



Article

Efficiency of Biofertilization and Spraying Nano-Iron on Some Mineral Content and Carbohydrates in Leaves of Pomegranate Transplants CVS. Halabja

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Abstract: The look at turned into finished at Lath-house of the College of Agricultural Technology / Northern Technical University. The experiment used pomegranate seedlings of the Halabja cultivar. The research was finished using the R.C.B.D. Three replications had been applied. There have been 4 seedlings in every replication. The experiment consisted of factors. The first element become the addition of *Bacillus subtilis* to the soil of the transplants. A remedy that lacked bacterial immunogen changed into additionally a part of the control. Every seedling turned into handled with a 10 ml of the bacterial answer once throughout the season. This manner became completed in April according to a predetermined treatment plan. The second factor was the utility of foliar sprays with 3 special doses of nano-iron (0, 20, 40 mg.L⁻¹), which had been sprayed two times for the duration of the season - the primary in April and as soon as a month later. On the other hand, the remedy utilized consisted of spraying with distilled water. The findings established that seedling which includes *Bacillus subtilis* exhibited higher ranges of nitrogen, phosphorus, potassium, and carbohydrate content in the leaves as compared to seedling without bacterial inoculation. The research indicated that the application of nano-iron foliar sprays had a massive effect on all of the attributes studied. Transplants handled with a awareness of 40 mg L⁻¹ had the highest reported values. All the attributes studied were strongly impacted through the interplay among *B. Subtilis* and foliar spray of nano-iron. The best values of all studied parameters had been executed with *B. Subtilis* and management of 40 mg L⁻¹ nano-iron.

Key words: *Punica granatum* L., Biofertilizer, nano fertilizer.

1. Introduction

Pomegranate *Punica granatum* L., belongs to the Punicaceae circle of relatives, is considered one of the economically essential deciduous fruit timber in tropical and subtropical areas (Chandra *et al.*, 2010). The range of pomegranate trees planted in Iraq, in line with figures from the Central Bureau of figures, reached more or less 6,495,705 timber with a production rate of 241,671. Tons of pomegranate fruits at a relative production charge of 37.20 kg in line with tree (Central Agency for Statistics and Information Technology, 2020). The nutritional significance of pomegranates comes from the fact that it carries distinctive dietary additives for one ripe fruit, including carbohydrates 14.5-16.4 mg.Kg⁻¹, nutrition C 4-14 mg.Kg⁻¹, protein 1.6-5 g.Kg⁻¹, fat 0.3%, ash 0.7%, and the share of water in it's far % 82.3-87 (Opera *et al.*, 2009). The monetary fee of pomegranates is represented within the lengthy length of the fruits' presence in the markets because they could resist long-distance transportation and the possibility of storing them for a long period whilst retaining their nutritional price (Al-Dulaimi, 1999). Pomegranates have fitness significance in treating a few diseases, including gout. It additionally strengthens the heart, and drugs are extracted from pomegranate peels to deal with a few kidney and digestive system diseases. It is an inhibitor of oxidation and atherosclerosis and is used to deal with leprosy, dysentery, and others (Moreno *et al.*, 2005).

