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Response of Pomegranate Transplants cvs. Halabja to Biofertilization and Spraying Nano-Iron on Vegetative Growth

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Abstract: The study was conducted at the Lath-house of the College of Agricultural Technology / Northern Technical University. The experiment concerned one-year-old transplants of the Halabja variety of pomegranates. The investigation was conducted using (R.C.B.D.). with 3 replications. Each replication had four transplants. The experiment had two factors. The first factor was the addition of *Bacillus subtilis* to the soil of the transplants. A treatment that lacked bacterial immunogen was also part of the control. The bacterial suspension was utilized once during the season in a volume of 10 ml per transplant. This procedure was conducted in April, following the specific treatment regimen. The second factor was the utilization of foliar sprays that contained nano iron in three different concentrations (0, 20, 40 mg. L^{-1}), applied both times during the season - the first time in April and a month later. The treatment that was used on the other hand was sprayed with water that was distilled. The outcomes demonstrated that the addition of *Bacillus subtilis* to the transplants increased the length, diameter, number of branches, and number of leaves of the plants, as well as their fresh and dry weight. These parameters were all significantly different than the controls. The utilization of foliar sprays containing nano-iron had a significant impact on all traits studied. However, transplants that were treated with a concentration of 40 mg L^{-1} had the greatest recorded values. The interaction between *Bacillus subtilis* and the nano-iron foliar spray had a significant impact on all traits evaluated. The administration of *Bacillus subtilis* and nano-iron in a concentration of 40 mg L^{-1} produced the greatest values for all the studied parameters.

Key words: Pomegranate, biofertilization, nano-iron fertilization.

1. Introduction

The pomegranate, *Punica granatum* L., a fruit belonging to the Punicaceae family, is native to temperate regions, particularly Central Asia and Persia, according to numerous sources. It is also believed that China, Iraq and India may be its first places of origin **(Bal, 2005, Stove**

and Mercure, 2007). The cultivation of pomegranates in Iraq has prospered due to their compatibility with the local environment, despite the need to shield them from sunlight during the summer. Several varieties are grown in Iraq, including the Halabja variety, which is popular in the northern part of the country **(Al-Jumaili and Abu Al-Saad, 1989)**. Pomegranate seedlings have a preference for different soil types, fertility and nutrient composition, and are grown in large quantities in soil on the margins of low fertility soil **(Raghupathi and Bharagava, 1998)**. Bio fertilizers and microorganisms are increasingly employed in agriculture to increase yield and quality. These beneficial microorganisms maintain ecosystems, improve soil quality, promote plant growth and increase productivity by altering the chemical, physical and biological properties of the soil. Crops containing Bacillus subtilis are significant to the soil's biological environment and can adapt to a wide range of pH values (5.5-8.5). **(Sahoo *et al.*, 2013)**. This procedure increases the availability of specific nutrients. The substance promotes the dissolution of phosphates, nitrogen, and the degree to which the soil interacts with nutrients, including potassium **(Al-Khalil, 2011 and Fadhl, 2010)**. A recent investigation by **Al-Zuhairi (2017)** the study revealed that the addition of 10 ml bacteria from *Bacillus subtilis* bacteria to *Citrus grandis* transplants grafts increased the growth of these plants when grafted onto two different citrus rootstocks. The increase in growth characteristics was attributed to the seedling's height, main stem's diameter, number of branches, and the dry weight of the shoot and root system. Nanotechnology is the study of materials with a diameter of 10^{-9} meters and properties that differ from the typical size (up to 100 nanometers). Health, research, engineering, and agriculture involve the protection of crops, the seeds that are planted, the nutrition of plants, and their growth. **(Saleh, 2015)** notes this Nanoparticles are employed in biotechnology, agriculture and industry because of their peculiar physical properties and their role in agriculture is crucial in order to promote sustainable development. Increasing the volume and quality of harvests fertilizers derived from nanotechnology have a large area of surface **(Singh *et al.*, 2016)**. This is caused by the small particle size, which causes them to be extremely soluble in water and other solvents. This property facilitates the entrance of nanoparticles into the plant's surfaces, including leaves and roots **(Qureshi *et al.*, 2018)**. Nano-chelated iron fertilizers are significant to plants because of their exceptional stability and long release of iron over a broad range of soil acidity (soil), their rich iron content, and their effectiveness as a fertilizer, another unique attribute of nano-chelated iron is the increase in the ratio of active to reactive ions of iron, these ions are essential for the formation of chlorophyll in plants **(Roosta *et al.*, 2015). Kumar *et al.* (2017)** acknowledged the effects of nano-chelated iron in their research (on the development of vegetative traits and the yield of a Chandler variety).

