Plant Archives Vol. 21, Supplement 1, 2021 pp. 1476-1480 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.232

EFFECT OF BIO-FERTILIZER AND NANOSCALE ELEMENTS ON ROOT NODULES FORMATION AND THEIR CHEMICAL CONTENT OF TWO *PHASEOLUS VULGARIS* L VARIETIES

Haifaa A.H. Al-Burki[1] and Saadon A.H.S. Al-Ajeel[2]
[1]Department of Hoticulture, college of Agriculture-Al-Qadisiya University, Diwaniya, Iraq
[2]Department of Biology, College of education for girls, University of Kufa, Njaf, Iraq
Corresponding E-mail: haifaa.abaas@qu.edu.iq

ABSTRACT

An experiment was conducted during the spring 2019 in one of experimental fields of Agriculture/University of Al-Qadisiyah, to study the response of two common beans *Phaseolus vulgaris* L varieties (Pole and Bush) to the bio-fertilization with *Rizobium phaseoli* sp with or without the presence of two mineral elements (iron and molybdenum) and their interactions, and the treatment effect on formation of root nodules and their chemical content. The experiment factors included two beans varieties (Pole and Bush), bio-fertilizer of *Rhizobium phaseoli* (inoculated and non-inoculated), nanoscale elements (iron 80mg.L^{-1}, molybdenum 10 mg.L^{-1}molybdenum) and all the possible interactions. The seeds were treated with bio-fertilizer before planting, while the Nano-scale elements treatments were applied one month of after planting. Measurements were taken at the end of the growing season, at harvest stage. The results showed that the use of bio-fertilizers and nanoscale elements improved the chemical content and the traits of the root nodules. The results showed that the plants treated with bio-fertilizer (inoculated) resulted in higher values and significantly differed from the untreated with bio-fertilizer (non-inoculated) by recording the highest averages for all the studied traits including number of root, nitrogenase enzyme activity, Amino acid tryptophan average, nitrates concentration ,iron concentration and molybdenum concentration . The addition Nano-elements also led to significant increase in the studied traits especially the combined treatment (iron 80 +molybdenum 10) mg.L^{-1} as the treatment with the mixture recorded the highest rates. In case of plant varieties, the variety Bush performed higher than the Pole in all the studied traits except for the molybdenum concentration in the nodule and the amount of nitrates in the nodule. Best results of were obtained from interaction of Nano-elements mixture and bio-fertilizer with Bush variety followed by the same interaction with pole.

***Keywords*:** Common bean, Biofertilization, Nano-elements, root nodules

Introduction

The *Phaseolus Vulgaris* is one of the plants belonging to the Leguminosae family, which are of three types in terms of growth, Bush type, Pole type and medium length type. Phaseolus is considered as the most common leguminous for human consumption for the amount of carbohydrates, proteins, fibers, vitamins, minerals, fats and calories it contains. Therefore, they are considered a good and healthy food and have many benefits for the body as they reduce the level of cholesterol, improve the functions of the digestive system and strengthen the immune system (2010, Mustafa).

The *P. vulgaris* was ranked the sixth in terms of global production compared to other vegetable crops, with an average production of about 270.8 thousand tons. The United States was ranked first in the world with an average quantity of exports estimated at 31 thousand tons (Metwaly, 2018). In Iraq, the cultivation of *P. Vulgaris* was not spread despite the many benefits and the increased consumption of it for reasons related to Iraqi soil, as well as for the plant itself, where it is sensitive to high and low ground humidity and soil PH, and it is also considered as one of the vegetable crops that are very sensitive to salinity, and the reason is that the bean seeds do not germinate in a temperature of less than 15 ° C or more than 35 ° C (2006, Al-Syed).

