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ORIGINAL ARTICLE

EFFECT OF BIO-FERTILIZER AND SALICYLIC ACID ON DRY WEIGHT AND LEAF CONTENT OF SOME NUTRIENT ELEMENTS OF FENUGREEK PLANT UNDER SALINE STRESS

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Abstract: A two factorial experiment of randomized complete block design with three replications was conducted at the experimental farm of the College of Agriculture, Anbar University, during the winter of 2020 using a plastic container. It was to study the effect of bio-fertilizer and spraying with salicylic acid in dry weight and the concentration of some elements in the fenugreek plant cultivated under saline stress. The first factor was bio-fertilizer and salicylic acid spraying on the shot as the following: without each of bio-fertilizer and spraying the Salicylic acid as control treatment, pollinated with bio-fertilizer, spraying with salicylic acid, pollinated with bio-fertilizer plus spraying with salicylic acid. While the second factor was the saline irrigation water with three saline levels *i.e.* 0.8, 5.0, and 9.5 ds m^{-1}. Increasing the salinity of irrigation water to 9.5 dsm^{-1} significantly decreased the rates of the studied characteristics except the nitrogen concentration in the leaves. Treatments for pollination with bio-fertilizer and spraying with salicylic acid differed significantly between them, where the interaction between biological pollination and spraying with salicylic acid superior to other traits except for proline, pollination and spraying with salicylic acid improved the functional condition of the fenugreek plant causing a significant decrease in the concentration of proline in the vegetative group reached to 1.33 mm g^{-1}. Significant decrease exceeded 11% compared to the control treatment that recorded 1.51. The interaction between irrigation water with a salinity of 0.8 dsm^{-1} and mixing of biological pollination and spraying with salicylic acid gave the highest values of dry weight and the concentration of elements in the vegetative part reached to 15.11g ,2.32,0.47 and 4.16 mg/g for each of dry weight and *NPK,* respectively.

***Key words*:** Fenugreek, Salinity, Bio-fertilizer, Salicylic acid.

Cite this article

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1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is grown annually in Mediterranean countries and Asia. Long ago, it had been used to feed human beings and animals and in addition, it has been used for the medicinal purpose [Zandi *et al.* (2015)] *e.g.* anticholestrolemic, antimicrobial, anthelmintic, antileprotic and antibronchitic [Mehrafarin *et al.* (2011)]. It characterizes as a fast and easy growing crop that needs less care thereby, it is inexpensive in monetary terms. In many countries, fenugreek is grown in arid and semi-arid regions that have salt-affected soils. In these regions, salinity is one of the considerable factors limiting seed germination, plant growth and development. Moreover, the quantity and quality of plant production of most species is delayed and reduced with a salinity increase. The plant responses may vary clearly based on species. The issue of agricultural soils salinity is a concern to the world and Iraq in particular. Since, the Iraqi agricultural soils are classified mostly as saline soils several strategies were used to reduce or control the salinity issue. However, most of these

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strategies are time-consuming and costly, where human resources and the amount of water are required. Recently, many studies have been carried out using various growth regulators to reduce the negative effect of salt stress on plants, due to its cost saving and production efficiency by reducing the water and nutrient requirement. Salicylic acid was found to reduce the adverse consequences of biotic and abiotic factors on the plant and showed a promising results [Farahbakhsh and Saiid (2011)]. Researchers also used bio-fertilizers, which was found to encourage growth in coexistence with salinity and increase the ability of plants to tolerate salt stress, as well as maintaining the soil fertility and being environment friendly. Bio-fertilizers contain different microbes that help to decompose the bio-matters for the plants to obtain nutrients. It also reduces the risk of environmental pollution and secretion of some important enzymes for the plant metabolism [Lucy *et al.* (2004)]. In addition to different plant defense techniques for the management of plant pathogens, others can be activated by using bio-fertilizers. Hence, this study was conducted to identify the effect of salicylic acid and bio-fertilizers on the concentration of some nutrient elements and some growth characteristics of fenugreek irrigated by saline water.

