J. Appl. Environ. Biol. Sci., 3(1)17-27, 2013

ISSN 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

The Effect of Plant Growth Promoting Rhizobacteria on Growth Parameters, Antioxidant Enzymes and Microelements of Canola under Salt Stress

Noorieh Baniaghil[*1], **M.H.Arzanesh**[2], **Mahlegha Ghorbanli**[3], **Maryam Shahbazi**[4]

[1]MSc, Gorgan Educational Office Gorgan, Iran
[2]PH.D, Dept. of Water and Soil, Golestan Agricultural and Natural Resources Research Center, Gorgan, Iran
[3]PH.D, Islamic Azad University , Gorgan Branch, Iran
[4]PH.D, Agriculture Biotechnology Research Institute of Iran (ABRII), Karaj, Iran

ABSTRACT

Plant Growth Promoting Rhizobacteria (PGPR) include bacteria (Rhizosphere and Rhizoplane) residing in roots that useful for plants in some conditions. *Azospirillum* and *pseudomonas* are some of these bacteria. The bacteria are responsible for physiological and morphological changes in plant. This study was carried out to investigate the effects of PGPR including three species of *Azospirillum*, *A.lipoferum* , *A.brasilense*, *Azosprillum lipoferum* sp. and two strains of *Pseudomonas*, *P. putida, Pseudomonas fluorescens* on growth parameters, Antioxidant enzymes and Microelements. The research was on two Canola cultivars; Hyola 401 and RGS 003 under two salinity levels, 80 and 160 mM NaCl based on randomize complete design as factorial with three replication under green house condition in Leonard medium with Hoagland solution in Department of Water and Soil in Golestan Agricultural and Natural Resources Research Center. The result showed that salinity stress had a significant effect on growth traits. Canola seeds inoculation with PGPR has recovered salinity effects on some growth parameters, and the most effects was related to *Azospirillium* strains specially *A.brasilense*. ANOVA results showed that the Maximum Fe and Zn were related to *A.lipoferum* and Maximum Mn was related to *A.lipoferum* Sp. Also, Antioxidant enzymes were increased under both salinity levels compare to control, and the increase was more under 160 mM NaCl, so that Canola inoculation with five strains of PGPR caused to decrease the level of enzymes, that *A.lipoferum Sp* and *A.lipoferum* had the most effects.

KEYWORDS: Plant Growth Promoting Rhizobacteria, Canola, Salinity, Azospirillum, Pseudomonas.

INTRODUCTION

Most of soils in arid and semi arid area in Iran are under salinity condition (18). Plants under salt stress confront with two processes: drought and ions toxicity (25). In saline soils, soil osmotic potential is negative and water absorption is very difficult. There is a difference between halophyts and glycophytes regarding salinity tolerance. Halophyts need salt stress to grow. For example, *Salicornia* needs some viscosity of $Na^+$for optimum growth. However, glycophyts growth reduces under salinity (1). Salinity may cause reduction of biomass and change in the compound of product (25). Oil function in oily plants is affected by reduction of seeds function and under salinity seed's oil percent (17). Safflower seeds oil can reach to 60% although increasing salinity usually can cause reduction of oil percentage. Increasing of salinity from zero to high salt viscosity is caused to reduce the oil percent from 38.4% to 34% (17).

However, it seems that in salt stress condition the fatty acid levels does not change a lot, but thickness of seeds cortex will increase (17). Salts create some general and special effects on plants that can affect their growth and production directly. Also, salts can affect some physical and chemical soil characteristics and; therefore, they can affect soil quality. Salt stress causes to accumulate reactive oxygen species (ROS) on the cell, that can damage the membrane and membrane processes of lipids, proteins and nucleic acids (28). Plant defense against ROS is related to antioxidant defense systems including catalase, peroxidase, superoxide dismutase, glutathione reductase, ascorbate peroxidase and nonenzymitic compounds that are included ascorbate, α-tocopherol and carotenoides, ascorbate, compounds have a vital rule to protect plants against ROS effects (4).

Due to salt stress antioxidant enzymes are activated as part of protection mechanism (25). Plants tolerance to salt stress is different based on their growth stages. Some plants like, barely, rice, wheat, corn, show resistance in the primary growth stages. Also, Sugerbeet tolerates salinity during its life cycle except for germination. Bearing in mind the effect of salinity on plants which are due to lack of water around roots and ions toxic effects, there are some mechanisms in salt tolerance that can recover lack of water in plants and prevent harming effects of extra toxic ions in the environment. Tolerance salinity mechanisms in different plants included using ions to osmotic regulation, K^+selected uptake to Na^+ and osmotic organic accumulation in cytoplasm (26). Another way to create tolerance in plants is using of genetic engineering method or inoculation with Plant Growth Promoting Rhizobacteria (PGPR). The benefits of inoculation with PGPR included the increase of various indexes like germination rate, root growth, yield, biotic control of pathogenesis

***Corresponding Author:** Noorieh Baniaghil, MSc, Gorgan Educational Office Gorgan ,Iran. neda_kh2003@yahoo.com, Address: Nasim – Esfand sharghi St. – Gorgan - Iran ,Tel and Fax: +98-1712231931

factors, leaf surface, chlorophyll content, dry tolerance, root and shoot weight and microbial activities (Lucy et al., 2004). Interaction between root and micro organisms in Rhizosphere causes essential nutrients uptake and prevents toxic compounds accumulation (29).

It is not obvious, which mechanism help develop the growth of the plants by Rhizosphere bacteria, but some of the most important of these mechanisms are ability to produce plant hormones ,nitrogen fixing as non-symbiosis confronting some plant patogenes by sidrophore production and antifungal compounds synthesis (14; 24; 27). Sidrophore production, phosphate solubilisation and other nutrients and ACC deaminase enzyme production are effective in reducing destructive affects of produced ethylene under stress (7; 11). Many PGPR bacteria by producing ACC deaminase causes to hydrolyze the precursor of ethylene production in plants, i.e. ACC, to ammonium and α – ketobutyrate (15; 16).

For example, wheat inoculation with *Pseudomonas fluorescens* caused increase in growth (22). A research showed inoculation canola with *P. putida* UW4 (ACC deaminase enzyme producer) had a significant increase in shoot biomass comparing to non- inoculation plants .Using biologic fertilizers from PGPR can increase quantity and quality of crop yield, efficiency of chemical fertilizers, water efficiency and tolerance of salt and dry stress as one of the proper ways to adapt to environment (6). Canola (*Brassica napus* L.) is from brassicacea family and it is one of the most important seed industrial oils in the world (23).There is a high demand for eating oils in Iran and high amounts of which is imported from other countries. In this regards, development of planting seed oil is very important. Canola has tolerance to salinity and it can be planted in soil with 10 $dsm^{-1}$and can tolerate 8 dsm^{-1} salinity with reducing yield (3; 28). About 33000 hectare of golestan province lands are planted by canola. High demand of canola to nitrogen and absence of micorhiza connection of this family with micorhiza fungus caused that using PGPR as completing the other fertilizers is put into consideration for researchers. Therefore, the aim of this research is to consider PGPR effects on growth parameters, microelements and antioxidant enzymes activities levels in tow canola cultivars under salt stress.

