



## EFFICACY OF ZINC FOLIAR APPLICATION FROM DIFFERENT SOURCES ON PRODUCTIVITY AND FRUIT QUALITY OF WONDERFUL POMEGRANATE TREES

[20]

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### ABSTRACT

Nanoparticles (NPs), especially from micronutrients, are recently motivated for replacing their common mineral counterparts. To evaluate their comparative efficacy, this investigation was conducted to estimate the impact of foliar application of zinc through different sources on productivity, fruit quality and improve marketable fruit of "Wonderful" pomegranate trees. The field experiment was performed during two seasons (2017 and 2018) on seven year old pomegranate trees "Wonderful" cv., cultivated in a private "Hegazi" farm located at 57 km. from Cairo on the road to Alex., Egypt. Four sources of zinc named "Zinc Sulphate, Zinc mannitol complex, Bio-Nano zinc (Bio Zn NPs) and Zinc Oxide nanoparticle (ZnO NPs) with four rates from each other were sprayed twice (the first before one week from full bloom and the second after a month from the first). So the experiment included seventeen treatments in a sample study spread in a randomized complete block design by five replicates. Results explicated that the greatest significant values of fruit set% were recorded by Bio Nano Zinc (Bio Zn NPs) treatments especially (400 ppm Bio-Nano Zinc (Bio Zn NPs)). Spraying with (3000, 4000 ppm Zn mannitol complex) and (300, 400 ppm Bio-Nano Zinc (Bio Zn NPs)) showed significantly the greatest values of productivity, improves marketable fruits and fruit quality of "Wonderful" pomegranate trees. So it could be recommended by spraying "Wonderful" pomegranate trees by 3000 ppm Zinc mannitol

complex or 300 ppm Bio-Nano zinc (Bio Zn NPs). Another important point is that the application of Bio Zn NPs fertilizer at around 10% from the commercial dose of zinc sulphate resulted in the same results without any change in the productivity, further researches are needed to study a further low level of Zinc Oxide nanoparticle (ZnO NPs) below (100 ppm Zinc Oxide nanoparticle (ZnO NPs)) which may be improving yield and fruit quality.

**Keywords:** Foliar Application, Pomegranate, Yield, Zinc sulphate, Zinc mannitol complex, Bio Nano zinc, Zinc Oxide nanoparticle

### INTRODUCTION

Pomegranate (*Punica granatum* L.) is a fruit shrub well adapted to arid & semi-arid zones, where the winter is cold and the summer is long, hot and dry. In Egypt, pomegranate cultivated areas reached about 35983.9 Hectares (85676 feddans) with fruit production of 381426 metric tons, according to (M.A.L.R.R. 2016).

Frequency of shortages of micronutrients into fruit trees have increased due to ultra-high density cropping in recent years, micronutrients leaching, increased purity of mineral fertilizers, soil erosion, use of marginal land (high pH and EC) for the production of crops and the climate change due to warm and dry weather may be another consequential reason for the disorders (**Zia et al 2006**). Zinc (Zn) deficiency is known in calcareous soils of arid & semi-arid zones, where pomegranate orchards are extensive. Zn is important for the normal and healthful growth of plants, humans and animals. It plays the main role in numerous key plant physiological pathways related to the formation of sugar and photosynthesis, protein and hormone synthesis, production of seeds and resistance to diseases (**Bayvordi 2006**). Zinc is needed for the synthesis of the amino acid tryptophan which is a precursor of IAA. (**Jamali et al 2011**). So, zinc deficiency reduced growth and yield of plants (**Hafeez et al 2013**). In traditional farming practices, Zn is applied as zinc Sulphate (ZnSO4) or EDTA-Zn through soil or foliar application.

Mannitol is mixed with the zinc element penetrating the tissues of plants easily. Zn-mannitolcomplex was considered as one of the most separated leaf fertilizers, it is easily absorbed by stomata with less energy consuming than other fertilizers such as amino acids. Mannitol helps stomata to absorb most of the fertilizer solution unlike other fertilizers and Mannitol is considered as a diffuser because of its capability to absorb water.

Many problems from commercial chemical fertilizers have been noticed like groundwater and atmospheric pollution, eutrophication, soil acidification, decreased soil fertility and biodiversity loss (Kourgialas et al 2017). Therefore, recently, there has been numerous effort to replace chemical fertilizers with environmentally friendly nano-fertilizers and biosynthesized nano-fertilizers (Liu and Lal 2015). One nanometer (nm) means10<sup>-9</sup> parts of a meter or one billionth. Nanotechnology, using nanoparticles (NPs), presents new plant nutrition approaches (Liu and Lal 2015). Nano-fertilizers at a scale (1-100 nm) greatly increase the points of influence due to their small size, in turn, micronutrient interplay and absorption in crop fertilization could be improved (Singh et al 2017). Nano-fertilizer foliar applications have confirmed that they are more effective compared to conventional fertilizers because they're provide plant nutrients in a controlled and gradual way, and also needs less quantities than conventional fertilizers (Davarpanah et al 2016 and Kah et al 2018). Nanotechnology will enable us make very high-quality, very fast and low-cost products (Liu et al 2003). Nanotechnology has many uses in plant breeding, biotechnology genetics, disease control, and fertilizer technology, etc. However, presently there is a limited understanding about using this new technology on human health and safety risks. Controlled implementation of the new technology will open chances for improving

new materials and methods to improve our capability to develop more efficiently, more sensitive and reliable analytical systems (Jha et al 2011).

