + foliar application of urea (R+M+U) and the lowest one belonged to the control treatment (C). Maximum dry weight of nodule (0.9 g) was obtained 90 days after planting and then it had a descending trend and reached its minimum 130 days after planting (Fig. 8). Highest number of nodules belonged to the treatments with Rhizobium + Mycorrhiza (R+M) and Rhizobium + Mycorrhiza + foliar application of urea (R+M+U) and lowest number of nodules belonged to control treatment (C).

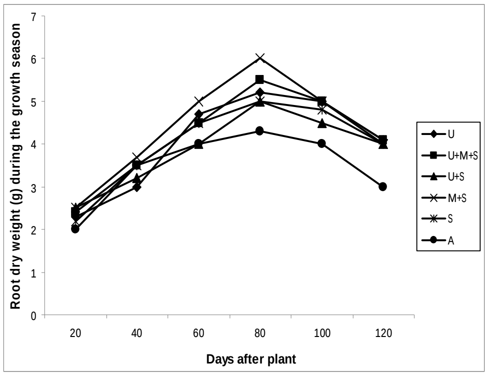

Fig. 7. Root dry weight (g) during the growth season: Control (C), Rhizobium (R), Rhizobium + Mycorrhiza (R+M), Rhizobium + foliar application of urea (R+U), Rhizobium + Mycorrhiza + foliar application of urea (R+M+U), foliar application of urea (U), Via LSD method ($p<0.05$).

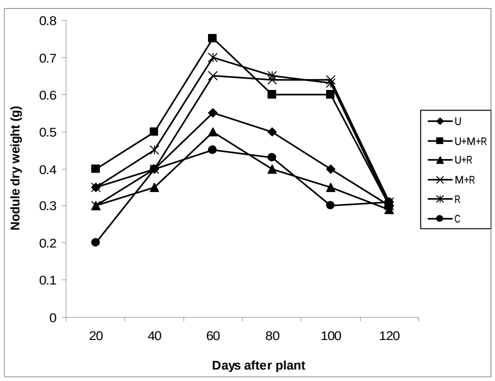

Fig. 8. Nodule dry weight (g) during the growth season: Control (C), Rhizobium (R), Rhizobium + Mycorrhiza (R+M), Rhizobium + foliar application of urea (R+U), Rhizobium + Mycorrhiza + foliar application of urea (R+M+U), foliar application of urea (U), Via LSD method ($p<0.05$).

Maximum number of nodules (45 nodules) obtained 115 days after growth and then it had a descending trend and reached its minimum 130 days after planting (Fig. 9). Mahmoud Taher *et al*. (2009) stated that seed inoculation with rhizobium along apply of phosphorus and nitrogen fertilizers increased number of nodules in root surface up to 70%. Ozer (2003) stated in harvest index is an important factor determining soybean yield in delayed planting.

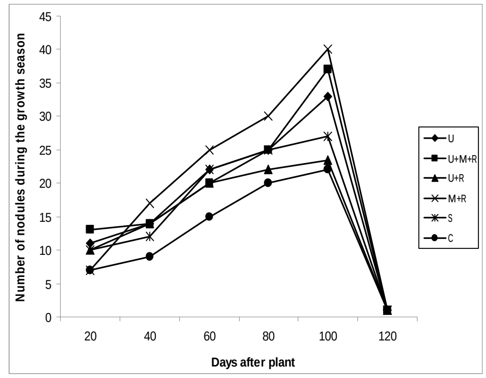

Fig. 9. Number of nodules during growth season: Control (C), Rhizobium (R), Rhizobium + Mycorrhiza (R+M), Rhizobium + foliar application of urea (R+U), Rhizobium + Mycorrhiza + foliar application of urea (R+M+U), foliar application of urea (U), Via LSD method ($p<0.05$).

Purcell *et al*. (2002) reported in delayed planting, due to reduction of plant life cycle by 13 to 25 days, rate of plant dry matter accumulation decreases and seed treatment can influence shortening of plant growth cycle. Nitrogen plays main role in biochemical and physiological processes in plants, so it is considered as one of main constructing components of proteins, nucleic acids, and chlorophyll molecules. Structure of most enzymes is from protein which reflects important role of nitrogen in plants. (Hause *et al*., 2007). Increase of clearing system activity of active oxygen species subject to defense mechanism stress is important. So consumption of bacteria contributes to clearance of active oxygen species (Demir, 2004). Azotobacter as a diazotroph bacterium need large amounts of usable elements for survival in soil and meets its need through root and seed (Gransee, 2001). It reported diazotroph bacteria improve plant growth by absorbing nutrients and increasing resistance to environmental stresses (Winkler *et al*., 2014).

CONCLUSION

Concurrent use of rhizobium and mycorrhiza and foliar application of urea increases the number of nodules in soybean. The increase of the number of nodules leads to the improvement of nitrogen fixation potential, plant growth improvement, increase of the number of grain per pod. Moreover, the increase of root dry weight, leaf area index, dry matter accumulation, efficiency of nutrients uptake in plants (nitrogen harvest index) and increase of protein and oil contents in soybean grain are the other positive effects of seed treatment with rhizobium and mycorrhiza and foliar application of urea (at flowering stage). Direct overuse of nitrogen fertilizers reduces the efficiency of rhizobium in soybean root, but foliar application of urea not only improves bacteria efficiency, but also has fewer negative effects. Therefore, applying and treating soybean seeds with rhizobium, mycorrhiza, and foliar application of urea improve soybean growth.

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