Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8152 - 8160
Received 16 February 2021; Accepted 08 March 2021.

Influence of Foliar Spraying with Potassium, Zinc, and Copper Nano-fertilizers on Some Vegetative Growth Characteristics and Anthocyanin Gene Expression of Pomegranate Transplants

Ali Saeed Atiyah Al-Janabi[1*], Wasen F.F. Alpresem[2], Doaa Ali Kadhem[3]

[1*]Horticulture and Landscape Department, Faculty of Agriculture, University of Kufa, Iraq.
E-mail: ali.aljanabi@uokufa.edu.iq
[2]Horticulture and Landscape Department, Faculty of Agriculture, University of Basrah, Iraq.
[3]Ministry of Agriculture, Najaf, Iraq.

ABSTRACT

This trial was carried out during the growing season of 2018/2019 to study the effect of nano-potassium, nano-zinc and nano-copper levels (0,1,2 g.L-1), (0,2,3 g.L-1) and (0,0.5,1 g.L-1) sprayed twice(15/3/2019 and 15/4/2019). The experiment was designed using R.C.B.D with three replicates. Results exhibited that the nano-potassium affected in Diameter increased, Leaves numbers increased/transplant, Leaves area, Total chlorophyll, P %, and K %. the nano-zinc affected Total chlorophyll for both concentrations reached 69.833 and 73.211100mg/g fresh weight, while nitrogen was highly significant when pomegranates transplant treated with nano-zinc by both concentrations 2 and 3 $g.L^{-1}$reached 1.95 and 1.97%. The percentage of zinc appeared high significant when pomegranate treated with nano-zinc for both concentrations (2 and 3 $g.L^{-1}$) reached 22.3 and 22.7 $mg.Kg^{-1}$ respectively. The high percentage of copper when pomegranate transplant treated with nano-copper (0.5 and 1 $g.L^{-1}$) reached 8.9 and 9.1 $mg.Kg^{-1}$ respectively. The expression of *ANSpg* gene was the best treatment when pomegranate transplant treated with potassium with both concentrations compared with control.

KEYWORDS

Pomegranate, Potassium, Zinc, Copper, Nano-fertilizers, Gene Expression.

Introduction

Pomegranate (Punica granatum L.) is one of the edible fruit crops and occupies the most commercial significance. It is a fruit tree belonging to the Punicaceae family, primarily cultivated in subtropical and tropical areas (Adsule and Patil, 1995; Naik and Chand, 2011). Pomegranate fruits are consumed in either processed or fresh manner providing an extent quality of antioxidant compounds, and balanced food for jams, jellies, syrups, and juices (Legua et al., 2012). Further, pomegranate could be consumed as a refined form (Alighourchi et al., 2008). The total fruitful trees of pomegranate in Iraq is approximately 3860139 trees with a production of around 219822 tons and the average yield per tree reached 34.58 $Kg.tree^{-1}$. The fruitful trees of pomegranate in Najaf, Iraq only is about 55766 trees with a production of around 119 tons and the average yield per tree reached 20.60 $Kg.tree^{-1}$ (CSOI, 2019). Minerals can affect fruit quality and production either directly or indirectly (Al-hadrawi and Al-janabi, 2020). The majority of indirect effects lead to altering vigor and energy, thus separating primary and secondary photosynthetic metabolites such as plant growth regulators, organic acids, carbohydrates, proteins, and flavor compounds which are critical for production (Taghliavini et al., 2000). The most substantial aim in agricultural systems to increase crop yield is the efficient use of fertilizers (Dong et al., 2005), but matching nutrient application to crop requirements is not straightforward. The capability of plant leaf canopy to absorb nutrients has contributed to the nutrients foliar spraying becoming a recurring trend for providing plants with nutrients (Weinbaum, 1988). The benefit of low usage rates, equal distribution of fertilizer resources and quick responses to applied foliar fertilization is that elements such as nitrogen, phosphorus, zinc, copper, boron, or potash play a physiological and vital role in boosting and promoting vigorous plant growth and development. The micro-nutrients for instance Fe, Zn, Mn, Cu, and B not only are essential, but they are equally crucial as macro-nutrients, considering their micro-quantity necessities. They are key components of plant production and growth (Das, 2014 and Yadav and Solanki,2015). Potassium (K) is an essential and important macronutrient for plants and pomegranate especially and K concentration compared with other macronutrients in peel and aril of pomegranate fruits was the highest (Al-Maiman and Ahmad, 2002; Mirdehghan and Rahemi, 2007). Zinc (Zn) is one of the significant and basic micronutrients for plants, and its decrease is prevalent in many crops (Swietlik, 1999; Marschner, 2012; Ojeda-Barrios et al., 2014). It is essential for the various enzymatic reaction, including dehydrogenases, transphosphorylases, polymerases of RNA and DNA, aldolases, isomerases, and is also contributed in tryptophan synthesis, cell division, photosynthesis, and membrane structure maintenance, also works as a regulatory cofactor for certain protein synthesis (Marschner, 2012). Copper has an influential role in the biological processes in plants by activating some enzymes, likewise the oxidizing enzymes of the polyphenol oxidase, and the role of transferring electrons in photosynthesis. Several studies revealed that about 70% of the total copper in the leaf tissues is present in the chloroplasts, and this confirms its bioactivity in the process of photosynthesis. Copper has long been used with other constituents as a mixture to tackle fungal diseases (Al-Sahaf, 1989). Most of the Iraqi soil is characterized by a high content of calcium carbonate that turns out to a basic medium. Also, by adding macro and micronutrients to the soil directly, they may be exposed to the processes of loss, fixation, and sedimentation, thus the plant does not utilize them, especially the micronutrients. This motivated researchers to explore efficient methods like foliar spray as an alternative to soil amendments (Al-salman et al., 1988). The current study aims to evaluate some vegetative growth indicators and gene expressions of pomegranate transplants under different doses of foliar spraying with nano-potassium, nano-zinc, and nano-copper.