The world now has tended to reduce the use of chemical fertilizers for their negative effects on the environment, and tended to use microorganisms as bio-fertilizers, that some of which fix nitrogen and some work to dissolve the important elements necessary for plants (Hanapi *et al*., 2013). Among the microorganisms which work to fix atmospheric nitrogen is the root nodule bacteria (*Rhizobium* sp). These bacteria are also considered as the organisms that are used as a bio-fertilizer as they fix the atmospheric nitrogen and provide the plant with the nitrogen element, in return they get their needs of organic carbon from plants in a symbiosis method, as they are characterized by their specialization with a specific legume host in addition to the type which is specialized with *Phaseolus vulgaris* (Taha, 2007). There is a high importance of the biological fixation of nitrogen, which is considered as an essential nutrient for plants for its role in the formation of important organic components for the plant cell. Other recent studies have tended to use nanoscale elements by manufacturing nanomaterials with particles sizes range between (1-100) nanometers (Liu and Lal, 2015), which were aimed to be used in various fields, including the field of agriculture, especially for plant protection and improving plant seed growth and germination (Kraeova and Jampilek, 2015). Among the used nanoscale elements is the iron element, which contributes to the vital processes in plants by

being a stimulant for enzymes of the process of respiration and electron transfer, and it is also included in the synthesis of chloroplast as well as many other enzymes (Barker and Stratton, 2015). Molybdenum is considered as one of the essential micro-nutrients important for plant growth, as it is present in various complexes in the soil and is more soluble in alkali soils as it is easily accessible by plants in the form of (Mo O4-) (2013, Fageria). Molybdenum and iron play an important role in the nitrogen fixation process, as it contributes into the synthesis of the enzyme nitrogenase and the enzyme of nitrate reductase, which reduces the conversion of nitrates to nitrites and this process is necessary for protein synthesis in plants (Singh *et al*., 2010). The plant species belonging to the *Phaseolus vulgaris* differ in terms of growth, as short plants grow nearly two feet long and without the need for support.

Recent studies have tended to increase the horizontal and vertical expansion in the production of this crop by using modern and environmentally friendly technologies. Therefore, the study aimed to use the rhizobia bacteria specialized with *Phaseolus vulgaris* as a bio-fertilizer to be chemical fertilizers alternative, iron and molybdenum nano-elements and evaluating treatments effects on root growth (root nodules),chemical content of it.

Materials and Methods

A pot experiment was conducted during the spring period of 2018-2019 in the experimental fields at the College of Agriculture / University of Al-Qadisiyah to determine the response of two varieties of *Phaseeolus vulgaris* L. (bush and pole) to bio-fertilization with rhizobia and with two elements (Fe and Mo) individually and their interactions in the formation of root nodules and their chemical content of the *P. vulgaris*. Plant seeds were imported from Beirut Agricultural Company / Lebanon by one of the agricultural offices in Diwaniyah.

The experiment was carried out in pots with a capacity of 12 kg plastic pots. The pots were filled with mixed soil from the riverbank, sterilized by the method of (solar pasteurization), which is the method used to sterilize protected cultivation soils, and no chemical pesticide was used to sterilize the soil. Samples of the experiment soil were taken before planting, for the purpose of conducting some physical and chemical analysis of the soil (Naseem *et al*., 2019).

Table 1 : Physical and chemical characteristics of the orchard experiment soil

pH	E.C. $ds.m^{-1}$	Organic matter %	N $mg.kg^{-1}$	P $mg.kg^{-1}$	K $mm\ ch.L^{-1}$	Clay	Silt	Sand	Soil texture
						$g.Kg^{-1}$			
7.8	2.4	1.10	37.2	2.13	20.3	79.76	72.84	87.4	Mixed