2. Materials and Methods

A two factorial experiment of Randomized complete block design (RCBD) with three replications was carried out at the experimental farm of the College of Agriculture, Anbar University, during the winter of 2020 using a plastic containers containing 9 kg soil. The soil was classified as sandy clay texture with electrical conductivity of 1.2 dsm^{-1}. The first factor consisted of bio-fertilizer and salicylic acid spraying on the vegetative part of plant, the first factor was applied as follows: without each of bio-fertilizer and spraying the salicylic acid as control treatment, pollinated with bio-fertilizer, spraying with salicylic acid, pollinated with bio-fertilizer plus spraying with salicylic acid. While the second factor was the saline irrigation water with three salinity levels *i.e.* 0.8, 5.0, and 9.5 dsm^{-1}. The salinity levels (5.0, and 9.5 dsm^{-1}) of water were prepared by mixing a saline water with electrical conductivity of 10 dsm^{-1} (drain water) with a river water with electrical conductivity of 0.8 dsm^{-1}. The Phosphate and potassium fertilizer were supplied with application rate of 90 and 150 $kg.h^{-1}$ *P* and *K,* respectively in one batch before planting. While the nitrogen was supplied with application rate of 40 kg *N* h^{-1} split into two doses, the first at planting while the second at the flowering stage. The seeds were washed several times using distilled water to remove the effects of sterilizer, then immersed in the bio-fertilizer that consist of Bacillus subtilis and Pseudomonas putidab and complex enzymatic systems Protese, Amylase, Chitinase and Lipase and biological stimulants gibberelin and cytokinins 0.3% for half an hour after adding a little sterile gum solution in a 10:1 ratio to increase the adhesion of the pollen to the seeds surface according to Bashan *et al.* (1993). The seeds were transported to sterile boxes for planting. The plants were sprayed with salicylic acid two weeks after plant exposure to salt stress. The solution was prepared with concentration of 50 ppm by dissolving 0.05 g of salicylic acid in a litre of distilled water, the tween 20 (emulsion T-20) was added as a diffuse with a concentration of 0.1% with the spray solution. The spraying process covered all plants parts after covering, the soil surface was mulched with a layer of plastic sheet during the spraying process to prevent the absorption of salicylic acid by the roots. A local variety (10 seeds) of fenugreek seeds were cultivated on 11/5/2019, after the emergence of seedlings (10 days after planting), they were thinned to 6 plants. All pots were irrigated with fresh water to rise up the soil moisture to field capacity conditions. Four weeks after planting, the plants were exposed to saline stress by irrigating them with saline water that previously prepared. Irrigation operations using saline water continued until the end of the experiment.

3. Results and Discussion

3.1 Dry weight of plant (g)

The results listed in Table 1 showed a reduction in dry weight with increasing the salinity levels, where the treatment EC 9.5 gave the lowest dry weight of 10.36 g while the treatment EC 0.8 recorded the highest dry weight of 12.40 g, which did not differ significantly from the treatment EC 5.0 that recorded 11.96 g. The decrease in the average dry weight was probably due to the inhibition of root growth at the high salinity levels of irrigation water, which reduced ability of the plant's roots to absorb water and the essential nutrients for the plant due to the osmotic effort, as well as inhibition of photosynthesis thereby non-transfer of metabolic products through plant tissue [Saberali and Moradi (2019)]. While the treatment of the bio-fertilizer plus spraying with salicylic acid significantly increased the average of dry weight up to 13.48 $g.plant^{-1}$ (Table 2), compared to the dry weight of both treatments of bio-fertilizer and spraying with salicylic acid that recorded 12.38 and 12.86 g.plant $^{-1}$, respectively. Probably due to the role of Pseudomonas putida in reducing the negative effect of salinity by increasing the uptake rate of k^+, Mg^{2+} and Ca^{2+} at the same time decreasing the absorption of Na^+ [Itelima *et al.* (2018)]. The dry weight per plant decreased to 8.02 g.plant^{-1} in the absence of both bio-fertilizer and salicylic acid (Table 2). This clearly shows the effect of bio-fertilizers that keep the soil environment rich in all kinds of nutrients (macro and micro nutrients) that led to increase the dry weight per plant, moreover, the high ability of Pseudomonas SPP to colonize the rhizosphere. Hence, its ability to produce some important enzymes, including the ketogenase enzyme and the ACC-deaminase enzyme, stimulates the hydrolysis of important intermediate compounds that reflected in increasing the volume and mass of the root system and then increasing the dry weight of the plant. In addition to the ability of these isolates to produce growth regulators including indole acetic acid IAA and gibberelin GA3 and the production of multiple amines that have an important role in reducing the harmful effects of salt stress and then improve the growth of the plant under salt stress [Naz and Bano (2010)]. The results of the interaction between salinity levels and pollination with bio-fertilizer and spraying with salicylic acid showed a significant effect on the dry weight of the plant where the combination of EC 0.8 with the bio-fertilizer and spraying with salicylic acid superior to the other treatments gave 15.11 g.plant^{-1} while the combination of EC9.5 and control treatment recorded the lowest rate of 6.39 g.plant^{-1} (Fig. 1).