Materials and Methods

Pomegranate (Punica granatum L.) transplants with two years old were planted in a private nursery located in Najaf, Iraq, setup in bags of polyethylene comprising 3Kg of soil. The site soil was analyzed to measure the properties of a soil-related table (1).

Table 1. Some chemical and physical properties of the site soil

Properties	Value
Sand %	41.2
Clay %	38.9
Silt %	19.9
Texture	Sandy Clay
F.C %	18.44
Ph	7.88
EC (dS/m)	0.78

The transplants were cultivated under nursery conditions from the sapling age till 1 June 2019. Two concentrations of nano-potassium (nK) 1 and 2 $mg.L^{-1}$, nano-zinc (nZ) 2 and 3 $mg.L^{-1}$, and nano-copper (nCu) 0.5, 1 $mg.L^{-1}$, were sprayed as foliar application plus the control treatment (without spray) and all doses repeated two times at 15/3/2019 and 15/4/2019. The experiment design was conducted in a Randomized Complete Block Design (R.C.B.D). Each experimental unit had three transplants then triplicated to provide 7 treatments for each foliar concentration (21 con.) and the total transplants for the whole trial were 105. The results were statistically analyzed and Duncan's multiple range test was used for mean comparison at 5% probability level (Montgomery, 2017). All data obtained were statistically analyzed by GenStat software computer-based.

1-Plant Growth Measurements

All measurements were recorded on 1st June 2019.

The mean of plant length increment (cm) was measured at the end of the experiment using measuring tape (after-before spraying). The increment in the mean of stem diameter (mm) was measured at 5cm above the soil surface was measured using vernier(after- before spraying). While the increment of leaf number was determined in three transplants randomly selected per treatment. The average leaf area **(**cm²**)** of the 6-10th leaves (Reisinaur, 1978), used the gravimetric method shown in Drovnic *et al*. (1965) that leave were selected from the height of transplants per treatment, afterward, the leaves weight were taken using a disc which(0.302 cm²) diameter for each leaf was sampled. The leaves dried using an oven at 70ºc for every weight fixing. The average leaf area calculated by the following equation:

A.L.A = A.L.D.W × a.d/d.w.d :A.L.A = Average leaf area (cm²),A. L.D.W = Average leaf dry weight (g) a.d =Average area of disc given from the leaves (0.302 cm²) d.w.d = Average dry weight of discs given from the leaves (g).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8152 - 8160
Received 16 February 2021; Accepted 08 March 2021.

The leaf fresh weight and dry weight (%) of the shoots were measured separately. Leaves were placed in an electrical oven at 70˚c until weight fixing (Al-janabi *et al*., 2016).). Leaf dry matter was calculated as follows: leaf dry matter (%) = 100 × Leaf Fresh Weight/ Leaf Dry Weight.

Acetone (80%) was used to determine total chlorophyll in leaves, the leaves extract was filtered using a paper filter and then centrifuged at 3000 $c.h^{-1}$ for five minutes. The leaves extract measured by light absorption using a spectrophotometer, determined at 663 and 645 nm and using the following equations: Total chlorophyll = 20.20 A645 + 8.02 A663. The A645, A663 was the spectrophotometer reading at 663 and 645 nm consecutively, (Mackinny, 1941).

2-Concentration of Macro and Micro-elements in Leaves

As in Chapman and Pratt (1961), pomegranate samples were prepared and the concentration of N was determined using the Kjeldahl process, P using spectrophotometry and K using photometry of flame emissions, while atomic absorption spectrophotometry (AAS) was used to measure Fe, Cu, and Zn concentrations (Arnon,1949).