Biofertilizers are substances that include microorganisms which can be useful to the soil and can deliver vegetation with part of the dietary necessities of plants. It also can be considered an additive derived from a biological source called microbial inoculants, on which many flora rely to supply flowers their nutritional requirements. They remodel materials (of their chemical activity) which can be already unused into materials that may be absorbed by way of flowers and that sell and growth the increase of plants, which includes: Plant hormones, consisting of auxin, are answerable for the fixation of atmospheric nitrogen via their symbiotic or non-symbiotic dwelling, and protective the plant host from some pathogens, this reduces the want for chemical fertilizers through approximately 25%, which in flip reduces the prices of agricultural processing and production (Al-Haddad, 2003 and Al-Badawi, 2008). In a preceding investigation, Al-Zuhairi *et al.* (2021) studied the consequences of treatment with a suspension of *Bacillus subtilis* bacteria that become 10 ml.Seedling⁻¹ of Citrus limon L., the concentrations of nitrogen, phosphorus and potassium in the leaves have been extended extensively, and the concentrations of these equal vitamins within the soil had been additionally extended. Nanofertilizers have bizarre residences due to their small length and big floor area, this increases the surface region available for absorption, which improves the procedure of photosynthesis and hence an boom in plant boom (Singh *et al.*, 2016). Iron is one of the most essential micronutrients for flora. When applied to the shoots, it enables their absorption and rapid movement in the plant, thereby stopping the accumulation of the substance on the soil's surface and as an alternative accomplishing the roots. It triggers enzymes associated with iron's hobby, and is one of the components that participate in the essential approaches in flowers. It's worried in the synthesis of numerous enzymes, along with catalase and peroxidase, in addition to the development of chlorophyll, the garage of redoxes, and the formation of several proteins, together with the synthesis of cytochromes and ferredoxins. Nano-chelated iron particles have a effectiveness this is 10 to 1,000 times extra than traditional fertilizers due to the fact they provide a wider variety of responses (Filipponi and Sutherland, 2013 and Al-Khalifawi, 2017). In a research with the aid of Alalaf *et al.* (2020) studied the outcomes of foliar software of 4 extraordinary concentrations of nano-iron (15, 30, 45 and 60 mg. L⁻¹) in addition to a contrast to the outcomes of a nine% Fe attention inside the leaves of seedlings Pomelo grafted on orange roots. The results demonstrated that the attention of 60mgFe.L⁻¹ had a tremendous impact at the leaves' content of nitrogen, phosphorus, potassium and iron.

2. Materials and Methods

The look at become achieved 1 March to 1 September, 2023 at the Lath House, Agricultural Technical College/Northern Technical University/Ministry of Higher Education and Scientific Research/Iraq, Mosul, Iraq, to look at the results of *Bacillus subtilis* biofertilization and spraying of numerous concentrations of nano-iron awareness in Halabja range pomegranate seedlings, that have

been planted in 5 kilograms of polyethylene plastic in a bag that became soil-based totally completely, as well as sure physical and chemical houses had been measured (Table 1).

Table (1). Some chemical and bodily houses of the transplant increase media before the commencement of the test

Adjective	Quantity	Measuring Unit
sand	303	gm . kg ⁻¹
silt	415	gm . kg ⁻¹
clay	282	gm . kg ⁻¹
Histology	Mixture
EC	0.17	dS . m ⁻¹
PH	7.05	
Organic matter	27	gm . kg ⁻¹
N	6.61	gm . kg ⁻¹
P	2.46	gm . kg ⁻¹
K	13.59	gm . kg ⁻¹
Fe	0.971	gm . kg ⁻¹

* The measurements have been carried out at the Central Laboratory of the Ministry of Agriculture and Forestry, University of Mosul, Iraq.

The experiment consisted of (R.C.B.D.) factors, three replications, and 4 transplants, depending on the experimental area. The first element included *Bacillus subtilis* vaccination and unvaccinated controls. The bacterial solution was carried out once consistent with season, with 10 ml. Transplant⁻¹ brought in April, similar to the April. The 2d factor consisted of programs of three concentrations of nano-iron (0, 20, 40 mg/L) at the leaves in the course of the season beginning in April. A second application become performed one month later. The manage answer in all spray answers changed into covered with water and a surfactant (Tween eighty). The pomegranate variety locally called Halabja changed into the most effective pomegranate range used in the test. The test consisted of 3 replications and four transplants consistent with unit ($2 \times 3 \times 3 \times 4 = 72$). The test was completed on 1 September 2023.