3.2 The chlorophyll content

The leaf content of chlorophyll significantly decreased with increasing salinity levels (Table 1), where the lowest chlorophyll content was 41.6 mg g^{-1} at the treatment EC 9.5, while the highest content of chlorophyll was recorded at the treatment EC 0.8 reach to 51.75 mgg^{-1}. Because of the inhibition of photosynthesis due to the negative impact of salinity on the fenugreek plant that led to break down the parts of the plastids, reducing their functional activity, breaking the bonds between the granules and protein, and reducing the amount of chlorophyll.

The results of the statistical analysis indicate that there was a significant difference in the rates of chlorophyll content due to the difference in the treatments of bio-fertilizer and spraying with salicylic acid in this study, where the bio-fertilizer plus spraying with salicylic acid treatments were superior and gave the highest rate of 50.50 mgg^{-1} compared to the control treatment that gave the lowest rate reached 39.20 mg g^{-1} (Table 2). The ability of the bio-fertilizer used to increase the concentration of chlorophyll can be attributed to the ability of bacteria (Bacillus subtilis and Pseudomonas putida) to produce the plant growth hormones, including auxin, gibberelin, cytokinin, and abscisic acid [Ansary *et al.* (2012)]. In terms of interaction, there was no significant differences between salinity levels, and spraying treatments, in the leaf content of chlorophyll (Fig.1).

3.3 Proline content

The content of proline increased with increasing salinity levels reach to 0.93, 1.31 and 1.65% at the salinity levels of 0.8, 5.0 and 9.5 dsm^{-1}, respectively (Table 1). In response to abiotic stresses, plants increased proline production through induction of the P5CS gene that is responsible for proline production, thereby proline plays an important role in inducing genes for tolerance of environmental stresses [Johari-Pireivatlou (2010)]. The results of Table 2 indicate that there were significant effects of the bio-fertilizer and salicylic acid in the leaf content of proline, where the ratios of proline were 1.51, 1.25, 1.33 and 1.10% for each of the following treatments control, bio-fertilizer, spray with salicylic acid and the bio-fertilizer plus bio-fertilizer, spray with salicylic acid, respectively, The reason can be attributed to the spraying with salicylic acid has improved the functional condition of the fenugreek plant, causing a significant decrease in the concentration of proline in the vegetative system. Salicylic acid has a role in maintaining and stimulating protein levels through high content of *K*, *P* and *N* elements in addition to chlorophyll content. The interaction between salinity levels and other treatments, namely bio-fertilizer and spraying with salicylic acid showed a significant difference in the leaf proline content, where the interaction between the treatment EC 9.5 and the control treatment gave the highest rate of 1.91%, while the interaction between EC 0.8 treatment and bio-fertilizer plus spraying with salicylic acid gave the lowest rate was 0.8%.

3.4 Shoot content of nitrogen, phosphorous and potassium

Table 1: Effects of salinity levels of irrigation water on the vital characteristics of fenugreek.

Water salinity (ds/m)	Dry weight (g)	chlorophyll (mg/g)	Proline	Conc.of *N*	Conc. of *P*	Conc. of *K*
0.8	12.4	51.75	0.93	1.76	0.32	4.01
5.0	11.96	44.65	1.31	1.99	0.28	3.44
9.5	10.36	41.6	1.65	2.11	0.24	2.25
L.S.D (0.05)	0.56	2.37	0.101	0.17	0.02	0.22

Table 2: Effects of bio-pollination and salicylic acid on the vital characteristics of fenugreek.