Biosynthesized Nano-fertilizers are up to date and most technically progressed method of fertilizing mineral nutrients to crops. The application of biosynthesized Nano fertilizers in agricultural maybe lead to sustainable development. Therefore, this leads to the sustainable agriculture by putting the least inputs and generating the least wastes, reducing nutrient losses, and release nutrients at a valid rate for plant need compared to traditional orchards. There are slight differences between Nano fertilizers and Nano-fertilizers biosynthesized depending on their methods of application, mechanisms in the plant and soil, application methods, optimum rates of addition and their impact on the environment (El-Ghamry et al 2018). Therefore, this study was carried out to compare the efficacy of the application of foliar Zn by Nano-fertilizers and conventional fertilizers on productivity, which improves marketable fruits and fruit quality of "Wonderful" pomegranate trees.

#### MATERIALS AND METHODS

The present investigation was carried out in two consecutive seasons (2017 and 2018) on seven year- old pomegranate trees "Wonderful" cv., planted at 3x 5m under drip irrigation system, at a private "Hegazi" farm located at 57 km. from Cairo on the road to Alex., Egypt. The orchard soil texture was sandy loam, the soil and water were analyzed according to (Wilde et al 1979) as presented in Table (1) and Table (2). To investigate this experiment, eighty-five trees were selected as mostly uniform in vigorous growth, healthy, fruitful, no visual nutrient deficiency symptoms and were subjected to the same agriculture practices adopted in the farm program. Four sources of zinc named "Zinc Sulphate, Zinc Mannitol Complex, Bio-Nano Zinc (Bio Zn NPs) and Zinc Oxide nanoparticle (ZnO NPs) with four rates from each other were sprayed in addition to the control treatment. So the experiment included seventeen treatments and was laid out in a sample study in a randomized complete block design with five replicates and each replicate was illustrated by one tree. Selected trees were sprayed twice (the first before one week from full bloom and the second after a month from the first) by the aqueous solution of different tested zinc materials until the point of runoff. Tween 80 at 0.1 percent was used as a wetting agent for all treatments. The control treatment was sprayed with tap water + tween.

## Efficacy of Zinc Foliar Application from Different Sources on Productivity and Fruit Quality of Wonderful Pomegranate trees

Table 1. Physical and chemical analysis of soil

| Soil                     | Surface         | 30 cm | 60 cm |  |  |
|--------------------------|-----------------|-------|-------|--|--|
| characteristics          | sample          | depth | depth |  |  |
| Particle size distrib    | oution %        |       |       |  |  |
| Sand (%)                 | 96.17           | 94.73 | 93.03 |  |  |
| Silt %                   | 1.51            | 3.11  | 3.58  |  |  |
| Clay %                   | 2.32            | 2.16  | 3.39  |  |  |
| Soil texture             | Sandy           |       |       |  |  |
| <b>Chemical characte</b> | ristics         |       |       |  |  |
| рН                       | 7.81            | 7.77  | 7.48  |  |  |
| EC(dsm-1)                | 2.79            | 2.55  | 2.63  |  |  |
| Soluble anions (me       | eq / 100g soil) |       |       |  |  |
| CO3=                     | -               | -     | -     |  |  |
| HCO3-                    | 1               | 0.7   | 0.9   |  |  |
| Cl-                      | 22.3            | 21.8  | 22    |  |  |
| SO4=                     | 4.62            | 3.06  | 3.42  |  |  |
| Soluble cations (m       | eq / 100g s     | oil)  |       |  |  |
| Ca++                     | 4.5             | 3     | 3.7   |  |  |
| Mg++                     | 1               | 1.1   | 1.2   |  |  |
| Na⁺                      | 22.12           | 20.13 | 20.41 |  |  |
| K+                       | 0.3             | 1.33  | 1.01  |  |  |

Table 2. Chemical characteristics of water

| Parameters              | Values |
|-------------------------|--------|
| рН                      | 8.4    |
| EC(dSm-1)               | 1.19   |
| Soluble cations (meq\l) |        |
| Ca++                    | 1.5    |
| Mg <sup>++</sup>        | 1.12   |
| Na⁺                     | 8.45   |
| K+                      | 0.89   |
| Soluble anions (meq\l)  |        |
| CO₃=                    | -      |
| HCO <sub>3</sub> -      | 1.4    |
| Cl-                     | 6.46   |
| SO4=                    | 4.1    |