2.1. Concentration of nutrients in leaves

Samples of leaves from seedlings had been gathered for each experimental institution. Adult leaves (12 leaves in line with experimental unit) were amassed from the middle of the growing branch, washed, and dried in an electric oven at 70 °C to a regular weight (48 hours), they then had been floor the use of a medium electric grinder. After grinding, 200 mg of each experimental unit become ingested in 5 ml of focused sulfuric acid in a Pyrex vessel for 24 hours according to the technique advocated via (Cresser and Parsons, 1979), then 1 ml of focused perchloric acid became integrated. The field for digestion was heated. The vapor turned into authorized to upward push until a clean, obvious solution changed into formed, and the solution was then reduced in temperature and made up to 50 ml with distilled water, the vitamins had been then estimated according to the identified technique as follows:

Nitrogen (%): It changed into predicted the usage of a Microkjeldahl tool and consistent with the method defined with the aid of (Al-Sahhaf, 1989).

Phosphorus (%): Ammonium molybdate and ascorbic acid had been applied and measured the use of a spectrophotometer at a wavelength of 882 nm as pronounced in (Apha, 1985).

Potassium (%): It changed into determined with a flame-photometer in line with the method stated via (Horneck and Hanson,1998).

Iron(ppm): The estimate was accomplished using an atomic absorption equipment according to the approach given in (Kalara, 1998).

2.2. Carbohydrate concentration in leaves (%)

The total quantity of carbohydrates inside the leaves that taken from the center of the shoot was determined using the approach of (Agrawal *et al.*, 2015) discussing the phenol technique of acidification. 100 mg of dried and ground plant samples had been accrued from every experimental unit and located into a test tube, then five ml of HCl (2.5N) changed into added to the tube, which turned into sealed and positioned in a 90°C water bathtub for 3 hours, then the temperature become decreased to room temperature, stable sodium carbonate changed into brought to neutralize the reaction. The volume became filled to one hundred ml with distilled water, the solution become filtered and 1 ml of the filtrate changed into reserved. 1 ml of 5% phenol and five ml of 96% sulfuric acid were included, the mixture changed into stirred for 10 minutes, and then placed in a 25-30 °C water bathtub for 20 mins. The absorption become then assessed the use of a spectrophotometer at a wavelength of 490nm. A fashionable graph for glucose become then created by way of taking the optical density values of the numerous concentrations of glucose referred to above and making use of the subsequent equation:

$$\text{Total carbohydrates (\%)} = \frac{\text{The amount of carbohydrates}}{\text{Sample volume}} \times 100$$

Data have been evaluated the usage of the Genstat software and as compared the usage of the Duncan's more than one range take a look at with a opportunity of zero.05 (Al-Rawi and Khalaf, 2000).

3. Results and Discussion

The findings in Figures (1-five) display that the pomegranate seedlings that had been inoculated with *Bacillus subtilis* and sprayed with numerous concentrations of nano-iron has a big impact on the leaf nitrogen, phosphorus, potassium, iron and carbohydrate content material. In the instance of inoculation with *Bacillus subtilis* micro organism, we observed that the seedlings injected with bacteria were a great deal better than the unvaccinated seedlings in all the capabilities studied. This may be thanks to the higher attention of those factors inside the soil after inoculation with micro organism, which promotes their absorption and accumulation inside the leaves. (Mengel *et al.*, 2001) mentioned that growing the attention of elements inside the soil answer results in improved absorption by using plant life through the manner of bacterial mineralization of complex organic count number present within the soil and through the mineralization of nitrogen into inorganic (mineral) ions, together with ammonia and nitrate (Qasim and Ali, 1989), as well as the formation of phosphorus humic compounds. These chemicals inhibit phosphorus precipitation, therefore making ready it for absorption (Tisdale *et al.*, 1997). In addition, these organisms perform a crucial role in enhancing the provision of nutrients in the soil via degrading chemical compounds. Complexes containing these elements may decrease the degree of soil touch and emit positive organic acids, consequently enhancing nutrient launch (Fadhl, 2010; Al-Khalil, 2011; Alwan and Al-Hamdani, 2012). The explanation may possibly be because the *Bacillus subtilis* utilized in the research is able to promote the release of siderophores to chelate various nutrients present in the soil, such as phosphorus, iron and other minerals, notably trace nutrients. In this way, they prevent interaction with the chemical components of the soil, thereby increasing their uptake by the plant and then increasing their concentration in plant tissues (Hayat *et al.*, 2010; Yadav and Sarkar, 2019), while increasing the vegetative growth of seedlings after biofertilization. The higher glucose content may be attributed to the increased leaf content of mineral components needed for development (Figures 1-4).