The *Rhizobium phaseoli* bacterial inoculant was obtained from the Agricultural Research Service / Al Za'franiya / Ministry of Science and Technology with a biological dencity of $2.1x10^7$ CFU/g prepared according to Beck *et al.* (1993). The method of applying the bacterial inoculant on the bearer (peat moss) that was adopted before (Mehboob, 2010), was readopted with a modification at 200 ml per kilogram of peat moss. The seeds to be inoculated were placed in a clean plastic container and then mixed with the inoculant and gum Arabic was added at a concentration of 10% in order to increase the adhesion of bacteria to the seeds, the treated seeds were left for 30mn to ensure seeds contamination with bacteria, then were planted directly, while non-inoculation seeds reserved as control. The cultivation was done on February 19, 2019, when the seeds were planted in the pots at a rate of 3-5 seeds in one pot, and after germination, the plants were reduced to two plants, and planting operations were carried out from irrigation and removing weeds manually whenever needed. Nanoscale concentrations (80^{iron}, $10^{molybdenum}$ and ($80^{iron} + 10^{molybdenum}$)) $mg.L^{-1}$ were added by soil application method after one month of planting according to the fertilizer recommendations leaflet, taking into consideration the weight of the pot, leaving the comparison treatment without addition. The experiment was complete randomized design CRD with three factors which are the bio-fertilization (inoculated and non-inoculated),two beans varieties (Bush and Pole) and the Nano-elements ($0,80^{iron}$, $10^{molybdenum}$ and amixture of $80^{iron} + 10^{molybdenum}$) $mg.L^{-1}$ with three replication. at the end of the growing season (at harvest), data were collected for all plants and all replications of each treatment and measurements were recorded including number of root nodules counted for each plant according to (Beck *et al*., 1993). and chemical content of root nodules as ,Iron concentration (mcg. g^{-1}) Iron element was determined by the Atomic absorption spectrophotometer according to Temmnghoff and Houba, 2004. The molybdenum concentration (ppm) was also determined by an atomic absorption spectrophotometer according to 1980, A.O.A.C., Nitrates concentration was measured according to the 2000, A.O. A.C mentioned under Henni *et* al., 2016, Measuring the effectiveness of the Nitrogenase enzyme was measured using the method described by Frederick and Weaver, 1982, using a Gas chromatography device and Amino acid tryptophan rate was determined according to the method used by Scriver *et al*., 2001. Using a Korean made amino acid analyzer. Data were analysis using the SAS program and analysis of variance ANOVA was performed. Means were compard according to the Duncan's Multiple Range Teset at aprobability level of 1% (Al-Rawi and Khalaf Allah, 2000)

Results and Discussion

Results in Table (2) indicate the effect of bio-fertilization ,mineral nano-elements, varieties and their interactions on the noumber of root nodules ,and some chimerical content parameters of root nodules of common bean plants .The of bio-fertilization led to a significant increase in number of root nodules, Nitrogenase enzyme activity and Nitrates concentration compared to control (non-bio-fertilizer).The result also show that the use of Nano-fertilizer (iron and molybdenum) a significant increase in the studied traits compared to the control. Interaction of bio-fertilizer and the mineral elements mixture resulted in the highest recorded values especially in case of Bush variety which more noticeable than the Pole variety in most studied traits except Nitrates concentration.

Table 2 : Effect of bio-fertilizer, nanoscale elements and plant varieties on the No. of root nodules, nitrogenase enzyme activity and nitrate concentration

Bio-fertilization X Nano-scale elements		No. of root nodules. Plant^{-1}		Nitrogenase enzyme activity		Nitrate concentration (ppm)	
		Pole	Bush	Pole	Bush	Pole	Bush
Non-inoculated	**0**	3.33l	4.33 hi	18.56 j	26.73 i	12.17 ef	12.21 ef
	Fe	8.66 efg	11.66 de	42.50 gh	50.96 f	12.55 def	12.73 cdef
	Mo	7.33 gh	8.00 fg	19.36 j	45.13 g	12.94 cdef	12.80 cdef
	Fe+Mo	11.00 edf	12.33 d	39.93 h	74.80 d	13.28 bcdef	12.97 bcdef
inoculated	**0**	10.66 defg	13.33 d	27.70 i	24.26 i	13.47 bcde	12.01f
	Fe	21.66 abc	21.00 bc	80.36 c	90.80 b	14.37 b	13.93 bcd
	Mo	18.33 c	22.00 ab	60.03 e	78.23 c	14.11 bc	14.00 bc
	Fe+Mo	20.66 bc	24.66 a	97.36 a	99.03 a	16.70 a	16.79 a
Average of varieties effect		12.70 B	14.66 A	48.22 B	61.12 A	13.69 A	13.43 A