3-Gene Expression Measurement

Total RNA extraction and cDNA synthesis: Total RNA extraction was prepared from pomegranate leaves samples selected from each treatment using the isolation kit (SV Total RNA, Promega, USA). Agarose gel electrophoresis was used to sampling RNA., to remove DNA from RNA samples used DNase I Mix from Promega, USA company (DNase I, $MnCl_2$, yellow core buffer). 16 µl of total RNA was prepared to synthesize the first-strand cDNA by (power Syntheses kit of cDNA, IntronBio. Inc. USA) with Oligo (dT) 15 primer, subsequent to the producer's manual then quantified via gel electrophoresis.

Quantitative Real-Time PCR (qRTPCR): Gene expression analysis was achieved using qRT-PCR applying a Mini Option's System real-time PCR and conducting (GO Taq Master Mix SYBR Green kit QPCR, IntronBio. Inc. USA). Primers for gene-specific amplification of pomegranate were designed to generate a product of 484 bp. The 27,786-bp region, comprising the 88-bp, revealed that these genes and genome-walked sequences ANSpg Gene Partial Sequences could be translocated into around 22-kb upstream region in a reverse direction. For primer design was used NCBI, Gene Bank website. The primer sequences *ANSpg* gene are responsible for anthocyanin synthesis in pomegranate. Primers for qRTPCR amplification were two parts (Forward5-AGAAGTACGCGAACGACCAG-3) (Reverse5-TGAAGGTTAGGGCGCTG ATG -3), T.m (60.11 -60.11), GC %55. PCR reactions proceeded twice on a plate. The reaction mixture (22.5 µl per well) comprised 2.5 µl primers (reverse and forward), 12.5 µl SYBR Green Master Mix, 2.5 µl cDNA, 7.5 µl DEPC-D.W. The program thermal cycling settings involved an initial denaturation stage at 95℃ for 10 min, followed-up by 40 cycles for 30 s, 60℃ for 1 min, at 95℃ and then for 30 s at 72℃. Agarose gel electrophoresis was to determine the diagram, molecular weight, and purity. Further, used Actin gene of pomegranate as a reference (housekeeping) gene (*pgActin*) with sequence primers to normalize (Forward5-AGTCCTCTTCCAGCCATCTC-3) (Reverse 5-CACTGAGCA CAATGTTTCCA-3), T.m (60.04-59.82), GC %55. GeneX program was used to analyze the real-time PCR data (Al-janabi and Al-rawi, 2018).

Results and Discussion

Mean of Plant Length increment (cm) and Diameter increment (mm): Table (1) showed high significance in length increment when pomegranate transplants treated with 2 and 3 $g.L^{-1}$ of nano-zinc reached 66.43 and 66.82 cm respectively. While the lowest length increment appeared when transplants treated with nano-copper and control. nano- potassium treatments were middle affected among high and low values. An increase in diameter appeared in 1 and 2 $g.L^{-1}$ of nano- potassium treatments reached 10.23 and 11.45 mm respectively. Further, the lowest significant value in increment diameter was in the control reached 5.44 mm, while the treated pomegranate transplant with nano-zinc and nano-copper were nonsignificant.

Table 2. Influence of potassium, zinc, and copper produced by nanotechnology on length (cm) and diameter (mm) increment of pomegranate transplants

Treatments		Length increment (cm)	Diameter increment (mm)
Control		26.50 c	5.44 c
nK	$1\ g.L^{-1}$	55.30 bc	10.23 a
	$2\ g.L^{-1}$	62.33 ab	11.45 a
nZn	$2\ g.L^{-1}$	48.23 c	7.30 b
	$3\ g.L^{-1}$	49.55 c	7.75 b
nCu	$0.5\ g.L^{-1}$	66.43 a	7.69 b
	$1\ g.L^{-1}$	66.82 a	7.44 b

The values in a single column with dissimilar letters indicate significant variance according to Duncan's multiple range test at 5% probability level.

Mean of Leaves Number Increment per transplants: Table(3) showed highly significant for leaf number when pomegranate transplants treated with 3 and 2 $g.L^{-1}$ nano-zinc reached 81.7 and 80.3 respectively. While the lowest signification of leave number appeared in control reached 44.2 and all nano-potassium and nano-copper treatments were middle among highest and lowest values.

Mean of Leaf Area (cm²): Table 3 indicated that the spraying of 2 $g.L^{-1}$ of nano-potassium, 0.5 and 1 $g.L^{-1}$ of nano-copper reached 10.74, 10.22, and 11.89 cm^2 respectively. While the minimum leaf area appeared in the control pomegranate transplants reached 6.40 cm^2. The nano-zinc treatments were middle in effects on leaf area.