The findings of the identical discern additionally reveal that foliar spraying of nano-iron had a large have an effect on on all of the functions investigated, as seedlings sprayed with both concentrations of nano-iron substantially exceeded the manage remedy, whilst the seedlings dealt with with 40 mg.L⁻¹ accomplished a whole lot higher than the manage therapy. The highest values for all traits, which include (nitrogen, phosphorus, potassium, iron, and carbohydrates), can be due to the high absorption rate of nano-iron fertilizers by way of the leaves because of the small particles and improved floor region of nano-iron fertilizers, which additionally requires the withdrawal of other important nutrients to establish a balance among nutrients within the plant to complete the system of photosynthesis and meet the plant's demand for vitamins synthesized via the leaves (Sekhon, 2014). The cause will also be linked to the truth that nano-chelated iron increases the efficacy of the metabolic conversion method of the mobile membrane, for this reason enhancing the access of nutrients into the plant. This serves to stimulate cellular department, boost improvement, and sooner or later increase the amount of nutrients (carbohydrates) generated inside the leaves (Figure five). This assures the regular need of the plant for vitamins obtained from the soil (Farahani et al., 2015). The aforesaid effects of the cutting-edge research are compatible with the findings of Alalaf et al. (2020) on grafted pomelo seedlings and Al-Zuhairi et al. (2021) on lemon seedlings.

It is likewise referred to from the consequences in Figures (1-5) that the interaction between biofertilization and nano- iron spraying had a enormous impact on all the developments studied, as the interaction remedy made the seedlings inoculated with *Bacillus subtilis* micro organism at the leaves sprayed with forty mg.L⁻¹ of nano- iron substantially higher and had the very best content material of leaf materials (nitrogen, phosphorus, potassium, iron and carbohydrates). From the investigation, we deduced that the simplest remedy become to infect the seedlings with *Bacillus subtilis* and spry the nano-iron with a concentration of 40 mg. L⁻¹, this caused an boom within the content of these additives within the leaves, which in flip brought about an growth within the increase of the seedlings.