## The experiment included the following seventeen treatments

- T<sub>1</sub>: spraying with tape water (control)
- T<sub>2</sub>: spraying with 1000 ppm Zn SO<sub>4</sub> 22% Zinc
- T<sub>3</sub>: spraying with 2000 ppm Zn SO<sub>4</sub>
- T<sub>4</sub>: spraying with 3000 ppm Zn SO<sub>4</sub>
- T<sub>5</sub>: spraying with 4000 ppm Zn SO<sub>4</sub>
- T<sub>6</sub>: spraying with 1000 ppm zinc mannitol complex 22% Zinc

 $T_7$ : spraying with 2000 ppm zinc mannitol complex  $T_8$ : spraying with 3000 ppm zinc mannitol complex  $T_9$ : spraying with 4000 ppm zinc mannitol complex

T<sub>10</sub>: spraying with 100 ppm Bio Nano zinc (Bio Zn NPs) 100% Zinc

- T<sub>11</sub>: spraying with 200 ppm Bio Nano zinc
- T<sub>12</sub>: spraying with 300 ppm Bio Nano zinc
- T<sub>13</sub>: spraying with 400 ppm Bio Nano zinc
- T<sub>14</sub>: spraying with 100 ppm Zinc Oxide nanoparticle (ZnO NPs) 80% Zinc
- $T_{15}$  spraying with 200 ppm Zinc Oxide nanoparticle

 $T_{16}$ : spraying with 300 ppm Zinc Oxide nanoparticle  $T_{17}$ : spraying with 400 ppm Zinc Oxide nanoparticle



Fig. 1. Zn-mannitol- complex  $(\underline{C}_6\underline{H}_{13}\underline{O}_6)_2$ zn. (Ming et al 2015)

#### Preparation of zinc mannitol complex

Preparing Zinc Sulfate was conducted by using Mannitol mixure in a ratio of 1.5 mol : 1.0 mol respectively, 36.44 gm of Mannitol and 88.83 gm of Zinc Sulfate heptahydrate were dissolved in 206 ml of distilled water, the clear solution was obtained from Zn-mannitol- complex ( $\underline{C}_{6}\underline{H}_{13}\underline{O}_{6}$ )<sub>2</sub>Zn. (Ming et al 2015).

One litter of different concentrations of zinc mannitol complex (1000ppm, 2000ppm, 3000ppm, and 4000ppm) were prepared by taking 4.12 ml, 8.24 ml, 12.36 ml, and 16.48 ml respectively from the above –mentioned stock solution in four different 1L measuring flasks. The volume of each flask was adjusted using distilled water. Each concentration in 1L volume was used as a treatment for a tree.

# Chemical synthesis of Zinc Oxide Nano Particles

ZnSO<sub>4</sub>. 7H<sub>2</sub>O and NaOH were used in the following preparation. Slowly add sodium hydroxide solution to the zinc sulfate aqueous solution. Drop wisely in a molar ratio of 1:2 under vigorous stirring, and the stirring will continue for 12 h. The precipitation collected will be filtered with deionized water and cleaned thoroughly. The precipitate is dried in a 100° C oven and ground to a fine powder using age mortar. (Mohan Kumar et al 2013). Finally, we obtain Nanoparticles of zinc oxide, average size 17 nm (range from 16 to 18 nm) as shown in Fig. (2).



**Fig. 2.** Nano particles of zinc oxide composition patent-protected, average size 17 nm (range from 16 to 18 nm).

#### **Bio synthesis of Zinc Nano Particles**

**Sampling**: 32 Arid soil samples from 11 different locations were collected in the Egyptian desert as shown in **(Table 3)**. These soil samples were used to isolate bacteria. Isolation was conducted by suspending 10grams of soil in 90 ml of sterile distilled water and serial dilution under sterile conditions. One ml of each suspension was spread to the surface of the MSM sterile mineral salt media. **(Schlegel et al 1961)** and incubated at 30  $\pm$ 2°C for 7, 14 and 21 days.

- Isolation, purification and identification of bacteria: Bacteria colonies grown on mineral salts media MSM (Schlegel et al 1961) were picked up and recultivated several times for purity. Based on their cultural and morphological characteristics, the purified bacteria isolates are named to the genus.