Treatment	Dry weight (g)	chlorophyll (mg/g)	Proline	Conc.of *N*	Conc. of *P*	Conc. of *K*
Control	8.02	39.2	1.51	1.29	0.23	2.74
Bio-fertilizer	12.38	47.57	1.25	1.85	0.28	3.46
Salicylic acid	12.86	46.8	1.33	2.16	0.27	3.16
Bio-fer.+salicylic acid	13.48	50.5	1.1	2.51	0.34	3.5
L.S.D (0.05)	0.67	2.81	0.154	0.16	0.023	0.36

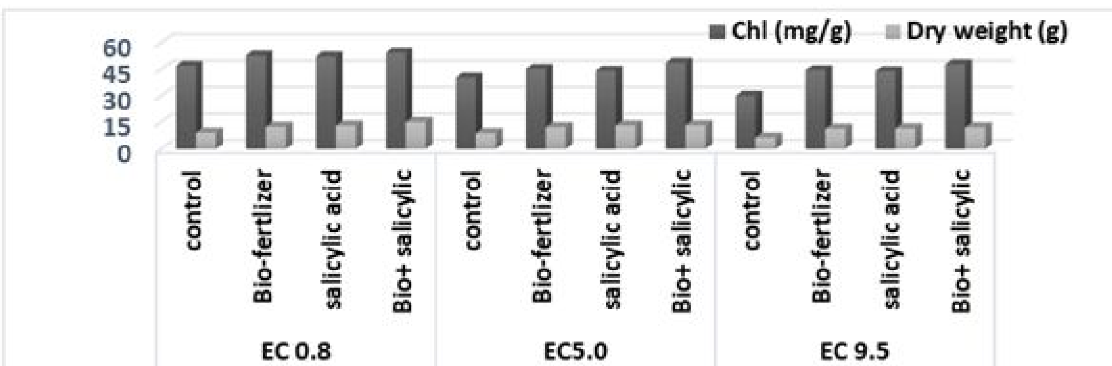

Fig. 1: The effect of the interaction between the levels of saline and both the bio-fertilizer and salicylic acid on dry weight and chlorophyll of fenugreek

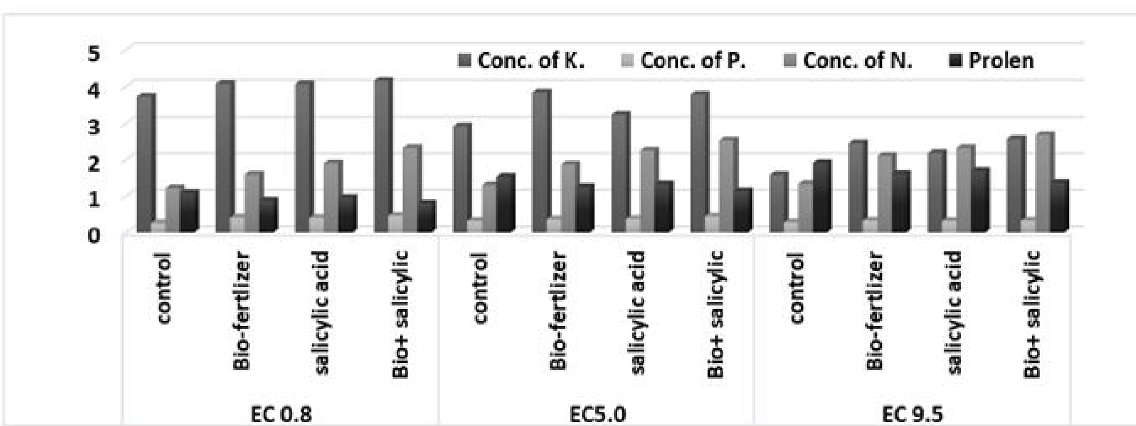

Fig. 2: The effect of the interaction between the levels of saline and both the bio-fertilizer and salicylic acid on concentration of *N, P, K* and proline of fenugreek