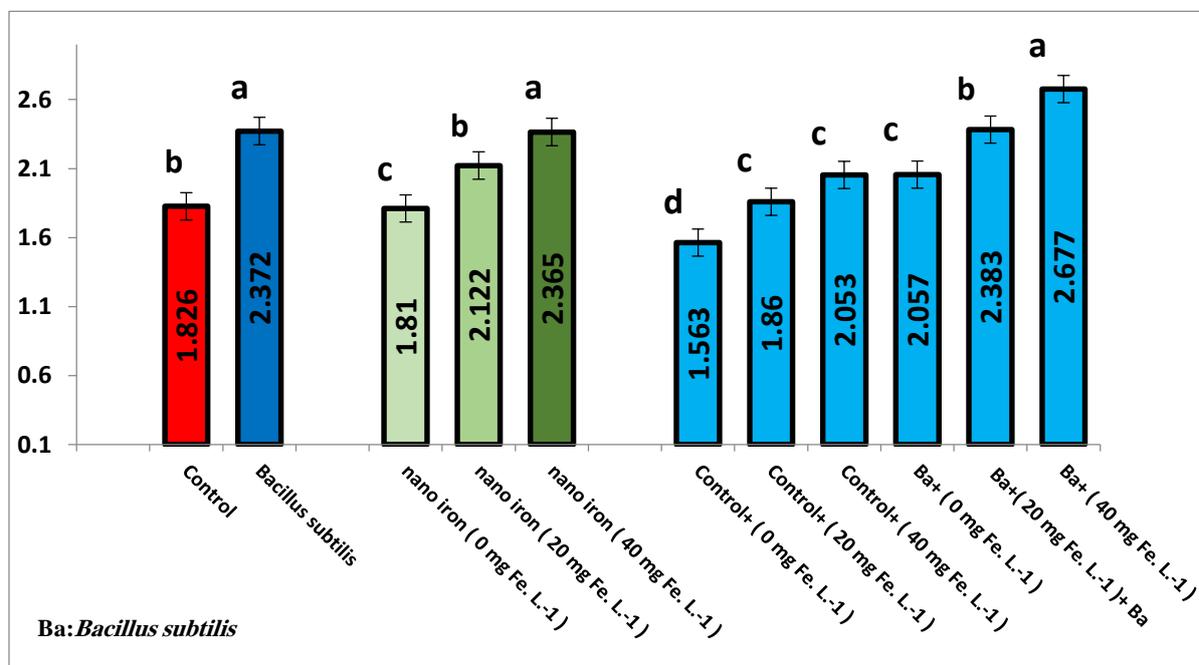


Figure (1). Effects of biofertilizer and nano-iron foliar fertilization and their interaction on nitrogen concentration (%) in pomegranate seedling leaves

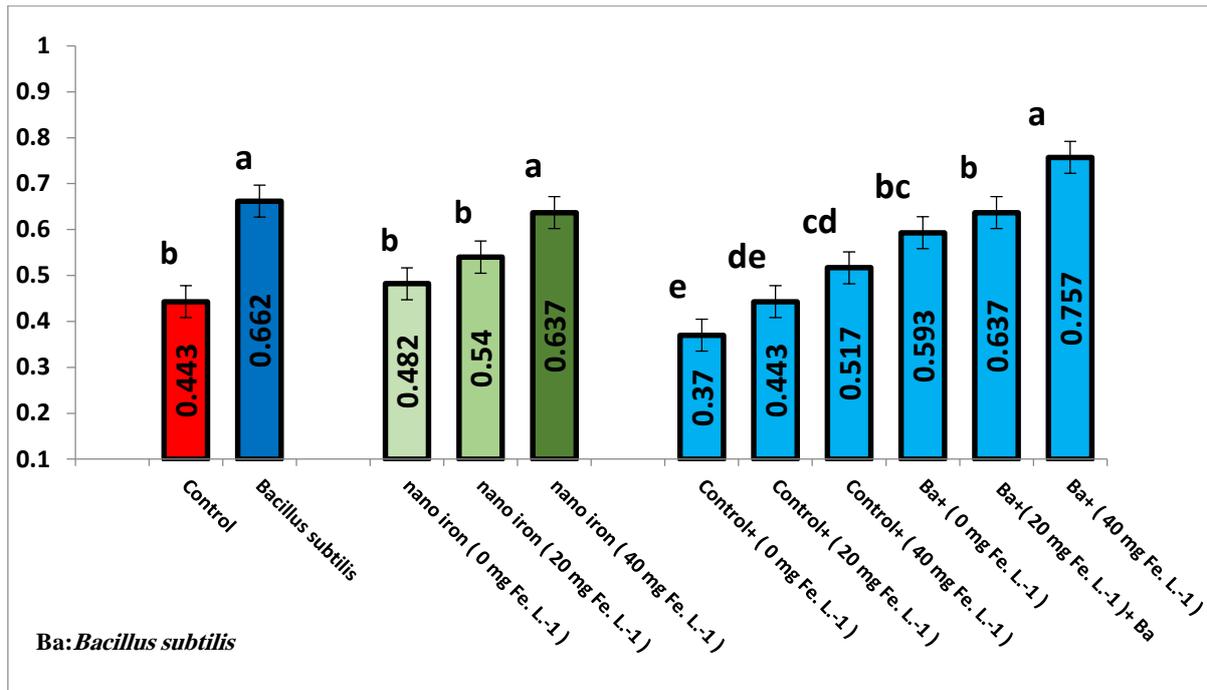


Figure (2). Effects of biofertilizer and nano-iron foliar fertilization and their interaction on phosphorus concentration (%) in pomegranate seedling leaves