| Sample No.      | Location            | No. soil<br>samples | Latitudes | Longitudes |
|-----------------|---------------------|---------------------|-----------|------------|
| New valley gove | ernorate            |                     |           |            |
| 1               | Black Desert        | 4                   | 28 °.386  | 27 °.608   |
| 2               | White Desert        | 2                   | 28 °.454  | 27 °.677   |
| 3               | Farafra 1           | 3                   | 27 °.984  | 27 º.219   |
| 4               | Major General Sabih | 3                   | 27 °.734  | 26 º.491   |
| 5               | Abohrirh            | 2                   | 27 °.650  | 26 °.499   |
| 6               | Abu mankar          | 2                   | 27 °.598  | 26 °.495   |
| 7               | Mountains Negev 1   | 2                   | 27 °.601  | 26 °.494   |
| 8               | Great Sand Sea      | 5                   | 27 °.665  | 26 º.537   |
| 9               | Mountains Negev 2   | 4                   | 27 °.667  | 26 °.493   |
| 10              | Paris 1             | 2                   | 31 º.281  | 28 º.113   |
| 11              | Harga Oases         | 3                   | 31 °.082  | 28 º.211   |

| Table 3. Si | te descriptic | ons of soil | s samples |
|-------------|---------------|-------------|-----------|
|-------------|---------------|-------------|-----------|

### Identification of D3 isolate

Gram stain conducted using the method described above (Collins and Patricia 1984). The isolate was identified using partial 16S rRNA gene analysis. The universal set of primer was 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (I) (GGTTACCTTGTTACGACTT). The sequence was analyzed using an http://www.ncbi.nlm.nih.gov BLAST algorithm and submitted to Gen Bank. Multiple alignments of sequences and evolutionary history were compared with other sequences downloaded from the NCBI database (http://www.ncbi.nlm.nih.gov). Evolutionary history was inferred from the Maximum Likelihood Method. and Tamura-Nei model (**Tamura-Nei model, 1993**). The phylogenetic tree was established with MEGA X (**Kumar et al 2018**).

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- Screening for zinc nanoparticles synthesis by D3 isolates: Ability of D3 isolates to produce zinc nanoparticles was tested by UV-visible spectrophotometer test, then by Zeta Sizer nano-series (Particle size measurement).

- Preparation of biomass: D3 isolates were grown according to (Waghmare et al 2011).

- **Spectrophotometry:** The reduction of metal ions was confirmed by a qualitative UV-visible spectrophotometer supernatant test. ZnNPs were characterized by a two-beam UV-visible spectrophotometer (Shimadzu-UV 1700) scan of the absorbance spectra in the 200-600 nm wavelength range. The spectra of the surface Plasmon resonances of zinc sulfate in the supernatants were estimated at different times during biosynthesis. The control (without D3 biomass) showed no change in the color of the aqueous solution when incubated in similar conditions.

- Zeta Sizer nano-series (Particle size measurement): Particle sizing measurements using laser diffractometry were performed using nano-series Zeta Sizer (Nano ZS). All measurements were estimated in the range between 0.6:6000 nm (Tarafdar et al 2012). All previous techniques were carried out in the central laboratory for nanotechnology and advanced materials, Agriculture Research Center, Giza, Egypt.

- **Detection of zinc nanoparticles:** A number of 2 methods were applied to detected zinc nanoparticles:

#### a. Transmission Electron Microscopy

To understand the surface topology and size of NPs synthesized, Transmission Electron Microscope (TEM) was used. According to (Fultz and Howe, 2007).

# b. Fourier transforms infrared spectroscopy (FTIR) spectrum analysis

The NPs samples were analyzed in FT-IR to identify the possible biomolecules (chemical functional groups) responsible for decreasing the concentration of zinc ions to Zn NPs by the cell filtrate, described by (**Prati et al 2010**). All previous techniques were applied to Nanotechnology and advanced materials central lab, Agriculture Research Center, Giza, Egypt.

### The following characteristics were studied

**1- The percentage of fruit set:** At full bloom (on the first of May) the total number of flowers was counted. Then after two weeks of full bloom, the fruit set was measured using the equation below:

### Fruit set (%) = [Total number of fruitlets / Total number of flowers] x 100

2- Yield: At maturity time (the first week of October) in each season, the average number of fruits / tree was counted and at the harvest time fruits of each tree (replicate) were weighted to get the average yield/tree (kg). Then, we separated and counted marketable and non-marketable fruits and calculated the percentage of each. Twenty- five fruits from every tree (replicate) were taken to get the average fruit weight.

**3-Fruit quality**: For every season, a sample of five fruits / tree was taken randomly to evaluate the fruit's physical and chemical properties: Arils weight and juice weight were determined and then we calculated the percentages of arils /fruit weight.

Total acidity (TA) was determined by titrating10 ml of juice with 0.1 mol/L NaOH to pH8.1 (AOAC 1984). The percentage of acidity was calculated as an anhydrous citric acid per 100 milliliters of juice. Total soluble solids (TSS) were determined as a percentage of juice by hand refractometer. The TSS / Acid ratio was calculated. The content of ascorbic was determined according to (AOAC 1995). Ascorbic acid was measured as mg/100 ml of juice. Total anthocyanins in juice samples were measured spectrophotometrically according to (Rapisarda et al 2000). Total polyphenol (TP) and tannin (TT) contents of juice samples were estimated by a colorimetric assay based on the procedures described by (Alsiede et al 2015).

