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EFFECT OF BIO-FERTILIZER AND NANO-ELEMENTS ON GROWTH AND YIELD OF TWO *PHASEOLUS VULGARIS* L. VARIETIES

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ABSTRACT

An experiment was conducted during the spring 2019 in one of experimental fields belong to the Faculty of Agriculture/University of Al-Qadisiyah, to study the response of two common beans *Phaseolus vulgaris* varieties (Pole and Bush) to bio-fertilization with *Rizobium phaseoli* sp with or without the presence of two mineral elements (iron and molybdenum) and their interactions, and the treatments effect on plant vegetative growth, chemical content and yield. The experiment factors included the two beans varieties (Pole and Bush), bio-fertilizer of *Rizobium Phaseoli* (inoculated and non-inoculated), Nano-scale elements of iron 80 mg.L^{-1}, molybdenum 10 mg.L^{-1}) and all the possible interactions. The seeds were treated with bio-fertilizer before planting while the nanoscale elements treatments were applied one month of after planting. Measurements were taken at the end of the growing season at harvest. The results showed that the use of bio-fertilizers and Nano-scale elements improved the traits of growth, yield and chemical content of the plant. 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 plant height, number of branches/plant, leaf area, leaf content of nitrogen and number of pods.plant^{-1}. The addition of Nano-scale mineral elements also led to a significant increase in the studied traits especially the combined treatment (iron 80 mg.L^{-1}+ molybdenum 10 mg.L^{-1}). as the treatment with the mixture of elements 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 plant height. Best results of the growth and yield were obtained from the interaction of Nano-scale elements mixture and bio-fertilizer with Bush variety followed by the same interaction with Pole.

***Keywords*:** Common bean, *Rizobium phaseoli*, Biofertilization, Nano-minerals

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 (Metwally, 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. There is a high importance of the biological fixation of nitrogen, which is considered as an essential nutrient for plants (Taha, 2007). 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 they contribute in the synthesis of enzymes nitrogenase and nitrate reductase, which reduces the conversion of nitrates to nitrites and this process is necessary for protein synthesis in plants (Singh *et al.*, 2010). 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 fertilizer alternative, iron and molybdenum nan-elements and evaluating treatments effects on vegetative growth, chemical content and yield of two beans varieties.

Materials and Methods

The 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 types of *Phaseeolus vulgaris* L. (bush and pole) to bio-fertilization with rhizobia and with the two elements (Fe and Mo) individually and their interactions in the growth and yield of the *Phaseeolus vulgaris*. Plant seeds were imported from Beirut Agricultural Company/ Lebanon by one of the agricultural offices in Diwaniyah. The experiment was carried out in 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 inoculum was obtained from the Agricultural Research Service / Al Za'franiya / Ministry of Science and Technology with a biological density of 2.1×10^7 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 Arabic gum 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 served 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. Nano-scale concentrations (80^{iron}, $10^{\text{molybdenum}}$ and ($80^{\text{iron}} + 10^{\text{molybdenum}}$) $mg\text{-}L^{-1}$ 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}, $10^{\text{molybdenum}}$ and a mixture of 80^{iron} and $10^{\text{molybdenum}}$) $mg.L^{-1}$ with three replications.

At the end of the growing season (at harvest), data were collected for all plants and all replications of each treatment and measurements of vegetative growth parameters were recorded including plant height (cm), number of branches ($branch.plant^{-1}$), plant leaf area ($dm^2.plant^{-1}$) using a planometer according to Lu *et al*. (2004). For the chemical contents, leaf samples were digested (Cresser and Parsons, 1979) to determine the percentage of nitrogen according to Bremner and Breitenbeck (1983) using Micro-Kjeldhal device. Plant yield characteristics were also recorded for number of pods/plant, number of seeds per pod. Data were analyzed using the SAS program and analysis of variance ANOVA was performed. Means were compared according to the Duncan's Multiple Range Tests at a probability level of 1% and (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 vegetative growth parameters of common bean plants. The use of bio-fertilization led to a significant increase in plant height, number of plant branch and leaf area compared to control (non-bio-fertilizer). The result also show that the use of Nano-fertilizer (iron and molybdenum) a significant increase in the vegetative growth 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.