The concentration of nitrogen, phosphorus and potassium in the vegetative part was affected by the high salinity of the irrigation water used (Table 2). The nitrogen concentration increased significantly with increasing salinity levels, where the treatment EC 0.8 gave the lowest nitrogen content reached 1.76%, while the treatment EC 9.5 gave the highest ratio reached 2.11% of the nitrogen concentration. The increasing in nitrogen content which is associated with increasing the salinity of irrigation water probably due to nitrogen is a mobile nutrient, therefore, it is available on the root surface, regardless of the level of salinity and root size [Langdale and Thomas (1971)], in addition to the nitrogen concentration increasing probably due to the decrease of dry matter of the plant results of increasing the salinity (Table 1). The treatments of bio-fertilizer and spraying with salicylic acid had a significant effect on the nitrogen leaf content (Table 2). The treatment of bio-fertilizer plus spraying with salicylic acid was superior to that of other treatments under study, where it recorded the highest percentage of 2.51% compared to control treatment, which gave 1.29%. Due to the role of salicylic acid in increasing vital activities within the plant system, including the absorption efficiency, from roots or vegetative parts, the transfer of absorbed elements and their collection in leaves, stems and modern vegetative growth increased the proportion of nutrients in them. There was no significant interaction between the salinity levels and the treatments of both the bio-fertilizer and the salicylic acid spray in the nitrogen leaf content. The leaf content of phosphorus and potassium decreased significantly with increasing salinity levels (Table 2), where the highest content of phosphorus and potassium was obtained from treatment EC 0.8 reached to 0.32 and 4.01, respectively. While the treatment EC 9.5 gave the lowest content of phosphorus and potassium concentration in the vegetative group reached to 24% and 2.25%, respectively, the reduction was due to the interaction between the K^+ and Na^+ ions and the P^- and Cl^- ions resulting from the competitive effect between them on the absorption sites on the roots because they have a similar electrical charge, meaning that the Antagonism process occurs between the similar charges. Since sodium and chlorine ions are available in high concentrations around the root zone, potassium and phosphorus absorption will decrease [Ma *et al.* (2008)]. In addition to saline water, irrigation causes nutrient unbalance due to the competition of Na^+ and Cl^- with K^+, Ca^+, P^-, NO^{3-} [Hu and Schmidhalter (2005)]. The bio-fertilizer and salicylic acid treatments had a significant effect on the leaf content of phosphorus and potassium (Table 2), where the bio-fertilizer plus spraying with salicylic acid treatment is superior to other treatments under study and gave the highest percentage of phosphorus and potassium reached 0.34% and 3.50% , respectively, compared to the control treatment, which gave 0.23% and 2.74% for phosphorus and potassium, respectively. This reflects the role of the biological fertilizer in increasing the concentration of nutrients nitrogen, phosphorous and potassium in the plants. The appropriate concentration of salicylic acid increased the plant's absorbing efficiency of both phosphorous and potassium thus increasing the elements in the leaves [Fahad and Bano (2012)]. The interaction between salinity levels and treatments for each of the bio-fertilizer and spraying with salicylic acid showed a significant difference in the leaf content of potassium, where the interaction of the treatment EC 0.8 with bio-fertilizer plus spray with salicylic acid gave the highest rate of potassium leaf content reached 4.16% (Fig. 2), while the treatment EC 9.5 with control gave the lowest potassium leaf content reached 1.58%. There was no significant interaction between the salinity levels and the treatments of both the bio-fertilizer and spraying with salicylic acid in the phosphorous leaf content.

4. Conclusion

The obtained results show that the absence of both bio-fertilizer and salicylic acid led to decrease the dry weight, which confirms the importance of bio-fertilizer and salicylic acid in reducing the negative effect of the salinity. In terms of interaction, the results show that the salinity levels and pollination with bio-fertilizer and spraying with salicylic acid had a positive significant effect on the dry weight of the plant, where the combination of EC 0.8 with the bio-fertilizer and spray of salicylic acid superior to the other treatments giving the highest dry weight. Also the leaf content of chlorophyll has an inverse relationship with salinity levels, where the leaf chlorophyll content significantly decreased with increasing the salinity levels. On the other hand, in response to abiotic stresses the proline content increased with increasing salinity levels. Regardless of the level of salinity the plant nitrogen content increased, due to nitrogen mobility. In addition , the nitrogen leaf content was positively affected by bio-fertilizer and spraying with salicylic acid, where the nitrogen leaf content was superior to other treatments under study. Likewise, the leaf content of phosphorus and potassium decreased significantly with increasing salinity levels. While the appropriate concentration of salicylic acid increased the plants absorbing efficiency of both phosphorous and potassium thus increasing the elements in the leaves.