**4-Statistical analysis:** The experimental data were statistically analyzed using the variance analysis as reported by **(Snedecor and Cochran, 1980)** applying Duncan's multiple range tests at 5 % **(Duncan, 1955)**.

### **RESULTS AND DISCUSSION**

### Effect on fruit set percentages and yield of wonderful pomegranate

Results in Table (4) show the effect of spraying by different sources and rates of zinc on fruit set percentages, fruit weight, fruit number and yield of wonderful pomegranate during two growing seasons (2017 and 2018). Generally, results showed that in the two seasons, fruit set percentages of Pomegranate were significantly affected by diverse treatments. In general, in the two seasons, all ZnO NPs treatments provided the least significant values of fruit set percent, followed by control and Zn SO4 treatments. On the contrary, the maximum significant values of fruit set percent were recorded by Bio-Nano Zn treatments especially T13 (400 ppm Bio- ZnNPs) whereas, Zn mannitol treatments gave intermediate values between the treatments stated above. Data from Eiada & Mustafa (2013) indicated that zinc and manganese spray had a significant increase in the pomegranate fruit set compared with the untreated trees in the two seasons.

The fruit weight data showed that, control treatment, Bio- Nano Zn treatments except T<sub>13</sub> and high levels of Nano Zn (300 and 400 ppm ZnO NPs) gave the least significant fruit weight values, particularly in the first season. On the contrary, the maximum significant values were gained from the treatment of Zn mannitol especially T<sub>8</sub> (3000 ppm Zn mannitol) and T<sub>9</sub> (4000 ppm Zn mannitol) followed by high levels of ZnSO<sub>4</sub> T<sub>5</sub> (4000 ppm Zn SO<sub>4</sub>).

Regarding fruit number/tree, in the two seasons,  $T_{17}$  (400ppm ZnO NPs) gave the least significant values of fruit number/tree followed closely by  $T_1$  (control treatment) in the second season only. In the two seasons, the second and third levels of ZnO NPs (200 and 300ppm) gave the greatest values of fruit number/tree some other treatments gave similar values but the trend differed from season to another.

Results revealed that, in the two seasons the least significant values of yield were recorded by the high level of ZnO NPs (400 ppm) followed by T<sub>1</sub> (untreated trees), T<sub>2</sub> (1000 ppm ZnSO<sub>4</sub>), T<sub>10</sub> (100ppm Bio-ZnNPs) and T<sub>11</sub> (200 ppm Bio-ZnNPs). On the other hand, in the two seasons the high yield was gained from T<sub>5</sub> (4000 ppm ZnSO<sub>4</sub>), T<sub>8</sub> (3000 ppm Zn mannitol), T<sub>9</sub> (4000 ppm Zn mannitol), T<sub>12</sub> (300 ppm Bio-ZnNPs) and T<sub>13</sub> (400 ppm Bio-ZnNPs). Besides, **Eiada & Mustafa (2013)** reported that the mixture of Zn, Mn and Fe affected Pomegranate trees, resulting in an increase in fruit set, fruit weight and the yield. On the other side, **Davarpanah et al** 

(2016) found that foliar spraying with Nano-Zn at 60 ppm + Nano-B at 6.5 ppm resulted in the maximum number of fruits per tree and the greatest yield compared with other treatments.

## Effect on marketable and unmarketable fruits of wonderful pomegranate

Results in **Table (5)** show the effect of spraying by different sources and rates of zinc on marketable and unmarketable fruits of pomegranate during 2017 and 2018 seasons. The trend was more clear in the first season than the second one. The results revealed that, the high values of marketable fruits and least values of unmarketable fruits were gained by the following treatments:  $T_5$  (4000 ppm ZnSO4),  $T_9$  (4000 ppm Zn mannitol),  $T_{12}$  (300 ppm Bio-ZnNPs) and  $T_{13}$  (400 ppm Bio-ZnNPs)

### Effect on some fruit physical properties of wonderful pomegranate

Results in **Table (6)** show the effect of spraying by different sources and rates of zinc on some physical properties of wonderful pomegranate in both 2017 and 2018 seasons.

Data concerning arils weight revealed that the maximum significant values were recorded by T9 (4000 ppm Zn mannitol) in the two growing seasons followed closely by T8 (3000 ppm Zn mannitol) and T12 (300 ppm Bio- ZnNPs) in the second season only.

Regarding juice weight the data indicated that, control treatment (T1) followed by T17 (400 ppm ZnO NPs) gave the lowest significant values of juice weight. On the other hand, the maximum significant values of juice weight were gained by T8 (3000 ppm Zn mannitol) and T9 (4000 ppm Zn mannitol) in the two growing seasons followed closely by T7 (2000 ppm Zn mannitol), T5 (4000 ppm ZnSO4) and T2 (1000 ppm ZnSO4) in the first season only and T12 (300 ppm Bio- ZnNPs) in the second season. In this respect, **Hasani1 et al (2012)** reported that the best treatment was the combination of manganese sulfate at 0.6% and zinc sulfate at 0.3% for pomegranate trees to increase the juice content of arils.