Total Chlorophyll Content(100mg/g F.W): Showed table (3) that the leaf content of total chlorophyll was significantly increased with applying nano-fertilizers by potassium, zinc, and copper in comparison with the control treatment. However, the lowest total chlorophyll was in the control pomegranate transplants reached 53.355 $mg.g^{-1}$ in fresh weight of control leaf samples.

Table 3. Influence of potassium, zinc, and copper produced by nanotechnology on Leaf number per transplant, leaf area (cm²), and total chlorophyll content (100mg/g F.W) of pomegranate transplants

Treatments		Leaves numbers increased /transplants	Leaves area cm^2	Total chlorophyll (100mg/g F.W)
Control		44.2 c	60.40 c	53.355 c
nK	$1\ g.L^{-1}$	80.3 a	8.52 b	65.443 ab
	$2\ g.L^{-1}$	81.7 a	10.74 a	71.691 a
nZn	$2\ g.L^{-1}$	66.2 b	9.06 b	69.833 a
	$3\ g.L^{-1}$	70.7 b	8.93 b	73.211 a
nCu	$0.5\ g.L^{-1}$	71.2 b	10.22 a	62.496 b
	$1\ g.L^{-1}$	70.8 b	11.89 a	68.588 a

The values in a single column with dissimilar letters indicate significant variance according to Duncan's multiple range test at 5% probability level.

Concentration of macro and micro-elements in leaves: Table(4) showed that the lowest nitrogen percent was in the control transplant leaves reached 1.52%. However, the highest nitrogen percent was significant when pomegranate transplants treated with nano-zinc by both concentrations 2 and 3 $g.L^{-1}$ reached 1.95 and 1.97% respectively. The nano-copper treatments provided a highly significant increase for nitrogen percent in leaves by both concentrations 0.5 and 1 $g.L^{-1}$ reached 1.86 and 1.84 consecutively. Phosphor percent was the maximum in pomegranate leaves when treated with $1g.L^{-1}$ of nano-copper reached 0.36% and $2g.L^{-1}$ and reached 0.41% when treated with 2 $g.L^{-1}$ of nano-potassium. These findings were in agreement with Khayyat et al. (2007) and Khayyat et al. (2012). The analysis of potassium percent of pomegranate leaves was highly significant when transplants treated

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8152 - 8160
Received 16 February 2021; Accepted 08 March 2021.

with both concentrations of nano-potassium (1 and 2 $g.L^{-1}$) provided 0.996 and 1,01 % consecutively. Whereas, the lowest potassium percent in pomegranate leaves was in the control reached 0.62%. The nano-zinc and nano-copper treatments were in the middle between high and low values.

Table 4. Influence of potassium, zinc, and copper produced by nanotechnology on N, P, and K percent of pomegranate transplants

Treatments		N (%)	P (%)	K (%)
Control		1.52	0.13 d	0.62 c
nK	1 $g.L^{-1}$	1.66	0.34 ab	0.96 a
	2 $g.L^{-1}$	1.78 b	0.41 a	1.01 a
nZn	2 $g.L^{-1}$	1.95 a	0.19 d	0.77 b
	3 $g.L^{-1}$	1.97 a	0.28 c	0.70 b
nCu	0.5 $g.L^{-1}$	1.86 ab	0.33 ab	0.71 b
	1 $g.L^{-1}$	1.84 ab	0.36 a	0.73 b

The values in a single column with dissimilar letters indicate significant variance according to Duncan's multiple range test at 5% probability level.

Table(5) showed that the percentage of iron in leaf pomegranate among all treatments was nonsignificant. The percentage of zinc revealed high significance when pomegranate transplants treated with nano-zinc for both concentrations (2 and 3 $g.L^{-1}$) reached 22.3 and 22.7 $mg.Kg^{-1}$ respectively. However, the other treatments were nonsignificant compared to the control. The leaf analysis showed a high percentage of copper when pomegranate transplant treated with nano-copper (0.5 and 1 $g.L^{-1}$) reached 8.9 and 9.1 $mg.Kg^{-1}$ respectively. These results were consistent with Umer et. al (1999) and Tehranifar and Mahmooditabar (2009).

Table 5. Influence of potassium, zinc, and copper produced by nanotechnology on Fe percent and Zn ($mg.Kg^{-1}$), Cu ($mg.Kg^{-1}$) of pomegranate transplants

Treatments		Fe (%)	Zn ($mg.Kg^{-1}$)	Cu ($mg.Kg^{-1}$)
Control		11.7 a	10.3 b	6.2 b
nK	1 $g.L^{-1}$	12.3 a	12.6 b	7.5 b
	2 $g.L^{-1}$	13.4 a	12.2 b	6.9 b
nZn	2 $g.L^{-1}$	12.9 a	22.3 a	7.0 b
	3 $g.L^{-1}$	12.8 a	22.7 a	7.3 b
nCu	0.5 $g.L^{-1}$	11.9 a	13.5 b	8.9 a
	1 $g.L^{-1}$	12.0 a	13.7 b	9.1 a

The values in a single column with dissimilar letters indicate significant variance according to Duncan's multiple range test at 5% probability level.