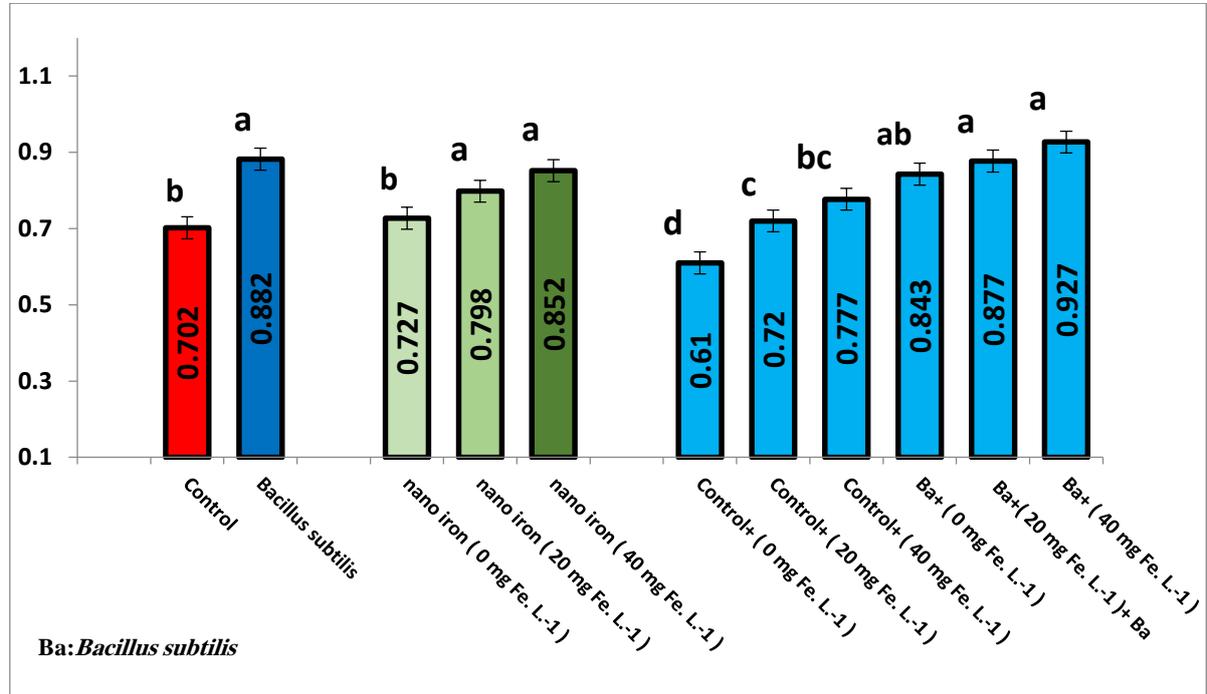


Figure (3). Effects of biofertilizer and nano-iron foliar fertilization and their interaction on potassium concentration (%) in pomegranate seedling leaves