From the results, it could be noticed that different Zn treatments gave lacked significance effect especially on arils/ fruit weight% in the first seasons whereas the trend was more clear in the second season than the first one and the maximum significant values for three physical properties percentage were recorded by T8 (3000 ppm Zn mannitol) and T12 (300 ppm Bio- ZnNPs).

| ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, | Fruit    | set%     | Fruit we  | ight (g) | Fruit num | bers/tree | Yield (k | g / tree) |
|--|----------|----------|-----------|----------|-----------|-----------|----------|-----------|
| l reatment                             | 2017     | 2018     | 2017      | 2018     | 2017      | 2018      | 2017     | 2018      |
| T1: Tap water (control)                | 38.4 ef  | 41.5 e   | 327.5 h   | 391.6 ij | 106.0a-d  | 86.6 b-d  | 37.4 gh  | 34.9 hi   |
| T2: 1000 ppm -Znso4                    | 39.7 ef  | 40.0 ef  | 391.6 fg  | 408.5 hi | 97.3 cd   | 106.3 a   | 37.7 f-h | 38.4 e-h  |
| T3: 2000 ppm -Znso4                    | 41.3 e   | 41.3 e   | 409.1d-g  | 452.0 g  | 107.0 a-d | 96.6 a-c  | 40.7 ef  | 42.5 c-e  |
| T4: 3000 ppm -Znso4                    | 43.4 de  | 41.0 e   | 466.6 bc  | 493.5 f  | 102.3 b-d | 94.6 a-c  | 43.9 cd  | 44.3 b-d  |
| T5: 4000 ppm -Znso4                    | 49.5 d   | 41.0 e   | 487.5 ab  | 585.8 c  | 103.0 b-d | 84.0 c-e  | 46.7 a-c | 45.6 a-c  |
| T6: 1000 ppm-Mannitol-zn               | 41.7 e   | 54.6 d   | 379.1 g   | 561.3 d  | 106.0 a-d | 72.6 e    | 38.1 f-h | 39.8 d-g  |
| T7: 2000 ppm-Mannitol-zn               | 49.3 d   | 54.7 d   | 442.5 cd  | 539.1 de | 94.6 d    | 79.6 de   | 40.7 ef  | 41.9 c-f  |
| T8: 3000 ppm-Mannitol-zn               | 59.0 c   | 60.5 cd  | 456.6 bc  | 654.0 ab | 112.6 ab  | 72.3 e    | 47.0 a-c | 45.5 a-c  |
| T9: 4000 ppm-Mannitol-zn               | 65.8 ab  | 67.5 c   | 519.1 а   | 674.0 a  | 102.3 b-d | 77.0 de   | 49.6 a   | 49.5 a    |
| T10: 100 ppm - Bio- ZnNPs              | 61.7 bc  | 78.3 b   | 375.8 g   | 365.6 k  | 103.3 b-d | 99.3 ab   | 38.7 fg  | 36.7 g-i  |
| T11: 200 ppm - Bio- ZnNPs              | 64.0 a-c | 95.5 a   | 385.8 g   | 520.5 e  | 108.6 a-c | 84.6 c-e  | 39.9 e-g | 42.2 c-e  |
| T12: 300 ppm - Bio- ZnNPs              | 64.3 a-c | 94.5 a   | 396.6 e-g | 636.1 b  | 117.6 a   | 77.6 de   | 46.0 bc  | 48.1 ab   |
| T13: 400 ppm - Bio- ZnNPs              | 68.9 a   | 91.2 a   | 436.6 c-e | 552.6 d  | 112.3 ab  | 89.6 b-d  | 48.2 ab  | 48.6 ab   |
| T14: 100 ppm - ZnO NPs                 | 33.5 f   | 34.3 e-g | 430.8 c-f | 444.3 g  | p-d 9.66  | 88.0 b-d  | 42.0 de  | 37.3 f-h  |
| T15: 200 ppm - ZnO NPs                 | 24.0 g   | 32.8 fg  | 437.5 c-e | 414.1 h  | 106.6 a-d | 100.3 ab  | 45.1 bc  | 40.2 d-g  |
| T16: 300 ppm - ZnO NPs                 | 22.8 g   | 30.8 g   | 403.0d-g  | 378.5 jk | 110.0 a-c | 96.3 a-c  | 41.9 de  | 34.6 hi   |
| T17: 400 ppm - ZnO NPs                 | 21.4 g   | 26.9 g   | 396.6 e-g | 365.8 k  | 94.3 d    | 89.3 b-d  | 35.5 h   | 32.4 i    |

Means having the same letter(s) within a column are insignificantly different at 5% level.