2. Materials and Methods

The study was conducted from 1.3.2020 to 1.9.2020 within the Lath-house of Mosul Agricultural Technical College/Northern Technical University/Ministry of Higher Education and Scientific Research/Iraq, to study the impact of biofertilization with *Bacillus subtilis* bacteria and the spraying of various concentrations of nano-iron for pomegranate seedlings, the Halabja variety, with almost uniform growth, so they have been planted in 5 kilograms of polyethylene plastic in a bag that was soil-based totally, as well as sure physical and chemical residences were measured (Table 1).

The experiment used (R.C.B.D.) with two factors, 3 repetitions, and 4 transplants in line with experimental area. The first factor involved inoculation with *Bacillus subtilis* in addition to a control that lacked inoculation. the bacterial answer turned into administered once according to season, and 10 ml. transplant^{-1} became included in April, which corresponded to the remedies in April. The second factor involved into the application of 3 concentrations of nano iron to foliage (0, 20, 40 mg/L) two times all through the season that started out in April, a month later, a second software was made. The remedy for control changed into coated with water and a surfactant (Tween 80) in all solutions that were sprayed. The pomegranate variety that is locally called Halabja is the only that was applied within the experiment. The experiment had three replicates and four transplants consistent with unit (2 × 3 × 3 × 4

= 72). The experiment changed into completed on September 1st, 2023, with the calculation increase the length (cm) from the soil's floor to the very best increase peak became determined the usage of a metric ruler. The diameter (mm) of Vernonia became measured at a peak of five cm from the soil surface, number of branches and leaves have been advanced at the transplant, as these parameters were documented twice: once on the start of the experiment and again at the quilt of the experiment. The discrepancy between the two values of each attribute was decided by taking the second reading and subtracting it from the first. The fresh weight of shoots and roots (g) became recorded using a sensitive balance that separated the shoots and roots from every other inside the crown area the usage of pruning shears. After being severed into smaller pieces, the vegetative elements (leaves, branches, stems) and the roots had been placed in paper bags with holes, those were dried in an oven at 70°C till the weight changed into decided and the stability turned into used to calculate the load. This approach became employed to calculate the dry weight of shoots (g) and roots (g). Data had been evaluated the usage of the Genstat program and as compared the usage of the Duncans multiple range test with a chance of error of 0.05 **(Al-Rawi and Khalaf, 2000)**.

Table (1). Some chemical and physical characteristics of the growing medium in which transplants grow before the start of the experiment

Adjective	Quantity	Measuring Unit
clay	279	gm. kg^{-1}
silt	416	gm. kg^{-1}
sand	305	gm. kg^{-1}
Histology	Mixture	**.....**
EC	0.16	dS. m^{-1}
PH	7.06	
Organic matter	28	gm. kg^{-1}
N	6.91	gm. kg^{-1}
P	2.86	gm. kg^{-1}
K	14.29	gm. kg^{-1}
Fe	0.982	gm. kg^{-1}

* The measurements were conducted in the central laboratory of the Department of Agriculture and Forestry, University of Mosul, Iraq.

3. Results and Discussion

From the information in Tables 2 and 3, it can be visible that the transplants that were inoculated with *Bacillus subtilis* had a widespread increase in all of the parameters evaluated: increase the length, diameter, number of branches and leaves, as well as the fresh and dry weight of the shoots and roots (while in comparison to non-inoculated transplants). These findings may be attributed to the enhanced efficiency of the photosynthesis system in the production of food, specifically carbohydrates, in addition to the utility of regulators like auxins, gibberellins, and various enzymes in the vegetative and root growth processes, which promote the division of cells and their proliferation, this complements the vegetative and root development characteristics of the plant. **(Jandiya, 2003 Sekhon, 2014, Al-Asadi and Ali, 2019)** in addition to the characteristics of the plant, as well as the bacteria utilized enhance the growth of the root system and increasing its density because they produce growth regulators, such as auxins. Thus, this enhances the plant's capacity to absorb water and nutrients from the soil solution surrounding its roots. **(Al-Zuhairi *et al.*, 2021, Spaepen, 2015, Bhat *et al.* 2019)**. There are several researchers who have pointed out the importance of biofertilizers in enhancing the growth of citrus transplants through improved vegetative and root development., including Al-Hadethi *et al.* 2017 on peach transplants and **Al-Zuhairi 2017** on Pomelo transplants.