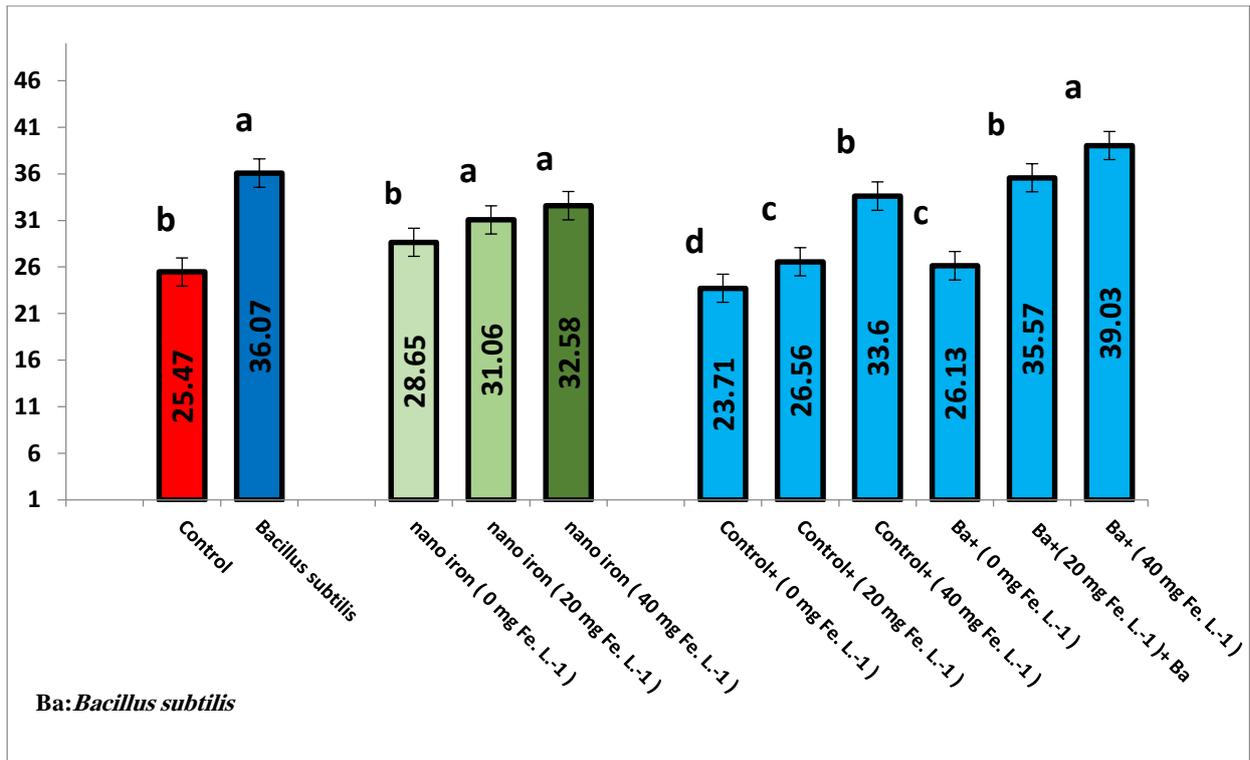


Figure (4). The effects of biofertilizer and the interplay between iron and the surroundings on the iron content of leaves in the seedlings of pomegranate