Findings also shows the effect of study factors on leaf content of nitrogen, average number of pods per plant and number of seeds/pod (Table 3). The effect of bio-fertilization was significant in increasing the percentage of protein and yield compared to the treatment without fertilizer. The use of Nano-scale elements (iron and molybdenum) resulted in a significant increase in the values 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 leaf content of nitrogen, average number of pods per plant and number of seeds/pod were recorded in the interaction of fertilizer, Nano-elements mixture and Bush variety followed by the same interaction with Pole variety compared to all the other treatments and interactions.

Table 2 : Effect of bio-fertilizer and mineral Nano-elements on vegetative growth of two common bean *Phaseolus vulgaris* varieties

Bio-fertilization X Nano-elements mg.L^{-1}		Plant height		No of branches. plant^{-1}		Leaves area of plant	
		Pole	Bush	Pole	Bush	Pole	Bush
Non-inoculated	0	98.3 d	46.6fg	3.33c	3.33c	38.00f	37.33f
	Fe	108.0c	48.6fg	3.33c	4.33abc	39.66ef	42.33def
	Mo	101.3d	52.3fg	3.66bc	3.66bc	40.33ef	40.33ef
	Fe+Mo	128.3a	53.0f	4.33abc	5.00ab	43.00de	53.00b
inoculated	0	110.0c	45.6g	3.33c	3.33c	39.66ef	39.66ef
	Fe	114.0c	49.6fg	4.33abc	5.33a	40.00ef	47.00cd
	Mo	120.3b	48.3fg	3.66bc	4.33abc	50.33bc	52.66b
	Fe+Mo	129.0a	59.6e	4.66abc	5.33a	48.00bc	67.33a
Variety effect		113.6A	50.5B	3.83B	4.33A	42.37B	47.45A

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 ($P \leq 0.01$)

Table 3 : Effect of bio-fertilizer and mineral Nano-elements on leaf content of nitrogen and yield parameters of two common bean *Phaseolus vulgaris* varieties

Bio-fertilization X Nano-elements mg.L^{-1}		Percent of nitrogen in the leaves		No. seeds of pods		No. pode of plant	
		Pole	Bush	Pole	Bush	Pole	Bush
Non-inoculated	0	0.42g	0.96f	4.33e	4.33e	18.66f	19.33f
	Fe	1.51de	1.62cde	7.33bc	7.00bcd	21.66def	25.00cde
	Mo	1.51de	1.46de	7.66abc	8.00ab	20.33ef	21.33def
	Fe+Mo	1.53de	1.76cd	8.00ab	8.25a	26.00cd	31.00b
inoculated	0	1.11ef	1.79cd	6.33cd	5.66ed	23.66def	29.00bc
	Fe	1.68cd	1.48de	8.33ab	8.00ab	37.66a	40.33a
	Mo	2.06c	2.13c	7.00bcd	7.66abc	37.33a	39.00a
	Fe+Mo	2.61b	3.59a	8.33ab	8.00ab	41.00a	41.66a
Variety effect		1.55B	1.85A	7.16A	7.11A	28.24B	30.83A