Table (2). The effects of biological reproduction and spraying nano-iron on the increment, Length transplants, Diameter transplants, Number of branches and leaves of Pomegranate transplants *

Bio-Fertilizer	Levels of nano iron (mg. L.$^{-1}$)			
	0	20	40	Means
	Length transplants (cm)			
Control	50.33 c	57.83 b	61.00 b	56.39 b
Bacillus subtilis	63.33 b	71.00 a	76.67 a	70.33 a
Means	56.83 c	64.42 b	68.83 a	
Diameter transplants (mm)				
Control	5.003 d	5.267 d	5.497 d	5.256 b
Bacillus subtilis	6.150 c	7.303 b	7.840 a	7.098 a
Means	5.577 c	6.285 b	6.668 a	
Number of branches (Branch. transplant^{-1})				
Control	8.67 c	12.67 b	12.33 b	11.22 b
Bacillus subtilis	13.33 b	16.33 a	18.33 a	16.00 a
Means	11.00 c	14.50 b	15.33 a	
Number of leaves (leaf. transplant^{-1})				
Control	150.3 d	170.7 cd	178.3 cd	166.4 b
Bacillus subtilis	226.7 bc	255.3 ab	296.7 a	259.6 a
Means	188.5 b	213.0 ab	237.5 a	

* Coefficients with identical letters in the same column are not substantially different according to Duncan's multiple ranges test at 0.05 level.

As glaring from Tables (2 and 3), the effect of nano-iron foliar application had a significant effect on all of the studied parameters. Transplants that were treated with nano-iron at both concentrations exhibited a significantly outperformed. The therapy with 40 mg L^{-1} concentration of nano-iron had the greatest means of these features when compared to other treatments, which outperformed other therapies by a substantial margin. Iron nano-fertilizers should have a position in the enhancement of those properties. It's critical to the formation of numerous non-heme molecules, the maximum enormous of which is ferredoxin, this molecule helps the switch of electrons in several important functions. Also essential for the improvement of nitrogenase, aconitase, and xanthine oxidase, which have different roles in the plant, including photosynthesis. **(Phogat *et al.*, 2016; Marschner, 1986)**. Increasing the vegetative growth and its activity in manufacturing nutrition enhances the availability of quantities to the roots of these materials, the statement leads to its further deepening and spread **(Kamiab and Zaman Ibahram Abadi, 2016)**. Iron will increase the speed and intensity of photosynthesis in multiple developmental approaches and increases the quantity of increase hormones (IAA) in the leaves, leading to increased cell division and expansion, thereby enhancing the vegetative and root growth characteristics of the plant **(Jandiya, 2003 and Sekhon, 2014**. There are many researchers who have pointed out the importance of iron in enhancing the growth of seedlings, specifically in terms of vegetative and root development, including **Abou El-Nasr *et al.* (2015)** in transplants of pear cultivar Le-Conte and **Alalaf *et al.* (2020)** in pumelo transplants grafted on orange rootstock and **Al-Zuhairi *et al.*, 2021** on lemon transplants grafted on orange rootstock.

It is also noted from the results that the interaction between inoculated with *Bacillus subtilis* and application with nano iron had a significant impact on all the traits studied. Specifically, the interaction between inoculated with *Bacillus subtilis* that were foliar application with 40 mg. L^{-1} nano iron was significantly. The finest will increase in growth rate had been determined for the length, diameter, the number of branches, the number of leaves, as well as the fresh and dry weight of shoots and roots.

Table (3). Effect of biofertilization and spraying nano-iron on the augmentation of Fresh weight shoot and root, dry matter shoot and root of Pomegranate transplants *

Bio-Fertilizer	Levels of nano iron (mg. L^{-1})			
	0	20	40	Means
	Shoots Fresh weight (g)			
Control	27.52 c	30.78 c	34.46 b	30.92 b
Bacillus subtilis	35.23 b	36.87 b	43.48 a	38.97 a
Means	31.38 c	33.83 b	38.97 a	
Shoot dry matter (g)				
Control	15.24 d	18.57 c	20.22 c	18.01 b
Bacillus subtili	22.43 b	24.13 b	28.34 a	24.97 a
Means	18.84 c	21.35 b	24.28 a	
Roots Fresh weight (g)				
Control	18.76 d	20.21 cd	22.52 bc	20.50 b
Bacillus subtilis	21.43 c	24.36 b	27.84 a	24.54 a
Means	20.10 c	22.28 b	25.18 a	
Root dry matter (g)				
Control	8.84 e	10.86 d	12.61 c	10.77 b
Bacillus subtilis	12.91 c	15.61 b	17.88 a	15.47 a
Means	10.88 c	13.23 b	15.25 a	

* Coefficients with the same letter in the same row are not significantly different at the 0.05 level according to Duncan's multiple range test.

The study showed that the best treatments were transplants inoculated with *Bacillus subtilis* bacteria, and foliar application with nan-iron at a concentration of 40 mg. L^{-1}, which resulted in better nutrition and root development in pomegranate type Halabja seedlings. Therefore, under conditions like this study, it is preferable to use pomegranate seedlings. It is fertilized with biofertilizer containing *Bacillus subtilis* bacteria and using nano iron fertilizer at concentrations close to the concentrations that were used in the study.

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