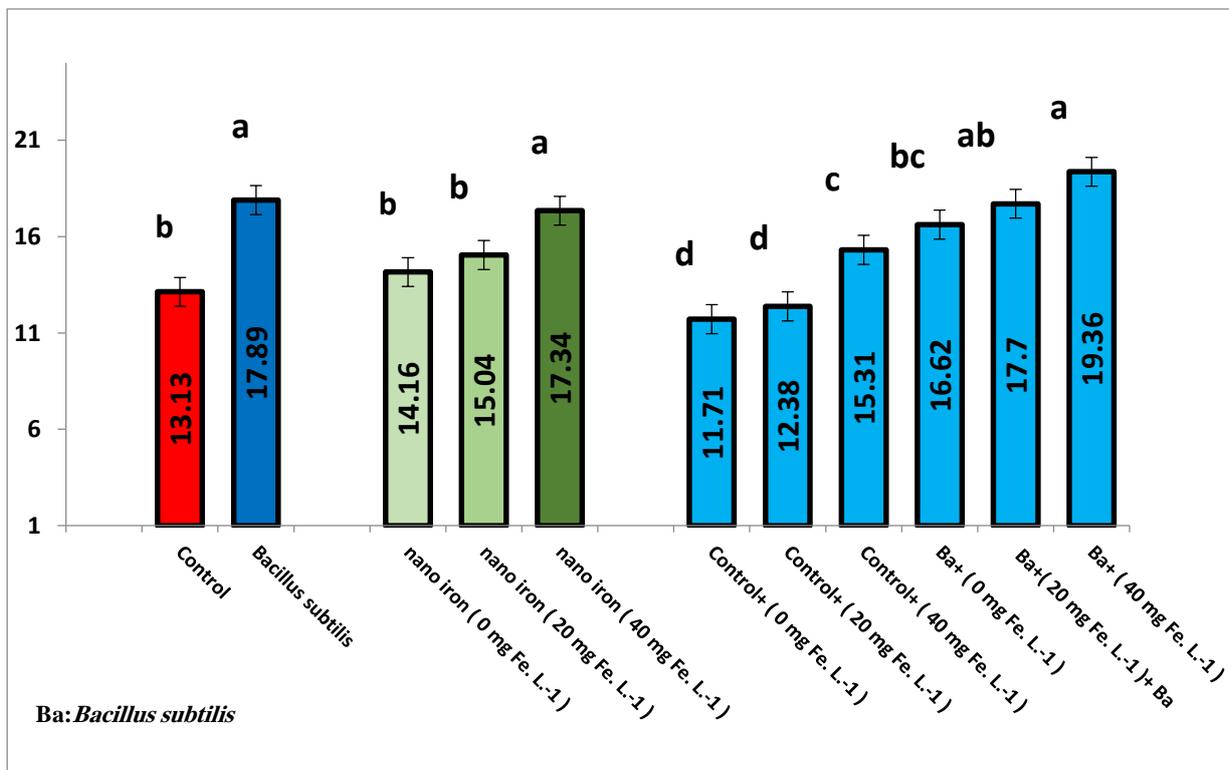


Figure (5). Effects of biofertilizer and nano-iron foliar fertilization and their interplay on carbohydrate awareness (%) in leaves of pomegranate seedlings

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كفاءة التسميد الحيوي والرش بالحديد النانوي في محتوى الاوراق من بعض العناصر المعدنية والكربوهيدرات لشتلات الرمان صنف حلبجة

فارس فيصل الزهيري - وسن وليد أحمد - مصطفى نذير مصطفى - أياد هاني العلاف

الخلاصة:

أجريت الدراسة في الظلة الخشبية في الكلية التقنية الزراعية/الجامعة التقنية الشمالية، استخدمت في التجربة شتلات رمان صنف حلبجة، اجري البحث باستخدام تصميم R.C.B.D، وتم تطبيق المعاملات بثلاث مكررات، وبأربع شتلات في كل مكرر، وتضمنت التجربة عاملين، العامل الأول هو إضافة بكتيريا BACILLUS SUBTILIS إلى تربة الشتلات إضافة الى معاملة الشاهد، اضيفت لكل شتلة ١٠ مل من المحلول البكتيري مرة واحدة خلال الموسم، اضيفت في أبريل، العامل الثاني هو استخدام الرش الورقي بثلاث مستويات من نانو الحديد المخليبي (٠، ٢٠، ٤٠ ملغم لتر⁻¹)، والتي تم رشها مرتين خلال الموسم - الأولى في أبريل والثانية بعد شهر، وقد أثبتت النتائج أن الشتلات التي اضيفت لها السماد البكتيري أظهرت مستويات أعلى من النيتروجين والفوسفور والبوتاسيوم والكربوهيدرات في أوراقها مقارنةً بالشتلات غير الملقحة بالبكتيريا، وأشار البحث إلى أن استخدام الرش الورقي بالحديد النانوي كان له تأثير كبير على جميع الصفات المدروسة، وقد سجلت الشتلات التي رشت بمستوى ٤٠ ملغم لتر أعلى القيم المسجلة، وتأثرت جميع الصفات المدروسة بالتداخل الثنائي بين السماد البكتيري والرش الورقي بالحديد النانوي المخليبي .

الكلمات الدالة : - *PUNICA GRANATUM L* ، سماد حيوي ، سماد نانوي .