Values are averages of three replicates. Means followed by the same letter(s) within parameters (lower case), or between the two varieties (upper case) are not significantly different according to the Duncan's Multiple Range Test at a probability level of ($P \leq 0.01$)

Finding also shows the effect of study factors on Table (3). The effect of bio-fertilization was significants in increasing the iron concentration, molybdenum concentration and Amino acid tryptophan average compared to the treatment without fertilizer.the used of Nano-scales elements (iron and molybdenum) resulted in a significant increase in the value of the studied traits ,as the highest values was recorded when treating with the elements mixture (iron +molybdenum) over the control. It was observed among all the treatments that the highest values of Iron concentration and Amino acid tryptophan average were recorded in the interaction of fertilizer, Nano-elements mixture and Bush variety except molybdenum concentration compared to all the other treatments and interactions.

Table 3 : Effect of bio-fertilizer, Nano- elements and varieties on chemical contents in root nodules of common bean

Bio-fertilization X Nano-scale elements		Iron concentration		Molybdenum concentration		Amino acid Tryptophan average	
		Pole	Bush	Pole	Bush	Pole	Bush
Non-inoculated	**0**	9.72 ef	9.59 f	1.14 de	0.96 e	20.08 j	24.16 b
	Fe	10.52 def	11.54 cd	1.45 cde	1.62 cde	20.76 m	28.95 c
	Mo	9.75 ef	9.65 ef	1.67 cd	1.77 cd	22.76 l	29.00 c
	Fe+Mo	10.71 de	12.00 bc	1.94 bc	1.73 cd	26.84 k	35.39 a
inoculated	**0**	10.38 ef	10.48 ef	1.09 de	1.21 de	22.45 i	49.90 f
	Fe	12.24 abc	12.61 ab	2.03 bc	2.01 bc	26.02 k	52.28 e
	Mo	10.38 ef	10.74 de	2.95 a	2.58 ab	27.76 h	52.84 e
	Fe+Mo	13.04 a	13.14 a	2.94 a	2.90 a	31.05 g	61.47 d
The average of varites effect		10.84 B	11.21 A	1.90 A	1.85 A	24.71 B	41.65 A

Values are average of three replicates. Means followed by the same letter(s) within parameters (lower case), or between the two varieties (upper case) are not significantly different according to the Duncan's Multiple Range Test at a probability level of ($P \leq 0.01$)

The use of bio-fertilizer or the bacterial inoculation had a significant effect on the number of the root nodules as well as the dry weight of the root nodule .The reason may be due to the increase in the number of Rhizobia bacteria in the soil and their ability to infect the host plant and penetrate the roots and therefore forming the root nodules and then increasing the dry weight of the nodule (Saad and Jabbar, 2014) and this was confirmed by (Hussain *et al*., 2014) on the Mung bean plant. The reason for the increase of nutrients iron and molybdenum in the root nodules is attributed to the fact that the bio-fertilizers that contain rizobia bacteria secrete growth regulators that improve the growth of roots and root hair, and when the growth of roots increases, the absorption of nutrients increases. The reason for the increase of iron may be attributed to the ability of bio-fertilizers to secrete siderophores, which work to chelate iron and increase its readiness for the plant. It may be due to the fact that nitrogen-fixing plants absorb higher amounts of cations compared to anions, which leads to a lower pH of the rhizosphere, thus increasing the readiness of iron and absorbing a higher amount of it by the plant, the increase in the molybdenum is attributed to the role of bacterial inoculation by increasing the density, depth and spread of the roots, which leads to an increase in the absorption of the element by the plant (Taiz and Zeiger, 2010). The increase may come by the specialized streptococcus bacteria forming the root nodules, and these structures greatly help in the uptake or absorption of molybdenum from the soil indirectly due to the need of the Bacteroid within the nodule for this element for the effectiveness of the nitrogenase enzyme, and therefore the more bacteria used as an inoculation of a high efficiency in nitrogen fixation, the more they tend to take larger quantities of molybdenum to meet their need (Biswas *et al*., 2009) and this agrees with the finding of (Makoi *et al*., 2013) when inoculating Phaseolus with rhizobia.