ANSpg **Gene Expression**

Figure 1 showed that extraction of RNA from pomegranate leaves under different treatments of nano-fertilizers were successful and high efficiency which gave RNA concentration ranged between 72ds to 79ds with purity reached 1.8 to 2 which used (SV Total RNA Isolation kit/ Bioneer-South Korea).

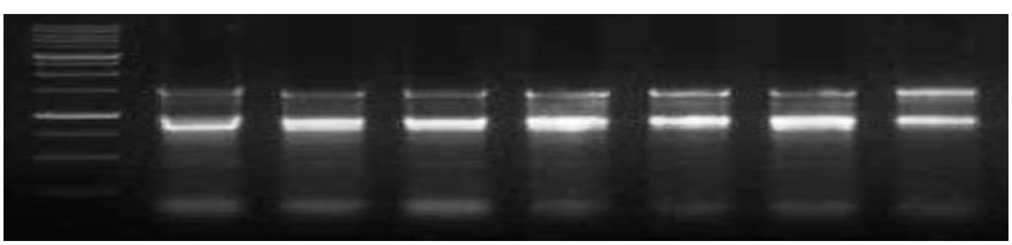

Figure 1. Total RNA extraction of the pomegranate leaves under Nano-fertilizers treatments on agarose gel 1.5%,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8152 - 8160
Received 16 February 2021; Accepted 08 March 2021.

and 100 voltage for 20 minutes, M:marker, con.: contorol, nK1and nK2:nanopotassium (1, 2 $g.L^{-1}$), nZn2 and nZn3: nanozinc (2,3 $g.L^{-1}$), nCu0.5 and nCu1 nanocopper (,0.5,1 $g.L^{-1}$)

The synthesis of cDNA for RNA of the pomegranate leaves under nano-fertilizer treatments was successful because used (cDNA Syntheses kit/ Bioneer -South Korea) that was high efficiency and specific (Figure 2).

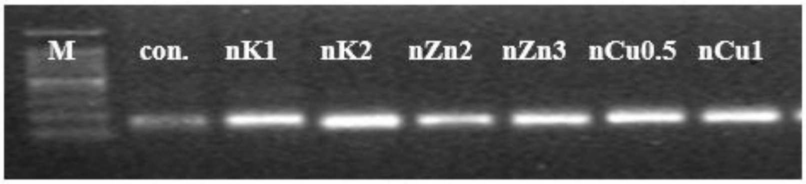

Figure 2. Synthesis cDNA for RNA of the pomegranate leaves under nano-fertilizers treatments on agarose gel 1.5%, and 100 voltage for 20 minutes.M:marker, con.: control, nK1and nK2:nano-potassium (1, 2 $g.L^{-1}$), nZn2 and nZn3: nano-zinc (2,3 $g.L^{-1}$), nCu0.5 and nCu1 nano-copper (0.5, 1 $g.L^{-1}$)

ANSpg gene is responsible for anthocyanin synthesis in pomegranate. The expression of *ANSpg* gene was high value when leaves treated with nano-zinc 1 and 2 $g.L^{-1}$ reached 31.13 and 33.53 fold respectively. While the treated pomegranate leaves with nano-zinc and nano-copper gave convergent results and mid among high and low values. The lowest value appeared in the control which reached 15.54 fold (Fig 3).