Values are average of three replicates. Means followed by the same letter(s) within parameter (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 improvement of the vegetative growth characteristics of common bean as a result of the use of bio-fertilizer may be due to the fact that the bio-fertilizer containing the *Rhizobia* bacteria produces phyto-hormone (growth regulators) such as Indole -3-acetic acide (IAA) and Cytokinin. These growth regulators stimulate the growth of the entire plant, as the indole acetic acid that stimulate plant growth by improving the growth of roots and leaves, and also participates in cell division and differentiation and formation of plant vascular system (Naseem *et al.*, 2019). Also due to the positive role of nitrogen through the nitrogen fixation process carried out by the bacteria, thus increasing the activity of meristematic tissues and cell division, in addition to the importance of nitrogen in building amino acids such as Tryptophan, which is an intermediate compound in the formation of (IAA) which stimulates cell elongation (Karumeyi, 2009). This was significantly reflected in the height of the plant as well as the leaf area of the plant, which are considered the criteria that determine the efficiency of the vegetative growth of the plant, as its increase works to absorb the largest amount of light rays which increases the carbon compounds produced in the leaves and the efficiency of the carbon representation process (Taiz and Zeiger, 2006; Lordanis *et al.*, 2013; Charjee *et al.*, 2012)

The increase of plant height, number of branches and leaf area as a result of adding the elements, is due to the fact that Nano-scale elements have unique characteristics for their high surface area and small particles which lead to an increase in their absorption. And the interaction of Nano-fertilizers affect the solubility and diffusion of nutrients, therefore the availability of these nutrients may result in an increase in the photosynthesis process. Iron contributes in many biological processes that occur in the plant, including stimulating the production of amino acids and enzymes activities involved in cell division and increase the activity of antioxidant enzymes and thus an increase in the growth rate of plant (Barker and Stratton, 2010). Nano-molybdenum has also roles in improving plant growth through its contribution to the formation and increase of chlorophyll and in many other biochemical processes, and this will lead to an increase in the efficiency of the photosynthesis process and an increase in the amount of carbohydrate manufactured materials in plants (Naseem *et al.*, 2009). This is reflected in the increase in the vegetative growth indicators and also, may be due to the role of molybdenum in forming root nodules, and this leads to increasing the biological nitrogen fixation process then leads to optimal plant growth (Karumeyi 2009). This will increase cell size and division as well as raising the efficiency of the activity of the photosynthesis process which is positively reflected on vegetative growth in general (Karimi *et al.*, 2014 and Tanou *et al.*, 2017)

With regard to the increase of the plant yield as a result of using bio-fertilizer, it is due to the ability of bacteria to fix nitrogen and the ability of the plant to benefit from nitrogen as well as increase the absorption capacity of the roots to the nutrients, as a good preparation of nitrogen leads to an increase in absorbing the nutrients required for the plant, and therefore reflected on the plant growth and the yield. Bio-fertilizers is important in fixation of atmospheric nitrogen and thus increasing nitrogen availability to the bacteria and eventually to the plant (Charjee *et al.*, 2012). Furthermore, plants especially leguminous ones, need molybdenum to participate in many biological processes, therefore adding molybdenum will help for a better growth of the plant, especially for plants inoculated with bacteria, and thus reflected on the increase of plant yield. The iron is used due to its chemical properties, for being more stable and its high ability to supply the plant with the iron element which is necessary for plant growth as well as its role in some qualitative and quantitative characteristics, including the leaves content of nitrogen and so plant yield (Naseem *et al.*, 2019; Karimi *et al.*, 2014). Considering the differences between the Pole and Bush varieties confirmed by the noticeable higher performance of Bush over the Pole common bean which is mostly due to genotype variation (Karumeyi, 2009).

References

Al-Rawi, K.M. and Abdul Aziz Muhammad Khalaf Allah (2000). Design and analysis of agricultural experiments. faculty of Agriculture. University of Al Mosul. Ministry of Higher Education and Scientific Research. Iraq.

Al-Syed, S.F. (2006). Vegetable production Technology inside the green houses and tunneles in the desert land. Egyption Library, Egypt, 478.

Barker, A.V. and Stratton, M.Z. (2015). Iron chapter II In: Barker, A.V. and Pilbeam D.J. (eds) Handbook of plant Nutrition –second edition CR press Taylor and Francis group .London New York, 399-426.