The addition of Nanoscales (iron and molybdenum) has a significant effect on increasing the number of root nodules, this may be attributed to the effect of iron on forming the lateral roots and its effect on the first steps of the infection process with streptococcal bacteria, and this is reflected in the weights of the root nodule. The importance of iron lies in activating the enzyme nitrogenase, as iron participates with thirty atoms in the synthesis of each of the enzyme molecules, as well as its importance in the synthesis of legume hemoglobin, ferredoxin, cytochrome and other enzymes involved in the process of nitrogen reduction and fixation within the bacterial nodule, and this is reflected in the increase of nitrogenous components in the nodule such as nitrates, and amino acids and the effect of molybdenum in activating the nitrogen enzyme, may be attributed to the role of molybdenum in forming the root nodules and through the conversion of the enzyme from the reduced form to the oxidized form and vice versa as a result of its loss and acquisition of.

The increase in the nutrient content of the plants by the effect of iron and molybdenum is due to the use of nanoscale elements as fertilizers that provide a larger surface area for different metabolism reactions in the plant, which increases the rate of photosynthesis and thus encourages the demand for mineral elements and results in an increase in dry matter and an increase in plant yield. It also preserves the plant from various biological and abiotic stresses (Singh *et al*., 2017). Kim *et al*., 2015 explained that adding or using nanoscale iron leads to an increase in the efficiency of the ATPase enzyme in the plasma membrane of the guard cells, which in turn leads to an increase in stomatal pores five times the normal state, which enhances the introduction of CO_2 and the increase the efficiency of the food-synthesis process, which leads to an increased demand by the plant for the nutrients that the plant takes up from the soil.

The differences between the pole type and bush type and the outperformance of the bush type in most of the studied traits may be attributed to the variation in their genotype. The increase in the studied traits as a result of the interaction of the experiment factors may be attributed to the existence of a synergistic relationship between the factors, i.e. between the bio-fertilizer and nanoscale elements.

References

A.O.AC. (1980) Official Methods of Analysis. 13th ed. Association of official analysis chemist. Washington D.C., USA.

A.O.A.C. (2000) Official method of analysis of d. Associatich of official Analytical Chemists to Gaithersburg, M.D. USA.

AL-Syed, sof. (2006) Vegetable production Technology Inside the greenhouse and Tunnels in the desert land. Egyptian Library, Alexandria, Egypt . pp 478

AL-Rawi, K.M. and Khalaf Allah, A.M. (2000). Design and analysis of agricultural experiments. faculty of Agriculture. university of Mosul. Iraq,

Barker, A.V. and Stratton M. L. (2015). Iron chapter II in Barker, A. v. and pilbeam, O.j. (eds) Handbook of plant Nutrition - Second Edition CRC press Taylor and Francis Group - London. New York, Pp: 399-426.

Beck, D.P.; Materon, L.A. and Fandi, A. (1993). Portical Rhizobium - legume Technology Manual. No. 19. I CARDA Aleppo, Syria.

Biswas, P.K.; Bhow mick, M.K. and Bhattacharya, A. (2009). Effect of molybdenum and seed inoculation on nodulation growth and yield in *Vigna munga* L. jo of crop and weed 5(1):141-144

Cresser, M.S. and Parsons, J.W. (1979). Sulphuric perchloric acid digestion of plant material for the determination of Nitrogen, phosphours, potassium, Calcium and magnesium. Analytric Chemical Acta. lod: 43-436.

Fageria, N.K. (2013). Mineral nutrition of Rice - florida CRC press.