MATERIAL AND METHODS

A factorial arrangement of treatments was conducted randomized completely design in a greenhouse of water and soil department of golestan agricultural and natural resources researches center. Factorial combinations of salinity treatments (0, 80 and 160 mM), two cultivars of canola (Hyola 401 and RGS 003) and 3 levels of bacterial suspensions (*Azospirillum brasilense, Azospirillum lipoferum, Azospirillum lipoferum, Pseudomonas putida* and *Pseudomonas fluorescens*) were the treatments of the experiment. Salinity treatments were created by adding 3.2 and 6.4 gr (NaCl) into 1 liter of distil water. Bacterial suspensions were made as described by lin et al. (20). Seeds which were the same in weight and size were washed three times with water and after hydration they were put into 96% alcohol for 15 seconds. Then, seeds were washed with distilled water and were put into 2.5% sodium hypochlorite solution for 5 minutes. After, seeds washed with distilled water 5 times and inoculated with bacterial suspensions, sowed in Leonard container filled by sand (sterilized by HCl). Salinity treatments performed by adding saline water to Hoagland solution.

The seedlings were under 20 ^{oc} days and 20 ^{oc} in nights and 12000 lux with 16 hours light and 8 hours darkness in a growth chamber in a period of 4 weeks. Fresh and dry root (gr), shoot weight (gr), leaf area (cm^2) and plant height (cm) measured in 21 days after planting).So, antioxidant enzymes activity and microelements concentration (Zn, Mn, Fe and Cu) were obtained in 21 DAP. Microelements concentration have been measured with atomic absorption and antioxidant enzymes activity of catalase, peroxidase, ascorbate peroxidase and superoxide dismutase enzymes in plant measured as described by Chance and Maehly (12), Arrigoni et al. (5) Weston method respectively. Data were analyzed using SAS software and means were adjudged by Duncan's Multiple Range Tests (DMRT).

RESULT AND DISCUSSION

Overall results showed that growth traits affected by salinity and the most negative effect of salinity relate to 160 mM. In the research, canola seed inoculation with different strains of PGPR was resulted to decrease salt stress effects (Table 1). Maximum of efficiency was related to *Azospirillum* specially *A.brasilense* . As the result revealed, the salinity had a negative effect on growth parameters (Table 2). In general, salts have general and specific effects on the plants that have direct effect on growth and yield. This results agreement with reportes of Bashan and Bashan (9). One of reasons of decreasing or stopping the growth under abiotic stress conditions such as salinity is the accumulation of ethylene in the plant (22; 25). Under this condition, ACC amount (ethylene precursor) increase in the plant consequently ethylene synthesis also increase in plant tissues (22).

Increasing of endogen ethylene in plant species, especially in most of dicotyledon can decrease germination and root growth. In Glick method it is offered that rhizosphere bacteria with ability of producing ACC deaminase enzyme can decrease ethylene amounts, and it could reduce destructive effects of salinity. Other researches indicated that rhizosphere bacteria increase growth and yield in the corn (14). Zaied et al.(30), demonstrated that strains of *Azospirillum* can provide a proper condition to grow corn, with secretion of plant hormones (28). Shahroona et al. studied on some different strains of *Pseudomonas* on growth in the corn with different levels of fertilizer and they showed that the strains can increase corn dry weight between 15.2 to 19.7 as compared with control (27). Abdul Jaleel et al.(2), reported inoculation of *Catharantus roseus* with *Psuedomnase* leads to increased leaf number, shoot and root length. However, other studies indicated that the

reaction of various plants to inoculation is different and consequently the effect levels differ. In other words, inoculation of canola with PGPR had significant effects on shoot microelements uptake under salinity levels.

results showed that the amount of growth parameters was decreased in two canola cultivars in that decrease was more in 160 mM Nacl. Inoculation of plant by PGPR caused to recover decreased effects of salt stress. *Azospirillum lipoferum* had the most effect on fresh shoot and root weight, dry shoot weight, leaf numbers, leaf area and plant height, in all salinity levels and cultivars (Figure 1-7). Also, the bacteria had a proper effect on dry root weight in Hyola under 80 mM NaCl and in RGS under 160 mM NaCl. The most amount of dry root weight in Hyola was related to *P.putida* under 160 mM NaCl and RGS was related *to A.lipoferum* and *A. brasilense* under 80 mM NaCl.

Also, the mean differences indicated maximum amount of two elements, Fe and Zn was related to the plants that had been inoculated with *Azospirillum lipoferum* and these amounts did not have a significant difference in two salinity levels (Figure 14 and 15). The maximum amount of Mn was related to *Azospirillum lipoferum* which did not have significant difference in two salinity levels (Figure 17). Plant inoculation with PGPR caused to facilitate microelements uptake. Microelements uptake, especially Fe, Mn and Zn, may be related to ability to produce plants sidrophore or microbial sidrophores. Sidrophores are organic compounds with low molecular weight and high and especial high affinity to complex with some cations such as Fe. Sidrophore production in PGPR such as *Psuedomonase*, *Azospirillum* sp. and *Azetobacter* has been demonstrated (6). Plants can use produced sidrophores by bacteria as a factor to provide their required Iron.

The results of this research indicated that antioxidant enzyme levels significantly increased in both Hyola 401 and RG003 cultivars under two salinity concentrations (80,160 mM).Therefore, the level of enzymes significantly decreased with inoculation and growth of bacteria. The maximum decrease of root catalase enzyme in Hyola was under *P.putida* and in RGS was under *Azospirillum lipofferum* Also inoculation with *Pseudomonas fluorescens* had the most decrease of leaf catalase enzyme in two canola cultivars (Figure 8). The most decrease of root peroxidase enzyme in Hyola was related to *P.putida* and RGS was under *A. lipoferum sp*.(Figure 9). Also the most decrease of shoot peroxidase enzyme in Hyola was related to *A. lipoferum sp* and in RGS related to *A. lipoferum* under salt stress condition. PGPR inoculation caused to decrease ascorbate peroxidase enzyme activity in two canola cultivars and the minimum of rate Hyola was under *A .lipoferum* and in RGS was related to *A.brasilense* (Figure 10).

A research showed that increase of salinity caused a significant increase in gloutation reductase and ascorbate peroxidase enzymes activity in leaf lettuce in comparison to control (Figure ecrease the effect of salt stress on the enzymes activity (13). Also *P.putida* had the most effect on superoxid dismutase in Hyola and in RGS it was related to *A.lipoferum sp* (Figure 13).This result conform our research. Research indicated that the activity of superoxide dismutase, catalase, glutation reductase and malondialdehyde increased under stress (13). Dai et al. (13), showed that the level of superoxid dismutase, catalase and peroxidase were increased in *Brassica napus* L. under stress. Generally, biofertilizers , especially bacterial, causes to increase elements uptake such as nitrogen and phosphorous, that interfere with Carbohydrates as non- enzymic antioxidants.These compounds are built faster and less energy consuming than enzymic antioxidants that are useful to optimum usage and energy saving .

REFERENCES

1-Abdolzadeh, A., Kazuto, S., Chiba, K. (1998). Effect of salinity on growth and ion content In *Lolium multiflorum*,L.I perenne and Festuca arundinacea. J.Jap. Soc.Reveget.Thec. 23,161-169.

2- Abdul Jaleel, C. (2007). Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in Catharanthus roseus seedlings under salt stress. South African J. Bot. 73, 190-195

3-Almond, J.A., Dawkins, T.C.K., Askev, M.F. (1986). Aspects of crop husbandry. In :Scarisbrick DH and daniels , R. W. (eds.) Oil seed Rape. Collins, London, pp. 127-175.

4-Amirjani, M.R. (2010). Effect of salinity stress on Growth, Mineral Composition, Poline Content, Antioxidant Enzymes of Soybean. American J. Plant Physiology. 5, 350-360.