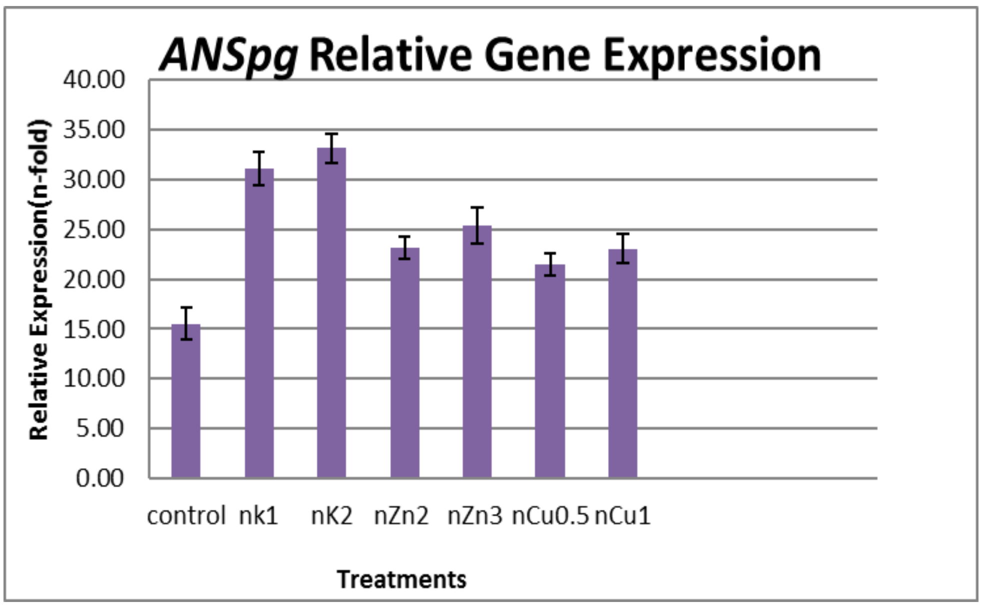

Figure 3. Relative gene expression for *ANSpg* gene under different treatments, control: without spray nK1and nK2: nano-potassium(1, 2 $g.L^{-1}$), nZn2 and nZn3: nano-zinc (2, 3 $g.L^{-1}$), nCu0.5 and nCu1 nano-copper (0.5, 1 $g.L^{-1}$)

Discussion

In promoting the growth and production of plants, macro-nutrients such as potash and micro-nutrients such as zinc and copper play a considerable and broad role. Nevertheless, their need for micro quantities, Zn and Cu are substantial and equally significant as macro-nutrients. They play crucial compositions in plant production and growth (Das, 2014 and Yadav and Solanki,2015). Moreover, potassium considers a key element in ameliorating fruit enlargement and cell turgidity by lowering carbohydrate contents (Fisher *et al*.,1959 and Marschner, 1986). The impacts of potassium on the influx of carbohydrates or the plant growth regulators synthesis in fruits and leaves may be related to enhancing fruit yield and quality. Likewise, optimum plant K levels can boost the loading of phloem, the transport of nutrients, and the unpacking of sucrose (Lester et al., 2005). Consequently, in terms of our findings, it is suggested that spraying nano potassium as a foliar application will be useful management practice enhancing fruit quality and declining harmful impacts of carbohydrates.

Zn fertilizers as new nano-materials can be used to develop plant parts, with the nano-particulate Zn characteristics (reactivity, rare surface area, and size) prompting the solubility, diffusion, and accessibility of Zn to plants (Subramanian and Sharmila Rahale, 2012; Mosanna and Khalilvand, 2015). The results obtained indicate that a low-quantity of foliar spray or Zn nano-fertilizers at (2 and 3 g.L-1) resulted in a medium increase in the pomegranate parameters. It is noteworthy that zinc is is one of the critical micronutrients for plants, besides Zn lack is predominant in many crops (Swietlik, 1999; Marschner, 2012; Ojeda-Barrios et al., 2014).). Additionally, the function of various enzymes usually requires cellular Zn concentration, including transphosphorylases, dehydrogenases, isomerases, aldolases. Zn is also involved in, DNA and RNA polymerases, tryptophan synthesis, membrane structure maintenance, cell division, and photosynthesis, as well as acting as a regulatory co-factor in protein biosynthesis (Marschner, 2012). Moreover, the impact of copper showed increase parameters of the pomegranate transplants may be due to increasing the enzymatic processes and reflecting an effective rate of photosynthesis, by which the compounds manufactured by leaves could increase the growth and improve the plant traits (Al-Qawami et al., 2002; Metep and Hasan, 2020). Also, applying these nutrients could increase the leaf content of chlorophyll (Table 3) promoting physiological plant activities which reflect positively the process of carbohydrate and protein biosynthesis, (Gobara, 1998 and Hasan et al., 2019).

Conclusion

Fernández *et al*. (2013) reported that foliar fertilizer sprays, including macro and micro-nutrients such as K, Zn, and Cu, are significantly convenient for field practice, due to their good efficacy and reactivity quickly to plants. Instead, many complications and difficulties in different fields of science and industry have been solved using nano-technologies (Scott and Chen, 2003), indicating that the nano-potassium increased stem diameter, number of leaves per transplant, Leaves area, total chlorophyll, P %, and K %. Besides, nano-zinc improved the total chlorophyll using both concentrations reached 69.833 and 73.211 mg/g fresh weight. However, high nitrogen percent in leaves were significant when pomegranate transplants treated with nano-zinc by both concentrations at 2 and 3 $g.L^{-1}$ reached 1.95 and 1.97% respectively. The percentage of zinc realized highly significant effects when pomegranate transplants treated with nano-zinc for both concentrations (2 and 3 $g.L^{-1}$) reached 22.3 and 22.7 $mg.Kg^{-1}$ consecutively. The expression of *ANSpg* gene was the best treatment when pomegranate transplants treated with potassium at both concentrations in comparison with the control.

References

[1] Abu, D., Youssef, M., & Al-Younes, M.A. (1988). Plant Nutrition Manual, Dar Al-Kutub Printing and Publishing Directorate, University of Mosul.