Hanapi, S.; Hassan, M.A.; Sheikk, I.U.; Ali, S.M.; Mohamad, R. and Sarmidi, R.A. (2013) Agriculture wastes conversion for bio fertilizer production using beneficial microorganisms for sustainable agriculture application. Malay. Je Microbiol. 9(1): 60-67.

Henni, C.; Silalahig, J.; Lux putra, E.D. and Satria, D. (2016). Analysis of Nitrite and vitrate in vegetables in Medan city. Der Pharma chemica, 8(24): 52-57.

Hussain, A.; Amjed, A.; Tasneem, K.; Ashfaq, A.; Zubair, A. and Muhammad, A. (2014) Growth nodulation and yield components of mung bean as affected by phosphorus in combination with rhizobium inoculation. Afri. J. Agri. Res., 9(30): 2319- 2323.

Kracova, K. and Jampilek, J. (2015) Application of nanotechnology in agriculture and food industry, its prospects and risks. Ecol. chem. Eng. 22(3): 321-361.

Temminghoff, E.J.M. and Houba, V.J.A. (2004). Plant Analysis procedures. Second Edition, Kluwer Academic publishers. Boston. London, Pp 90-179.

Kim, J.; Oh, Y.; Yoon, H.; Hwang, I. and Chang Y. (2015) Iron Nanoparticle. Induced Activation of plasma Membrane H^+-ATpase promotes stomatal opening in Arabidopsis thaliana. Environ. Sci. Technala, 49 (2): 1113-1119.

Liu, R. and Lal, R. (2015). Potentials of engineered nanoparticles as fertilizers for increasing agronomic production. A review. Science of the Total Environment 514:131-139.

Makoi, J.H.J.R.; Bambara, S. and Ndakidemi, P.A. (2013). Rhizobium inoculation and the supply of molybdenum and lime affect the uptake of macro elements in common bean- plants - Australian j. of crop Sciences 7(6): 784-793.

Metwally, S. (2018) Agriculture. Egypt ranks tenth in the world in the export of https: /www.agri 2 day.com.

Mehaboob, I. (2010). Plant growth promoting activities of Rhizobium with non-legumes. Athesis submitted in soil science, institute of soil and environmental Science, university of Agructure, Faisalabad, pakistan.

Mustafa, M-A.A-F. (2010). Vegetable (Food-prevention-Medication). Knowledge Library grove , Egypt - Pp 552.

Naseem, M.J.; Hussein, M.A.A.M. and Mohammed, W.H. (2019). Basics of plant Nutrition. Alexandria University. Faculty of Agriculture. saba Pasha.

Singh, M.D.; Chirag, G.; Prakash, P.; Mohan, M.H.; Prakash, G. and Vishwajith, K. (2017). Nano-fertilizers is a new way to increase Nutrients use Efficiency in crop production. International journal of Agriculture sciences, 9(7): 381-3883.

Saod, T. and S-jabbar (2014). The effect of inoculation with rizobia bacteria (Rhizobium leguminosarum and levels of phosphate rockin the growth and yield of the mung

bean plants. AL- Muthanna journal of Agricultural sciences, 201: 123-1280.

Singh, A.L.; Jat, R.S.; Chaudhari, V., Bariya, H. and Sharma, S.T. (2010). Toxicities and tolerance of mineral elements boron, cobalt, molybdenum and nick and nickel in crop plants. Plant stress, 4: 31-56.

Taiz, L. and Zeiger, E. (2010). Plant physiology 5th ed. Sinauer Associates - Inc. publishers Sunderland, Massachusetts - AHS - U.S.A .PP: 782.

Taha, S.M.R. (2007). Biofertilizer and organic agriculture healthy food and atunels in the desert land. Egyptian Library, Alexandria, Egypt. Pp 478.

Weaver, R.W. and Frederick, L.R. (1982). Rhizobiumin methods of soil analysis Part 2, chemical and microbiological properaties 2nd ed page, A.L, Miller, R.H. and Keeny D.R.(eds). Soil. Sci. of Am. madison WI-USA.