5-Arrigoni, O., Calabrese, G., De Gara, L., Bitonti, M.B., Liso, R. (1997). Correlation between changes in cell ascorbate and growth of *Lupinus albus* seedling. J. Plant Physiology. 150, 302-308.

6-Arzanesh, M.H., Alikhani, H.A., Khavazi, K., Rahimian, H.A., Miransari, M. (2009). In vitro growth of wheat (*Triticum aestivum* L.) seedlings, inoculated with *Azospirillum sp*., under drought stress. Int. J. Bot. 5, 244–249

7-Arzanesh, M.H. (2008). Investigating the function of some *Azospirillum* strains on wheat (*Triticumaestivum L.*) yield in different drought levels. Unpublished Ph.D. dissertation, Uni. Tehran, 208p

8-Ashraf M, Mcneily T (2004). Salinity tolerance in Brassica oil seeds.CRC press LLC. 23, 157-174.

9-Bashan, Y., de-Bashan, L.E. (2010). How the plant growth-promoting *bacterium Azospirillum* promotes plant growth-a critical assessment. Adv. Agron. 108, 77-136

10-Bashan, Y., Holguin, G., De-Bashan, L. (2004). *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances. Can. J. Microbiol. 50, 521–577.

11-Cattelan. AJ., Hartel, P.G., Fuhrmann, J.J. (1999). Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Sci.Soc.Am. J. 63,1670–1680.

12-Chance, B., Maehly, C. (1995). Assay of catalase and peroxidase. Methods Enzymol. 11, 764-755.

13-Dai, Q, Chen, C., Feng, B., Liu, T., Tian, X., Gong, Y., Sun, Y., Wang, J., Du, S. (2009). Effects of different NaCl concentration on the antioxidant enzymes in oilseed rape (*Brassica napus* L.) seedlings. Plant Growth Regul. 59, 273-278.

14-Egamberdiyeva, D. (2007). The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Appl. Soil. Eco. 36,184-189.

15-Glick, B.R., Penrose, D.M., Jiping, L.I. (1998). A model for the lowering of plant ethylene concentration by plantgrowth- promoting bacteria. J. Theor. Biol. 190, 63-68.

16-Glick, B.R., Todorovic, B., Czarny, J., Cheng, Z., Duan, J., McConkey, B. (2007). Promotion of plant growth by bacterial ACC deaminase. Crit. Rev. Plant. Sci. 26, 227-242.

17-Hans-Henning, M., Blackshaw, R.E., Byers, J.R., Huang, H.C., Johnson, D.L., Keon, R., Kubik, J., McKenzie, R., Otto, B., Roth, B., Stanford, K. (2004). Safflower production on the Canadian prairies. Agriculture and Agri-Food Canada. Lethbridge, Alberta. 43p

18-Kafi, M., Rostami, M. (2007). Yield characteristics and oil content of three safflower (*Carthamus tinctorius*) cultivars under drought in reproductive stage and irrigation with saline water. Iranian J. Field Crops Res. 5, 121-132.

19-Koca, H., Bor, M., zdemir, F.O¨., Türkan, I. (2007). The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ. Exp. Bot. 60, 344-351

20-Lin, W., Okon, Y., Hardy, R.W.F. (1983). Enhanced mineral uptake by *Zea mays* and *Sorghum* bicolor roots inoculated with *Azospirillum brasilense.* App. Environ. Micro. 45, 1775-1779

21-Lucy, M., Reed, E., Glick, B.R. (2004). Applications of free lving plant growth-promoting rhizobacteria. Antonie van leeu wen hook. 86, 1-25.

22-Mayak, S., Tirosh, T., Glick, B.R. (2004). Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol. Biochem. 42, 565–572.

23-Moarefi, A., Latifi, N. (1998). Investigating the effects of phospore consuming, Nitrogen, consuming time of Nitrogen, on yield of canola. The 5th congress of Agriculture and plant breeding in Iran. 448p.

24-Mrkovacki, N., Milii, V. (2001). Use of *Azotobacter chroococcum* as potentially useful in agricultural application. Ann. Microbiol. 51,145–158.

25-Munns, R., Tester, M. (2008). Mechanisms of Salinity Tolerance. Annu. Rev. Plant Biol. 59, 651-681.

26-Munns, R., James, R.A., Lauchli, A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. J. Experi. Bot. 57, 1025-1043.

27-Shaharoona, B., Arshad, M., Zahir, Z.A., Khalid, A. (2006). Performance of Pseudomonas spp. containing ACC-deaminase for improving growth and yield of maize (Zea mays L.) in the presence of nitrogenous fertilizer. Soil. Biol. Biochem. 38, 2971–2975.

28-Shahbazi, M., Arzani, A., Saeidi, G. (2011). Effect of NaCl treatments of seed germination and antioxidant activity of Canola (*Brassica Napus*) Cultivars. Bangladesh. J. Bot. 41, 67-73.

29-White, P.J. (2003). Ione transport. In: Thomas B, Murphy DJ, Murray BG, eds. Encyclopaedia of applied plant science. London: Academic press, pp. 625-634.

30-Zaied, K.A., Abd El-Hady, A.H., Sharief, A.E., Ashour, E.H., Nassef, M.A. (2007). Effect of Horizontal DNA Transfer in *Azospirillum* and *Azotobacter* Strains on Biological and Biochemical Traits of Non-legume Plants. J.Appl. Sci. Res. 3, 73-86.

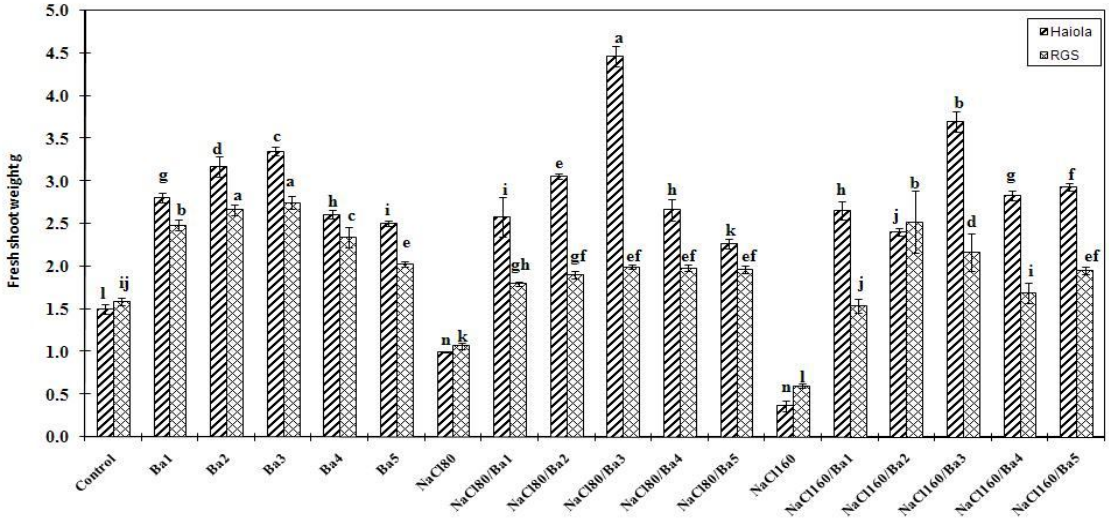

Figure 1: Fresh shoot weight (3 *Azospirillum* strains: Ba1=45-4 , Ba2=1-46 , Ba3=7-49 and 2 Pseudomonas strains: Ba4=P-flou , Ba5=P-put)