Conflict interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Ansary, M.H., H.A. Rahmani, M.R. Ardakani, F. Paknejad, D. Habibi and S. Mafakheri (2012). Effect of Pseudomonas fluorescent on proline and phytohormonal status of maize (*Zea mays* L.) under water deficit stress. *Annals of Biological Research*, **3(2)**, 1054-1062.

Bashan, Y., G. Holguin and R. Lifshitz (1993). *Isolation and characterizationof plant growth-promoting rhizobacteria.* In: Glick BR, ThompsonJ E (eds) Methods in plant molecular biology and biotechnology.CRC Press, Boca Raton.

Fahad, S. and A. Bano (2012). Effect of salicylic acid on physiological and biochemical characterization of maize grown in saline area. *Pak J Bot*, **44(4)**, 1433-1438.

Farahbakhsh, H. and M.S. Saiid (2011). Effects of foliar application of salicylic acid on vegetative growth of maize under saline conditions. *African Journal of Plant Science*, **5(10)**, 575-578.

Hu, Y. and U. Schmidhalter (2005). Drought and salinity: a comparison of their effects on mineral nutrition of plants. *Journal of Plant Nutrition and Soil Science*, **168(4)**, 541-549.

Itelima, J.U., W.J. Bang, I.A. Onyimba and E. Oj (2018). A review: biofertilizer; a key player in enhancing soil fertility and crop productivity. *J. Microbiol. Biotechnol. Rep*, **2**, 22-28.

Johari-Pireivatlou, M. (2010). Effect of soil water stress on yield and proline content of four wheat lines. *African Journal of Biotechnology*, **9(1)**, 36-40.

Langdale, G.W. and J.R.Thomas (1971). Soil Salinity Effects on Absorption of Nitrogen, Phosphorus, and Protein Synthesis by Coastal Bermudagrass. *Agronomy Journal*, **63(5)**, 708-711.

Lucy, M., E. Reed and B.R. Glick (2004). Applications of free living plant growth-promoting rhizobacteria. *Antonie van Leeuwenhoek*, **86(1)**, 1-25.

Ma, W., Z. Mao, Z. Yu, M.E.F. Van Mensvoort and P.M. Driessen (2008). Effects of saline water irrigation on soil salinity and yield of winter wheat-Maize in North China Plain. *Irrigation and Drainage Systems*, **22(1)**, 3-18.

Mehrafarin, A.A., S.H. Rezazadeh, B.H. Naghdi, G.H. Noormohammadi, E. Zand and Q.A. (2011). A review on biology, cultivation, biotechnology of fenugreek as a valuable medicinal plant and multipurpose. *Journal of Medicinal Plants*, 10(37), 6-24.

Naz, I., and A. Bano (2010). Biochemical, molecular characterization and growth promoting effects of phosphate solubilizing Pseudomonas sp. isolated from weeds grown in salt range of Pakistan. *Plant and Soil*, **334(1-2)**, 199-207.

Saberali, S.F. and M. Moradi (2019). Effect of salinity on germination and seedling growth of Trigonella foenum-graecum, Dracocephalum moldavica, Satureja hortensis and Anethum graveolens. *Journal of the Saudi Society of Agricultural Sciences*, **18(3)**, 316-323.

Zandi, P., S.K. Basu, L.B. Khatibani, M.O. Balogun, M.O. Aremu and M. Sharma (2015). Fenugreek (*Trigonella foenum-graecum* L.) seed: A review of physiological and biochemical properties and their genetic improvement. *Acta Physiologiae Plantarum*, **37(1)**, 1714.