Table 4. Effect of spraying some sources and rates of Zinc on yield and Productivity of "Wonderful" pomegranate trees in 2017 and 2018 seasons

**Table 5.** Effect of spraying some sources and rates of Zinc on Marketable fruits and unmarketable fruits

 percentage of "Wonderful" pomegranate trees in 2017 and 2018 seasons.

| Turada                           | Marketabl | e fruits% | unmarketa | ble fruits% |
|----------------------------------|-----------|-----------|-----------|-------------|
| Ireatment                        | 2017      | 2018      | 2017      | 2018        |
| T1: Tap water (control)          | 66.6 c    | 64.8 ef   | 33.3 a    | 35.1 ab     |
| T2: 1000 ppm -Znso4              | 66.3 c    | 67.4 de   | 33.6 a    | 32.5 bc     |
| T3: 2000 ppm -Znso4              | 68.3 c    | 68.1 de   | 31.6 a    | 31.8 bc     |
| T4: 3000 ppm -Znso4              | 79.9 b    | 74.9 c    | 20.0 b    | 25.0 d      |
| T5: 4000 ppm -Znso4              | 84.1 ab   | 74.8 c    | 15.8 bc   | 25.1 d      |
| T6: 1000 ppm-Mannitol-zn         | 69.0 c    | 75.1 c    | 30.9 a    | 24.8 d      |
| T7: 2000 ppm-Mannitol-zn         | 72.6 c    | 83.0 b    | 27.3 a    | 16.9 e      |
| T8: 3000 ppm-Mannitol-zn         | 80.5 b    | 82.0 b    | 19.4 b    | 17.9 e      |
| T9: 4000 ppm-Mannitol-zn         | 88.8 a    | 84.9 b    | 11.2 c    | 15.0 e      |
| T10: 100 ppm <b>-</b> Bio- ZnNPs | 72.9 c    | 82.2 b    | 27.0 a    | 17.7 e      |
| T11: 200 ppm <b>-</b> Bio- ZnNPs | 67.8 c    | 82.4 b    | 32.1 a    | 17.5 e      |
| T12: 300 ppm <b>-</b> Bio- ZnNPs | 85.2 ab   | 93.3 a    | 14.7 bc   | 6.6 f       |
| T13: 400 ppm <b>-</b> Bio- ZnNPs | 83.6 ab   | 87.2 b    | 16.3 bc   | 12.7 e      |
| T14: 100 ppm - ZnO NPs           | 71.7 c    | 68.9 c-e  | 28.2 a    | 31.0b-d     |
| T15: 200 ppm - ZnO NPs           | 72.1 c    | 71.7 cd   | 27.8 a    | 28.2 cd     |
| T16: 300 ppm - ZnO NPs           | 72.1 c    | 71.8 cd   | 27.8 a    | 28.1 cd     |
| T17: 400 ppm - ZnO NPs           | 70.5 c    | 59.0 f    | 29.4 a    | 40.9 a      |

Means having the same letter(s) within a column are insignificantly different at 5% level.

**Table 6.** Effect of spraying some sources and rates of Zinc on some fruit physical properties of "Wonderful"

 pomegranate trees in 2017 and 2018 seasons

| Transforment                     | Arils we  | eight (g) | Juice w   | eight (g) | Arils / f | ruit (%) |
|----------------------------------|-----------|-----------|-----------|-----------|-----------|----------|
| Ireatment                        | 2017      | 2018      | 2017      | 2018      | 2017      | 2018     |
| T1: Tap water (control)          | 193.1 gh  | 219.6 gh  | 108.8 f   | 134.3 i   | 54.4 c    | 56.0 b-e |
| T2: 1000 ppm <b>-</b> Znso4      | 220.8 e-h | 228.1 fg  | 194.0 ab  | 170.3 gh  | 56.3 a-c  | 55.8 b-e |
| T3: 2000 ppm <b>-</b> Znso4      | 229.1 ef  | 248.6 e   | 155.8c-e  | 187.6 ef  | 56.0 a-c  | 55.0 de  |
| T4: 3000 ppm <b>-</b> Znso4      | 286.5 ab  | 268.8 d   | 181.5bc   | 195.0 e   | 61.3 a    | 54.4 e   |
| T5: 4000 ppm <b>-</b> Znso4      | 274.1 bc  | 325.3 b   | 192.5ab   | 253.0 b   | 56.3 a-c  | 55.5 b-e |
| T6: 1000 ppm-Mannitol-zn         | 222.5 e-h | 306.5 c   | 155.8 с-е | 226.5 c   | 58.6 a-c  | 54.6 de  |
| T7: 2000 ppm-Mannitol-zn         | 263.3 b-d | 292.5 c   | 183.3 a-c | 212.0 d   | 59.4 a-c  | 54.2 e   |
| T8: 3000 ppm-Mannitol-zn         | 273.3 b-d | 372.0 a   | 210.0 a   | 297.3 a   | 59.8 a-c  | 56.8 a-d |
| T9: 4000 ppm-Mannitol-zn         | 314.1 a   | 374.3 a   | 185.8 ab  | 303.3 a   | 60.4 ab   | 55.5 b-e |
| T10: 100 ppm <b>-</b> Bio- ZnNPs | 207.5 f-h | 211.3 h   | 150.8de   | 164.0 h   | 55.2 bc   | 57.8 ab  |
| T11: 200 ppm <b>-</b> Bio- ZnNPs | 189.1 h   | 304.3 c   | 139.1 e   | 233.3 c   | 48.9 d    | 58.5 a   |
| T12: 300 ppm <b>-</b> Bio- ZnNPs | 239.6 d-f | 366.5 a   | 168.1b-d  | 301.5 a   | 60.3 ab   | 57.6 a-c |
| T13: 400 ppm <b>-</b> Bio- ZnNPs | 252.5 с-е | 305.6 c   | 166.6 b-d | 222.1 ed  | 58.0 a-c  | 55.3 с-е |
| T14: 100 ppm <b>-</b> ZnO NPs    | 249.1 с-е | 250.1 e   | 151.6 de  | 178.8 fg  | 57.8 a-c  | 56.2 a-e |
| T15: 200 ppm - ZnO NPs           | 251.1 c-e | 239.1 ef  | 173.3 b-d | 182.1 e-g | 57.4 a-c  | 57.7 ab  |
| T16: 300 ppm <b>-</b> ZnO NPs    | 226.6 e-g | 212.6 h   | 157.6 с-е | 164.0 h   | 56.1 a-c  | 56.1 b-e |
| T17: 400 ppm - ZnO NPs           | 222.5 e-h | 204.8 h   | 135.8 e   | 157.1 h   | 56.0 a-c  | 55.9 b-e |