Beck, D.P.; Materon, L.A. and Fandi, A. (1993). Partical Rhizobium – legume technology Manual No.19. ICARDA Aleppo, Syria.

Bremner, J.M. and Breiten Beck, G.A. (1983) Asimple method for determination of ammonium in semimicro-kjeldahl analysis of soil and plant material using ablock digester .communication in soil science and plant analysis 14(10): 905-913.

Charjee, R.; Bhatta, B.; Jourand, P.; Chaintreuil, C.; Dreyfus, B.; Singh, A. and Mukhopadhyay, S.N. (2012). Indole acetic acid and ACC deminase .production *Rhizobium leguminosarum* bv.trifoli SN 10 promote rice growth and in the process undergo colonization and chemotaxis. Biology and fertility of soil. 48(2): 173-182.

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. Analytic chemical Acta. 109 : 43- 436.

Fageria, N.K. (2013). Mineral Nutrition of Rice – florida :CRC press.

Hanapi, S.H.; Awad, M.; Undinsheikh, S.I.; Sitiltajar, A.; Sarip, M.; Sarmidi, M.R. and Aziz, R. (2013). Agriculture Wastes conversion for Biofertilizer production using beneficial microorganisms for sustainable agriculture application. Malay. J. Microbiol., 9(1): 60-67.

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

Kandil, H.; Nadia, G. and Abdelhamid, M.T. (2013). Effect of different rates of phosphorus and molybdenum application on two varities common bean of *Phaseolus vulgaries* L journal of agriculture and food Technology, 3(3): 8-16.

Karimi, Z.; Pourakbar, L. and Feizi, H. (2014). Comparison effect of nano iron chelate and iron chelate on growth parameters and Anti oxidant enzyme activity of mung bean. Adv. Env. Biol. 8(13): 916-930.

Karumeyi, B.S. (2009). Effect of rhizobium inoculation ,molybdenum and Lime on the growth and N2 fixation in *Phaseolus vulgaris* L MSC. Thesis in Horticulture in the faculty of applied sciences,Cape peninsula university of technology Cape town Pp95.

Liu, H.Y.; Liu, C.T.; Wei, M.L. and Chan, L.F. (2004) Comparison of different Models for Non-destructive leaf Areas estimation in Taro. Agron. J., 448-453.

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.

Lordanis, C.; Anastassive, K.V. and Wilhelm, H. (2013). Commission on genetic –resources for food and agriculture, Background study paper. FAO.64:2-23.

Mehaboob, I. (2010). Plant growth promoting activities of Rhizobium with non-legumes. A thesis submitted in soil science, institute of soil and environmental science, university of agriculture, Fais alabad, Pakistan.

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

Mustafa, M.A.A.F. (2010). Vegetable (food–prevention–Medication) Knowledge Library grove, Egypt. 552.

Naseem, M.J.; Hussein, M.A.A-M. and Mohammed Ali, W.H. (2019). Basics of Plant Nutrition. Alexandria University - Faculty of Agriculture - Saba Pasha.

Single, A.L.; Jat, R.S.; Chaudhari, V.; Bariya, H. and Sharma, S.T. (2010). Toxicities and Tolerance of mineral elements boron ,Cobalt ,Molybdenum and nickel in crop plants. Plant stress, 4: 31-56.

Taha, S.M.R. (2007). Biofertilizers and organic agriculture (Healthy food and a Clean Environment).Dar Arab Thought, Cairo. Egypt. Pp200.

Taiz, L. and Zeiger, E. (2006). Plant physiology. 4th ed. Sinour Associates. Inc. Publisher sunder land, Massachus. AHS. USA. Pp764.

Tanou, G.; Ziogas,V. and Molassiotis, A. (2017). Foliar Nutrition ,Biostimulants and Prime. LikeDynmics in fruit tree physiology. New Insights on an old topic frontiers in plant science, 8(75): 1-9.