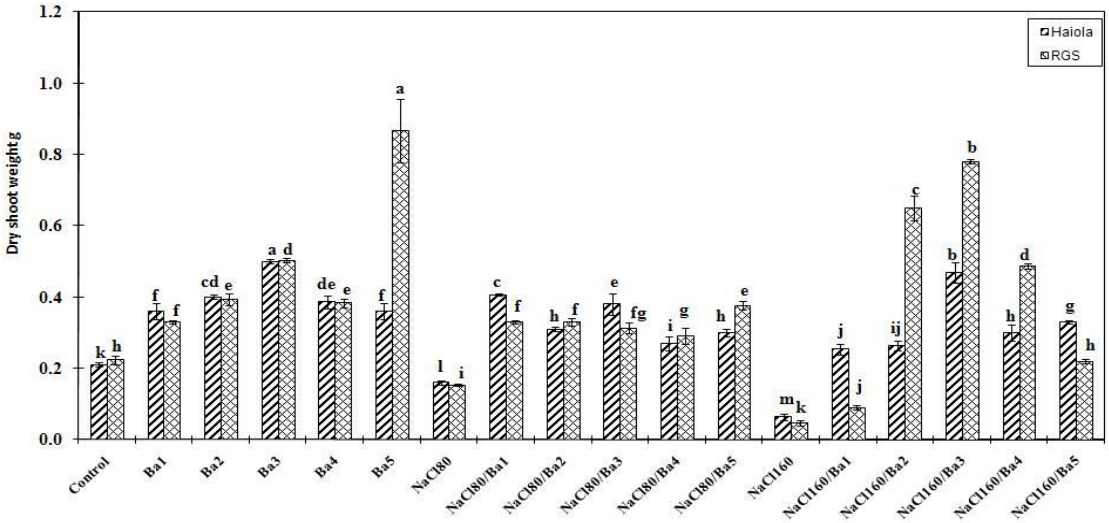

Figure 2: Dry shoot weight (3 *Azospirillum* strains: Ba1=45-4 , Ba2=1-46 , Ba3=7-49 and 2 Pseudomonas strains: Ba4=P-flou , Ba5=P-put)

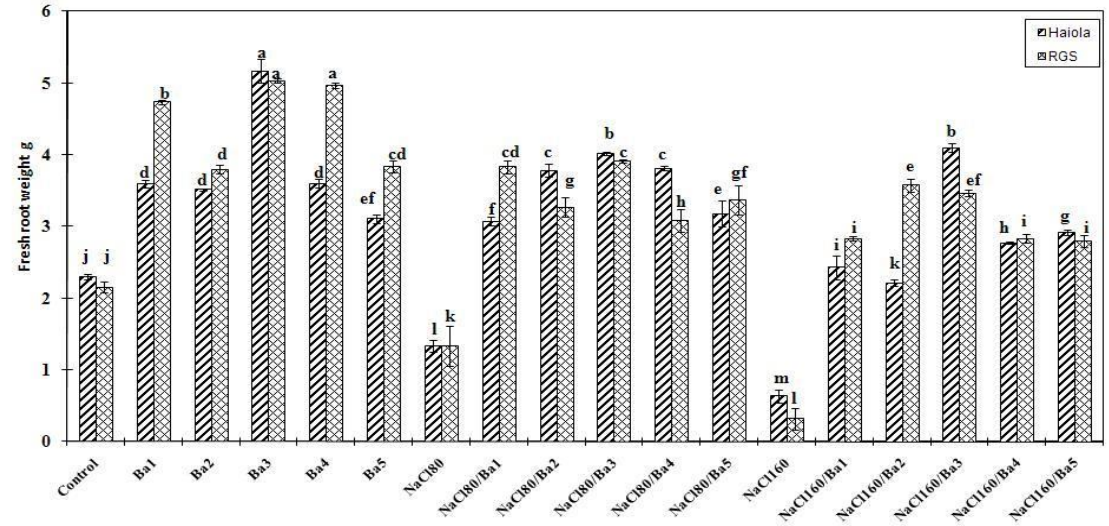

Figure 3: Fresh root weight (3 *Azospirillum* strains: Ba1=45-4 , Ba2=1-46 , Ba3=7-49 and 2 pseudomonas strains : Ba4= P-flou , Ba5=P-put)

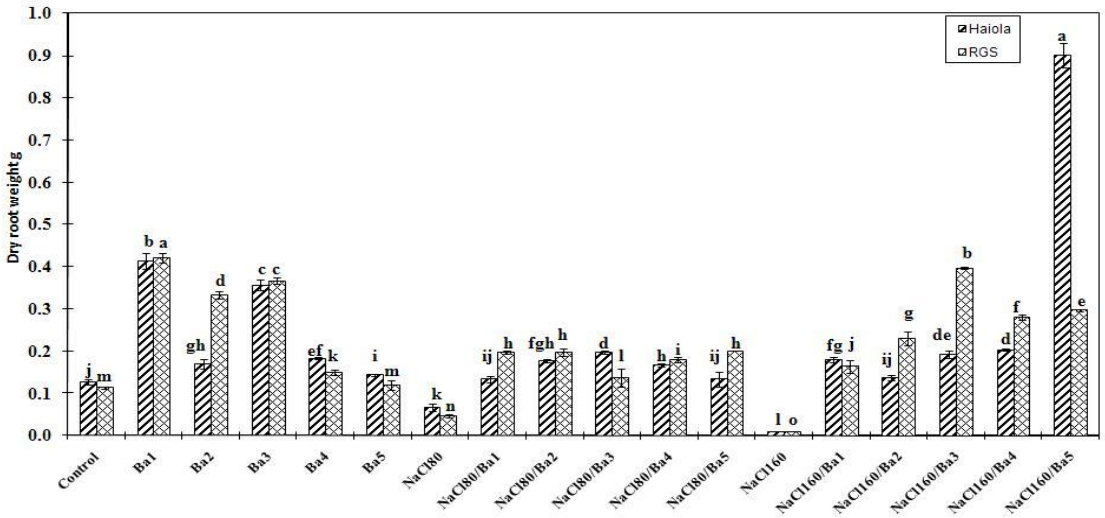

Figure 4: Dry root weight (3 *Azospirillum* strains: Ba1=45-4 , Ba2=1-46 , Ba3=7-49 and 2 pseudomonas strains : Ba4= P-flou , Ba5=P-put)

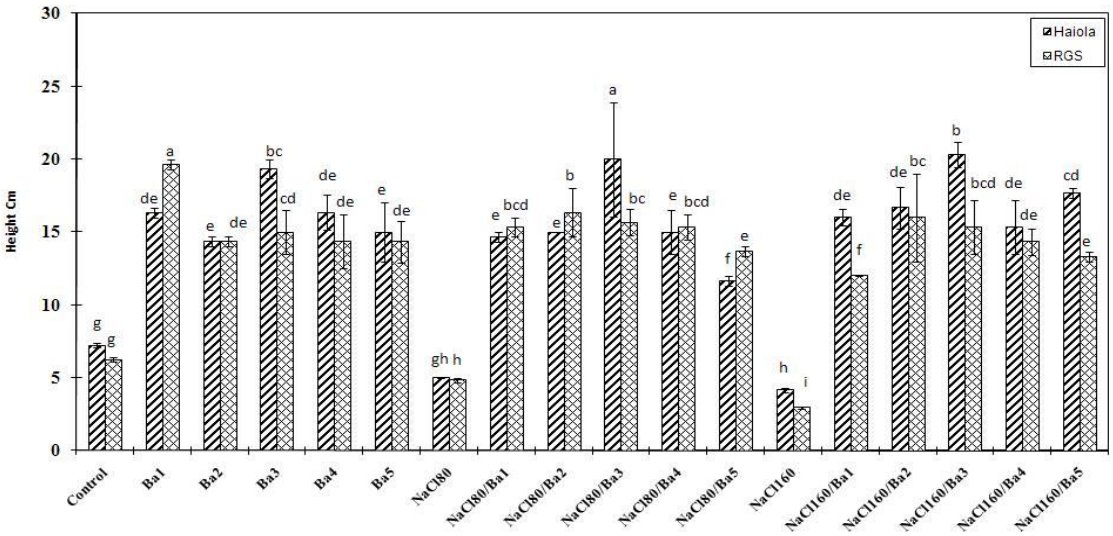

Figure 5: Plant height in 21 DAP (3 *Azospirillum* strains: Ba1=45-4, Ba2=1-46, Ba3=7-49 and 2 pseudomonas strains: Ba4=P-flou, Ba5=P-put)