[2] Adsule, R.N., & Patil, N.B. (1995). *Handbook of fruit science and technology.* In: Salunkhe, D.K., Kadam, S.S. (Eds.), Pomegranate. Marcel Dekker Publishers, New York, 455–464.

[3] AL-hadrawi, D.A.K., & Al-janabi, A.S.A. (2020). *Analysis FCPOXS Gene Expression Responsibility on Peroxidase Synthesis for Two Fig Cultivars Growing Under Salt Stress and Treated with Proline and Neutra-Sol. Plant Archives*, *20*(2), 1411-1420.

[4] Alighourchi, H., Barzegar, M., & Abbasi, S. (2008). Effect of gamma irradiation on the stability of anthocyanins and shelf-life of various pomegranate juices. *Food chemistry*, *110*(4), 1036-1040.

[5] AL-Janabi, A.S.A., Hasan, A.K., & Neamah, S.S. (2016). Effect of Biofertilizer (EM-1) and Organic fertilizer (Acadian) on Vegetative Growth of Many Cultivars of Apricot seedling (Prunus armeniaca L.). *Euphrates Journal of Agriculture Science*, *8*(4), 23-32.

[6] Al-Maiman, S.A., & Ahmad, D. (2002). Changes in physical and chemical properties during pomegranate (Punica granatum L.) fruit maturation. *Food Chemistry*, *76*(4), 437-441.

[7] Al-Qawami, S.M.J., Salman, A.M., & Salman, M.A. (2002). The effect of spraying with Fe, Zn, N, GA3 on some physiological traits of fig fruits (Ficus carica L.) Diyala black cultivar. *Iraqi Journal of Agricultural Sciences*, *33*(4), 71-76.

[8] Al-Janabi, A.L.I.S., & Al-Rawi, T.K. (2018). Effect of Irrigation Times and Termination on Gene

Expression of Oedgat1, Oefad2. 1, and Oefad6 and Oil Quality and Quantity in Olive Cultivars. *Sabrao Journal of Breeding and Genetics 50*(3), 329-343.

[9] Al-Sahaf, H.F. (1989). *Applied Plant Nutrition, Ministry of Higher Education and Scientific Research.* Baghdad. University. Bayt Al-Hikma. Iraq, 260.

[10] Al-Salman, M.A. (1988). *Propagation of horticultural plants. Baghdad University.* Ministry of Higher Education and Scientific Research, Iraq.

[11] Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant physiology*, *24*(1), 1.

[12] Carrasco-Gil, S., Rios, J.J., Álvarez-Fernández, A., Abadía, A., García-Mina, J.M., & Abadía, J. (2016). Effects of individual and combined metal foliar fertilisers on iron-and manganese-deficient Solanum lycopersicum plants. *Plant and soil*, *402*(1-2), 27-45.

[13] Chapman, H.D., & Pratt, P.F. (1961). *Methods of Analysis for Soils, Plants and Water.* University of California, Berkeley, CA, U.S.A.

[14] CSOI (2019). Central Statistic Organization Iraq.

[15] Das, S.K. (2014). Role of micronutrient in rice cultivation and management strategy in organic agriculture. A Reappraisal. *Agricultural Sciences, 5*, 765-769.

[16] Dong, S., Cheng, L., Scagel, C., & Fuchigami, L. (2005). Timing of urea application affects leaf and root N uptake in young Fuji/M. 9 apple trees. *The Journal of Horticultural Science and Biotechnology*, *80*(1), 116-120.

[17] Fernández, V., Sotiropoulos, T., & Brown, P.H. (2013). *Foliar fertilization. In: Scientific Principles and Field Practices.* International Fertilizer Industry Association, Paris.

[18] Fisher, E.G., Parker, K.G., Luepschen, N.S., & Kwong, S.S. (1959). The influence of phosphorus, potassium, mulch, and soil drainage on fruit size, yield, and firmness of the Bartlett pear and on development of the fire blight disease. *Proceedings of the American Society for Horticultural Science, 73*, 78-90.

[19] Gobara, A.A. (1998). Response of Le-conte pear trees to foliar application of some nutrients. *Egyption Journal of Horticulture, 25*(1), 55-70.

[20] Hasan, A.M., Mohamed Ali, T.J., & Al-Taey, D.K. (2020). Effects of winter foliar fertilizing and plant growth promoters on element and carbohydrate contents on the shoot of navel orange sapling. *International Journal of Fruit Science*, *20*(4), 682-691.

[21] Khayyat, M., Tafazoli, E., Eshghi, S., & Rajaee, S. (2007). Effect of nitrogen, boron, potassium and zinc sprays on yield and fruit quality of date palm. *American-Eurasian Journal of Agricultural & Environmental Sciences (JAES), 2*, 289-296.

[22] Khayyat, M., Tehranifar, A., Zaree, M., Karimian, Z., Aminifard, M. H., Vazifeshenas, M. R., & Shakeri, M. (2012). Effects of potassium nitrate spraying on fruit characteristics of 'Malas Yazdi'pomegranate. *Journal of plant nutrition*, *35*(9), 1387-1393.

[23] Legua, P., Melgarejo, P., Abdelmajid, H., Martinez, J.J., Martinez, R., Ilham, H., & Hernández, F. (2012). Total phenols and antioxidant capacity in 10 Moroccan pomegranate varieties. *Journal of food science*, *77*(1), C115-C120.

[24] Lester, G.E., Jifon, J.L., & Rogers, G. (2005). Supplemental foliar potassium applications during muskmelon fruit development can improve fruit quality, ascorbic acid, and beta-carotene contents. *Journal of the American Society for Horticultural Science*, *130*(4), 649-653.

[25] Mackinney, G. (1941). Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry, 140*(2), 315-322.

[26] Marschner, H. (1986). *Mineral Nutrition in Higher Plants.* New York: *Academic Press.*

[27] Marschner, H. (2012). *Mineral Nutrition of Higher Plants.* Academic Press Limited Harcourt Brace and Company, Publishers, London, 347–364.

[28] Metep, S.S., & Hasan, A.M. (2020). Response of apricot trees for spraying by antioxidants compounds and foliar fertilizers. *Ecology, Environment and Conservation*, *26*, 62-69.

[29] Mirdehghan, S.H., & Rahemi, M. (2007). Seasonal changes of mineral nutrients and phenolics in pomegranate (*Punica granatum* L.) fruit. *Scientia Horticulturae, 111*(2), 120–127.

[30] Montgomery, D.C. (2017). *Design and analysis of experiments.* John Wiley & Sons.

[31] Mosanna, R., & Behrozyar, E.K. (2015). Morpho-physiological response of maize (Zea mays L.) to zinc nano-chelate foliar and soil application at different growth stages. *Journal on New Biological Reports*, *4*(1), 46-50.

[32] Naik, S.K., & Chand, P.K. (2011). Tissue culture-mediated biotechnological intervention in pomegranate: a review. *Plant cell reports*, *30*(5), 707-721.

[33] Ojeda-Barrios, D.L., Perea-Portillo, E., Hernández-Rodríguez, O.A., Avila-Quezada, G., Abadía, J., & Lombardini, L. (2014). Foliar fertilization with zinc in pecan trees. *HortScience*, *49*(5), 562-566.

[34] Reisinauer, H.M. (1879). Soil and Plant-Tissue Testing in California, Division of Agricultural Sciences, University of California. *Bulletin*.

[35] Tagliavini, M., Zavalloni, C., Rombola, A.D., Quartieri, M., Malaguti, D., Mazzanti, F., & Marangoni, B. (1998, August). Mineral nutrient partitioning to fruits of deciduous trees. *In 25 International Horticultural Congress, Part 2: Mineral Nutrition and Grape and Wine Quality 512*, 131-140.

[36] Tehranifar, A., & Mahmooditabar, S. (2009). Foliar application of potassium and boron during pomegranate (*Punica granatum*) fruit development can improve fruit quality. *Horticulture & Environmental Biotechnology*, 50(3), 1–6.

[37] Umar, S., Bansal, S.K., Imas, P., & Magen, H. (1999). Effect of foliar fertilization of potassium on yield, quality, and nutrient uptake of groundnut. *Journal of plant nutrition*, *22*(11), 1785-1795.

[38] Weinbaum, S.A. (1988). Foliar nutrition of fruit trees. *Plant growth and leaf applied chemicals*, 81-100.

[39] Scott, N., & Chen, H. (2003). *Nanoscale Science and Engineering for Agriculture and Food Systems: A Report Submitted to Cooperative State Research, Education and Extension Service, the United States Department of Agriculture: National Planning Workshop, November 18-19, 2002, Washington, DC*. USDA.

[40] Subramanian, K.S., Manikandan, A., Thirunavukkarasu, M., & Rahale, C.S. (2015). Nano-fertilizers for balanced crop nutrition. In *Nanotechnologies in food and agriculture*, *Springer, Cham*, 69-80.

[41] Swietlik, D. (1999). Zinc nutrition in horticultural crops. *Horticultural reviews*, *23*, 109-180.

[42] Talaie, A., Badmahmoud, A.T., & Malakout, M.G. (2001). The effect of foliar application of N, B and Zn on quantitative and qualitative characteristics of olive fruit. *Journal of Agricultural Sciences Iran*, *32*(4), 726-736.

[43] Yadav, M.K., & Solanki, V.K. (2015). Use of micronutrients in tropical and sub-tropical fruit crops: A review. *African Journal of Agricultural Research*, *10*(5), 416-422.