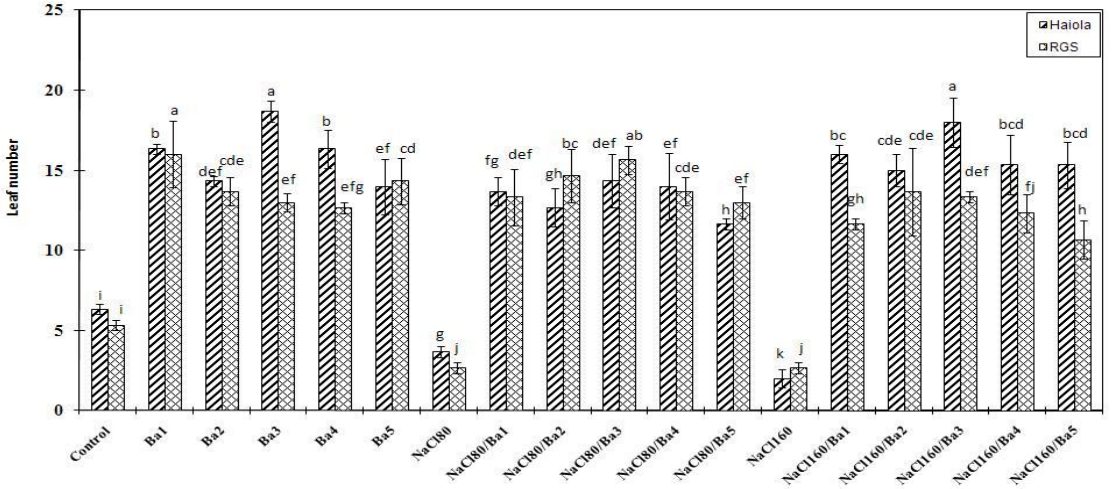

Figure 6: Leafnumbers in 21 DAP(3 *Azospirillum* strains: Ba1=45-4, Ba2=1-46, Ba3=7-49 and 2 pseudomonas strains: Ba4=P-flou, Ba5=P-put)

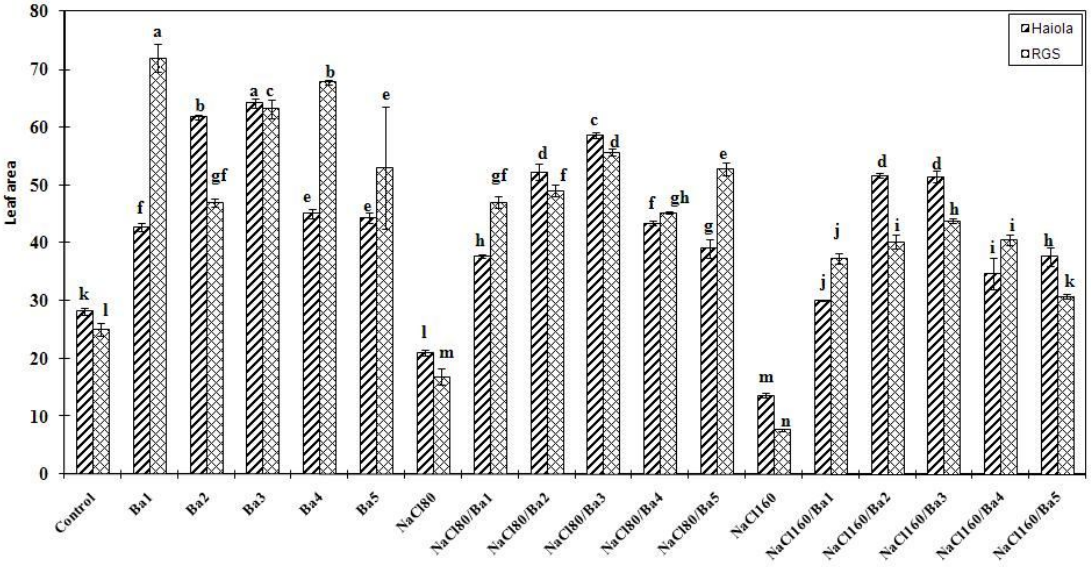

Figure 7: Leaf area (3 *Azospirillum* strains: Ba1=45-4, Ba2=1-46, Ba3=7-49 and 2 pseudomonas strains: Ba4=P-flou, Ba5=P-put)

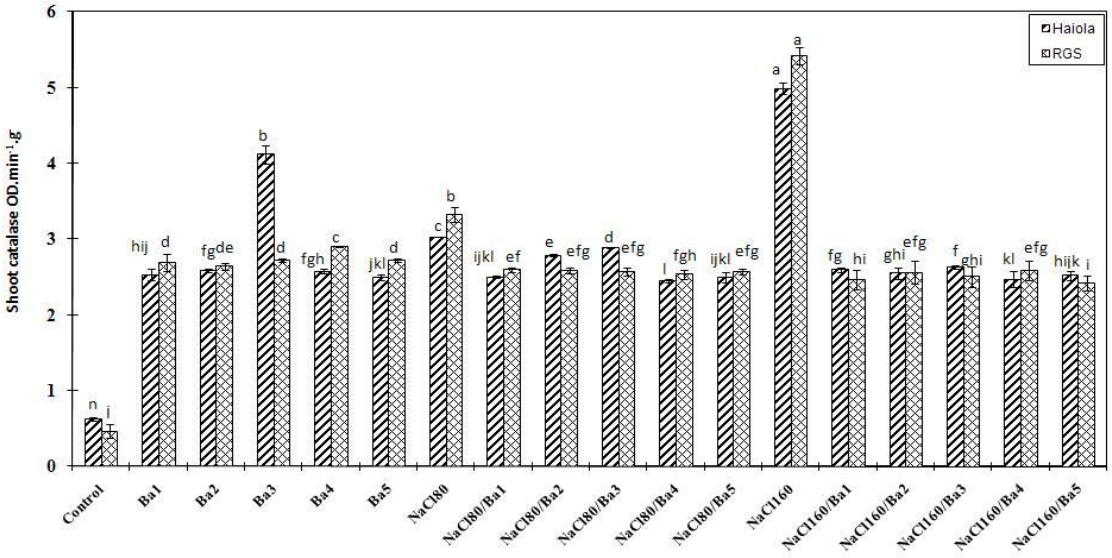

Figure 8: Shoot catalase enzyme (3 *Azospirillum* strains:Ba1=45-4, Ba2=1-46, Ba3=7-49 and 2 pseudomonas strains: Ba4=P-flou, Ba5=P-put)

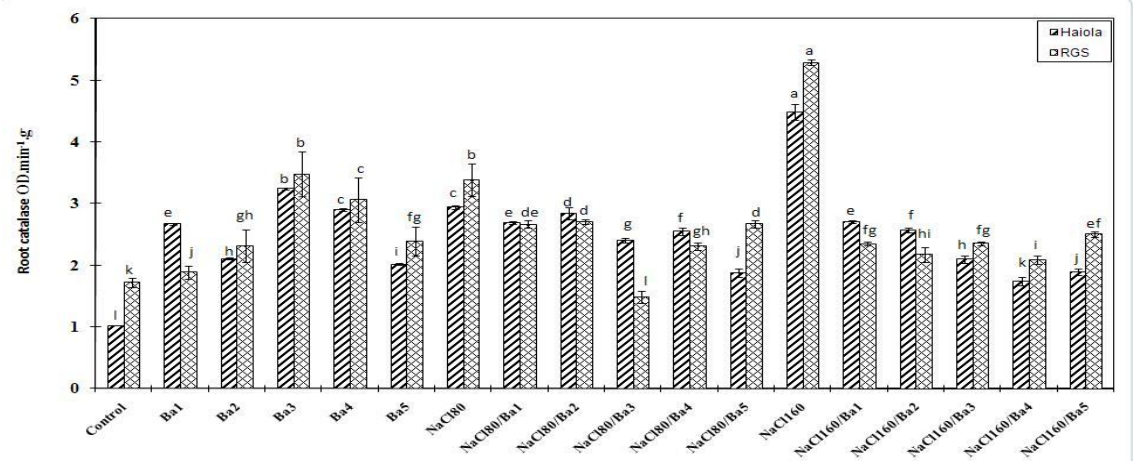

Figure 9: Root catalase enzym (3 *Azospirillum* strains:Ba1=45-4, Ba2=1-46, Ba3=7-49 and 2 pseudomonas strains: Ba4=P-flou, Ba5=P-put)

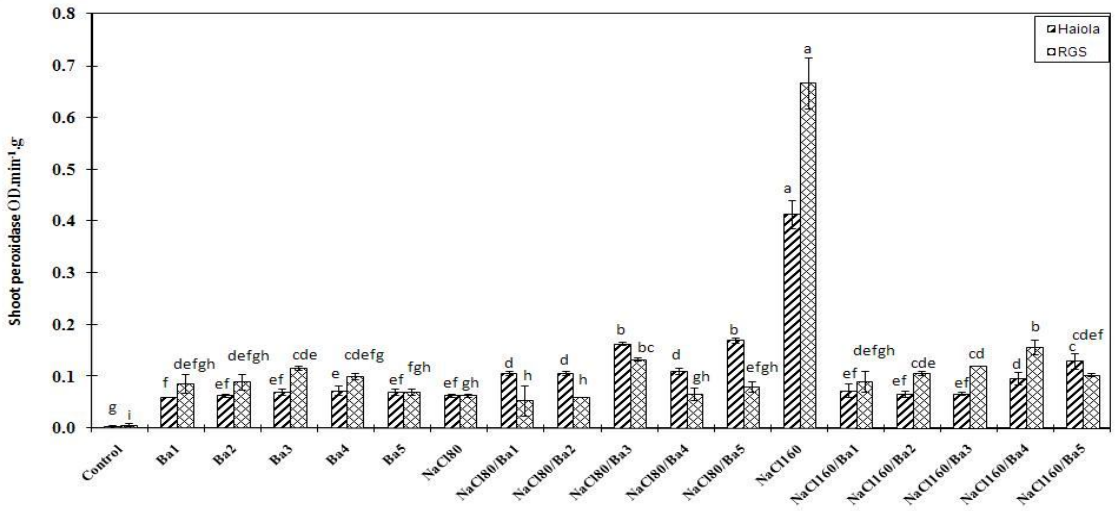

Figure 10: Shoot peroxidase enzym (3 *Azospirillum* strains:Ba1=45-4, Ba2=1-46, Ba3=7-49 and 2 pseudomonas strains: Ba4=P-flou, Ba5=P-put)

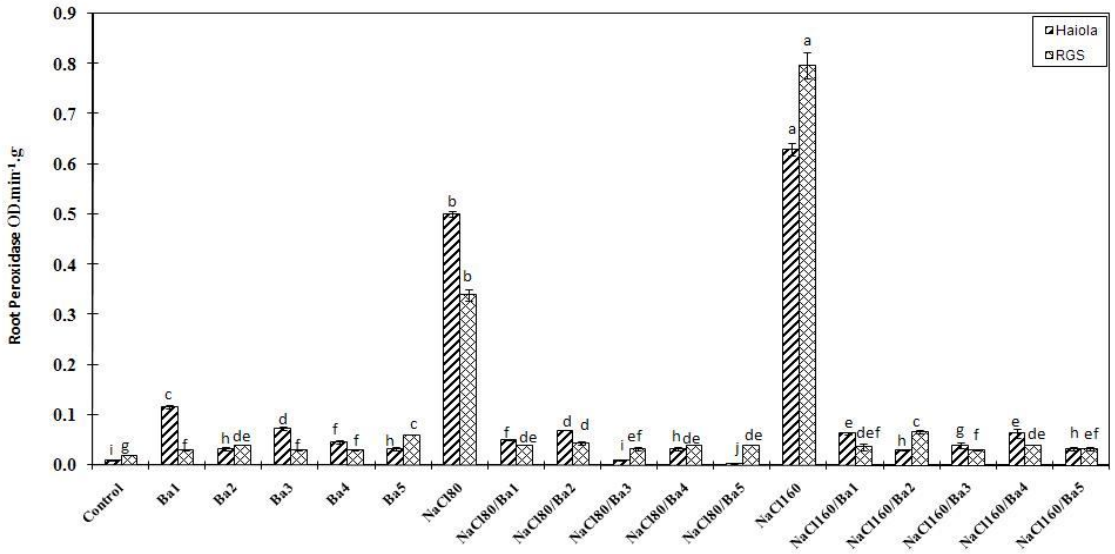

Figure 11: Root peroxidase enzym (3 *Azospirillum* strains:Ba1=45-4, Ba2=1-46, Ba3=7-49 and 2 pseudomonas strains: Ba4=P-flou, Ba5=P-put)

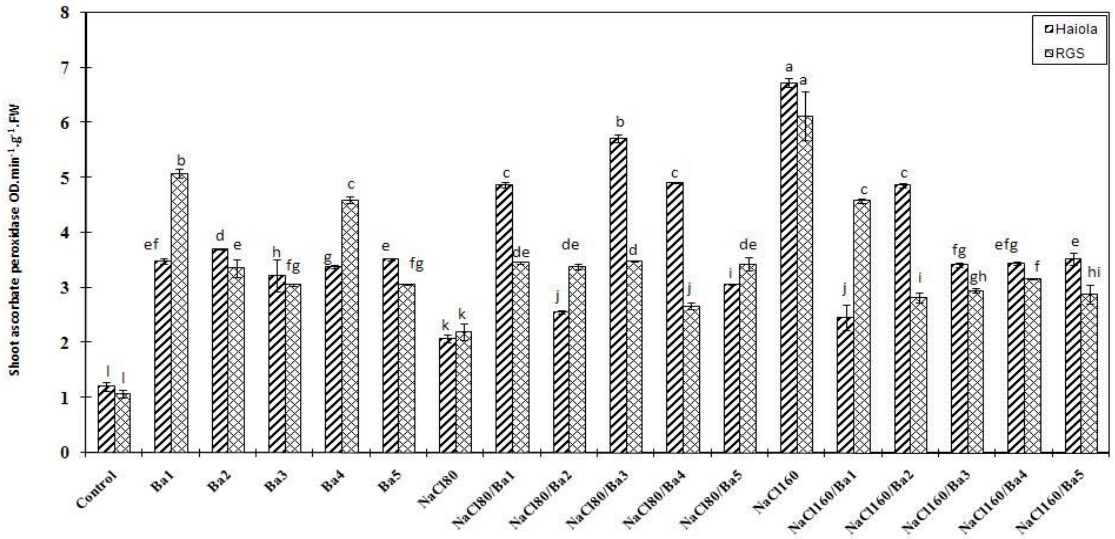

Figure 12: Shoot ascorbate peroxidase enzym (3 *Azospirillum* strains:Ba1=45-4, Ba2=1-46, Ba3=7-49 and 2 pseudomonas strains: Ba4=P-flou, Ba5=P-put)

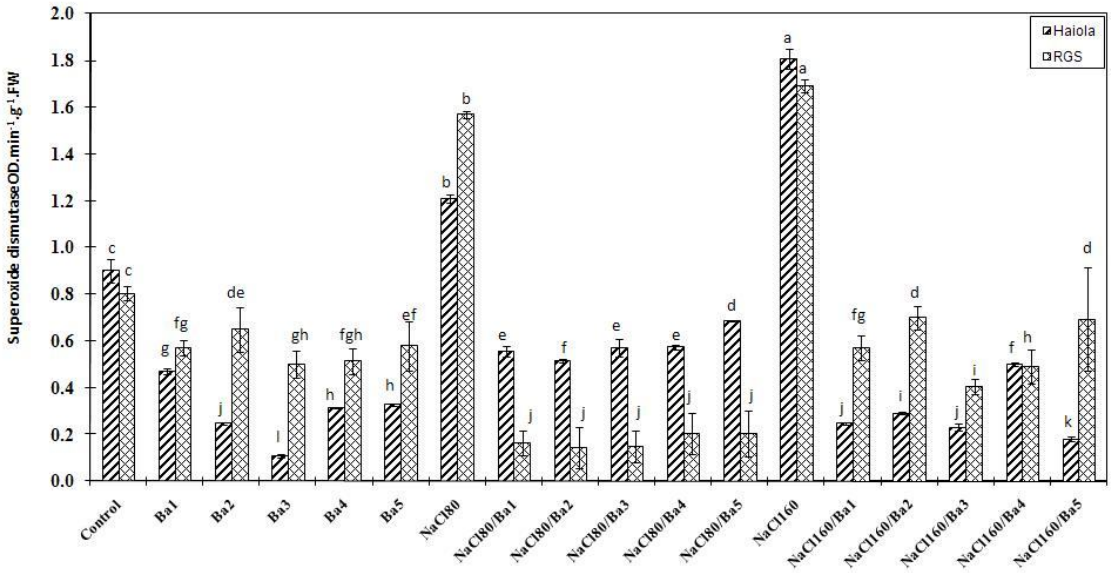

Figure 13: Superoxide dismutase enzym (3 *Azospirillum* strains:Ba1=45-4, Ba2=1-46, Ba3=7-49 and 2 pseudomonas strains: Ba4=P-flou, Ba5=P-put)

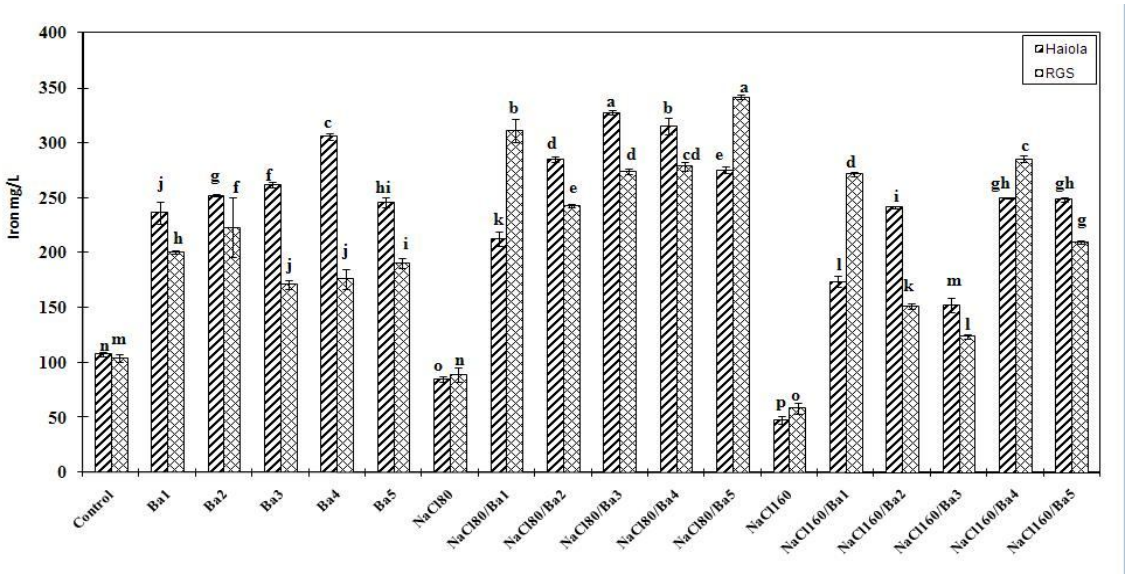

Figure 14: Iron levels (3 *Azospirillum* strains:Ba1=45-4, Ba2=1-46, Ba3=7-49 and 2 pseudomonas strains: Ba4=P-flou, Ba5=P-put)

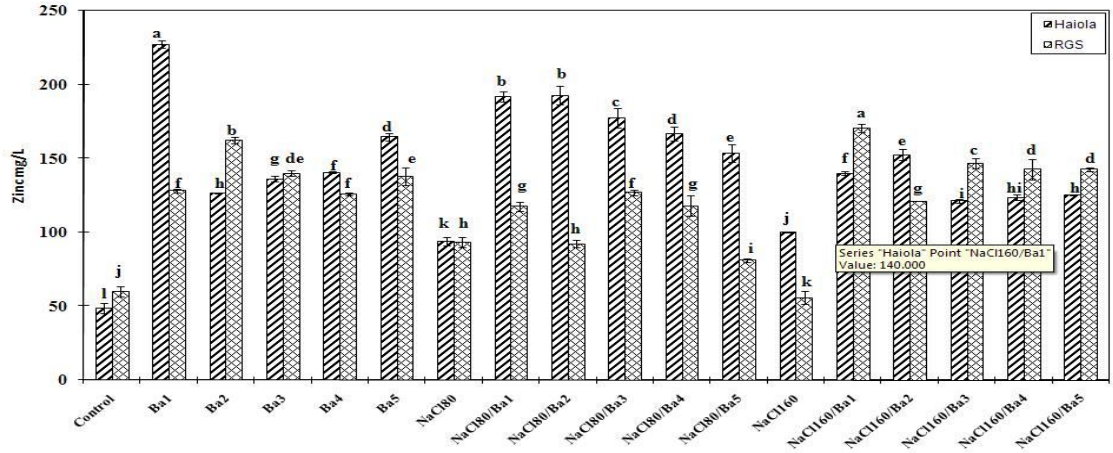

Figure 15: Zinc levels (3 *Azospirillum* strains:Ba1=45-4, Ba2=1-46, Ba3=7-49 and 2 pseudomonas strains: Ba4=P-flou, Ba5=P-put)

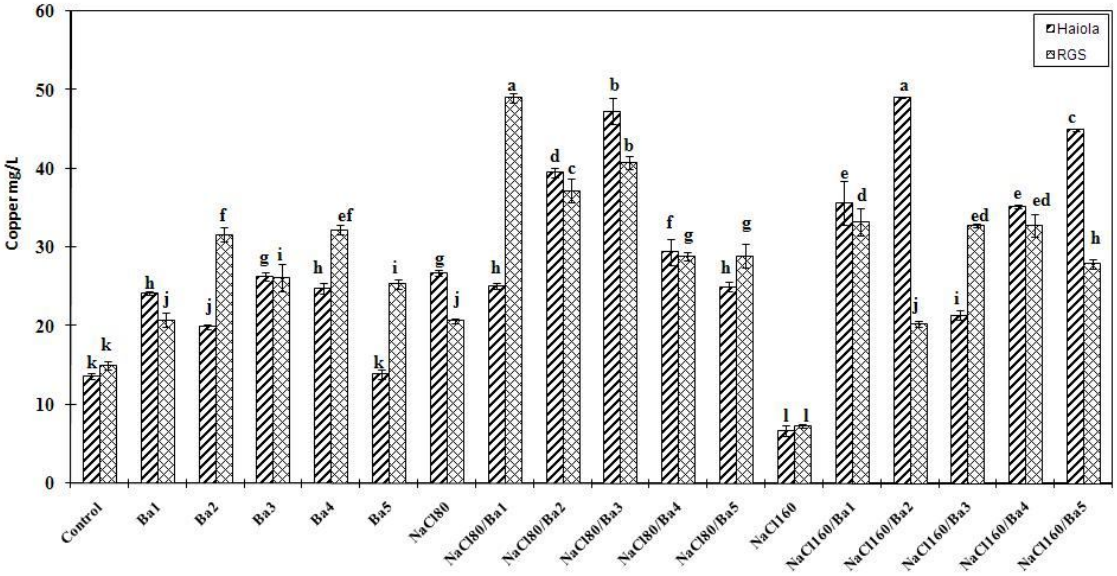

Figure 16: Copper levels (3 *Azospirillum* strains:Ba1=45-4, Ba2=1-46, Ba3=7-49 and 2 pseudomonas strains: Ba4=P-flou, Ba5=P-put)

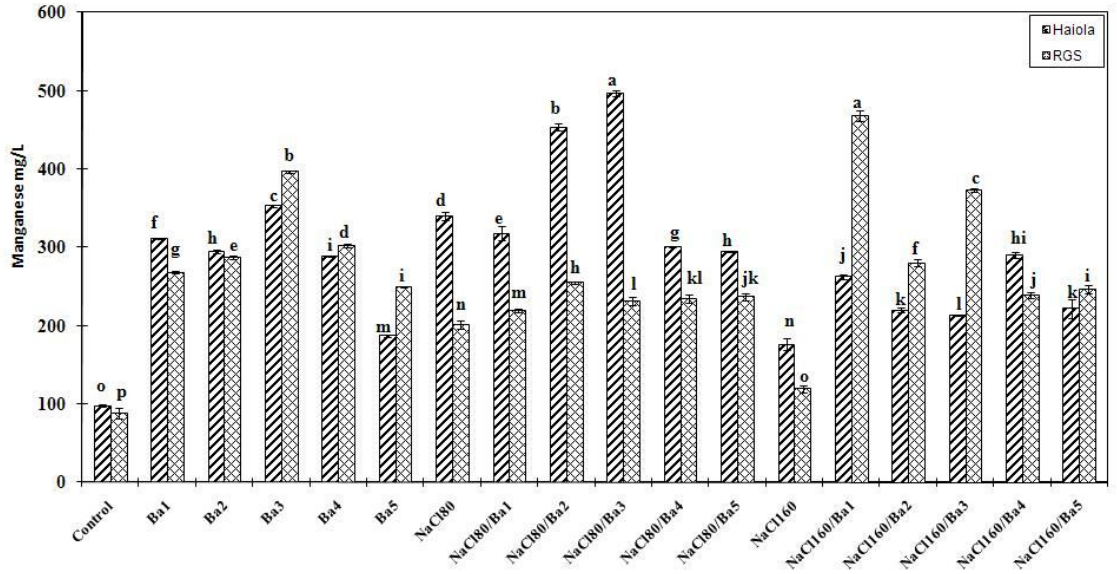

Figure 17: Manganese levels (3 *Azospirillum* strains:Ba1=45-4, Ba2=1-46, Ba3=7-49 and 2 pseudomonas strains: Ba4=P-flou, Ba5=P-put)

Table 1: Results of variance analysis (mean squares) for peroxidase root enzyme (OD/min.gr), Height (cm), Fresh weight root (gr), Fresh weight shoot (gr), Leaf area (cm^2), Cu, Zn, Mn and Fe (mg/l).

Peroxidase root	Height	Fresh weight root	Fresh weight shoot	Leaf area	Cu	Zn	Mn	Fe	df	Vs
0.0006*	26.205**	1.06**	11.64**	116.31**	0.381	14696.96**	15003.01**	8515.92**	1	C
0.509**	11.28*	13.92**	1.27**	2366.97**	978.86**	294.19**	18067.36**	44393.05**	2	S
0.005**	23.76**	0.86**	0.69**	228.68**	306.38**	8718.45**	92803.47**	10700.83**	2	C*S
0.98**	341.79**	17.21**	8.41**	2972.31**	827.65**	15663.31**	66712.68**	81387.38**	5	B
0.002**	7.66	0.65**	1.28**	421.36**	76.561**	1046.60**	5336.95**	8280.92**	5	C*B
0.52**	10.704**	0.43**	0.33**	53.54**	228.41**	1127.86**	16153.98**	5870.20**	10	S*B
0.008**	4.60	0.55**	0.38**	85.204**	241.001**	2722.09**	12151.65**	3645.30**	10	***S** C*S*B
0.0001	3.52	0.031	0.032	12.46	2.81	36.85	58.35	8611.87	72	Error
7.41	13.63	5.52	7.91	8.19	5.92	4.604	2.801	5.097		CV

Table 2: Results of variance analysis (mean squares) for Superoxide dismutase enzyme (OD/min.gr), Catalase root enzyme (OD/min.gr), Peroxidase shoot enzyme (OD/min.gr), Catalase leaf enzyme (OD/min.gr), Ascorbat enzyme (OD/min.gr), leaf number, Dry weight shoot (gr) and Dry weight leaf (gr).

Supeeoxide desmutase	Catalase root	Peroxidase shoot	Catalase leaf	Ascorbat	Leaf number	Dry weight shoot	Dry weight leaf	df	Vs
0.06*	0.34**	0.005	0.025	1.89**	53.48**	0.0002	0.09**	1	C
0.74**	0.73**	0.52**	7.404**	4.33**	23.25**	0.104**	0.11**	2	S
0.72**	0.11	0.026**	0.07*	2.47**	27.06**	0.009**	0.02**	2	C* S
5.24**	1.73**	0.35**	3.32**	1.53**	339.08**	0.12**	0.25**	5	B
0.02	0.79**	0.007*	0.39**	1.47**	5.88	0.04**	0.03**	5	C*B
0.74**	4.73**	0.47**	12.26**	10.01**	3.47	0.104**	0.07**	10	S*B
0.13**	0.23**	0.004	0.18**	2.61**	5.73	0.04**	0.05**	10	C*S*B
0.01	0.047	0.002	0.016	0.042	4.27	0.0003	0.0013	72	Error
16.52	8.53	31.72	4.56	5.81	16.54	8.21	10.43		CV