Means having the same letter(s) within a column are insignificantly different at 5% level.

### Efficacy of Zinc Foliar Application from Different Sources on Productivity and 283 Fruit Quality of Wonderful Pomegranate trees

### Effect on some fruit chemical properties of wonderful pomegranate

Results in Tables (7.a and 7.b) present the effect of spraying some sources and rates of zinc on fruit chemical properties of "Wonderful" pomegranate during two growing seasons (2017 and 2018). Generally, results have shown that in the two seasons, all fruit chemical properties of pomegranate were significantly affected by diverse treatments. Results in Table (7a) point out that, in the two growing seasons T1 (control treatment) gave the least significant values of TSS% followed by T6 (1000 ppm Zn mannitol). Contrary, T9 (4000 ppm Zn mannitol), T11 (200 ppm Bio- ZnNPs) and T12 (300 ppm Bio- ZnNPs) gave the maximum significant values of TSS% in 2017 and 2018 seasons. El-Khawaga (2007) reported that foliar spraying with 4000 ppm zinc sulfate at the first week of June increased the total soluble solids percentage.

Results revealed that, spraying high levels of Zn irrespective the source (T5, T9 and T13) gave the greatest significant values of total acidity in the two growing seasons. On the other hand, the second and third levels of Bio- ZnNPs (T11 and T12) gave the least significant values of total acidity especially, in the second season. Additionally, **EI-Khawag** (2007). The foliar application of zinc sulfate (2000 and 4000 ppm) on pomegranate trees improved the acidity of 'Manfaluty' pomegranate fruit.

Concerning TSS/acid ratio, the least significant values of TSS/acid ratio were gained by T5 (4000 ppm ZnSO4) in the 2017 and 2018 seasons followed by T3, T4, T6 and T16 in 2017 season. Contrary, it seemed that T12 (300 ppm Bio Nano Zn) gave the maximum significant values of TSS/acid ratio in the two seasons followed by T14 (100 ppm ZnO NPs) in the first season and T11 (200 ppm Bio-ZnNPs), T16 (300 ppm ZnO NPs) in the second season. Moreover, **Hasani1 et al (2012)** reported that the ZnSO4 at both levels (0.3 and 0.6%) had significant effects on the jucie TSS/TA ratio of pom-egranate.

The maximum content of zinc in pomegranate juice samples were recorded by T9 (4000 ppm Zn mannitol) in 2017 and 2018 seasons followed by T5 (4000 ppm ZnSO4) and T8 (3000 ppm Zn mannitol) in the first and second seasons, respectively. The dietary reference amount of zinc required by men is 15 mg/day, 12 mg/day for adult women, 5 mg/day for formula-fed infants and 10 mg/day for preadoles-cent children., **UNICEF (1996)**.

Results in **Table (7b)** show that, the least significant values from both of ascorbic acid and anthocyanin were gained by control treatment (T1) in the two growing seasons. Generally, ascorbic acid and anthocyanin content were increased by increasing the rate of spraying Zn irrespective the source expect with the high level of ZnO NPs in the first season which gave the least significant value of anthocyanin content. In the two growing seasons, it seemed that T9 (4000 ppm Zn mannitol) gave the maximum significant values of ascorbic acid and anthocyanin.

Tannins content was significantly affected by diverse treatments in two seasons. The trend was varied slightly from season to another. In the first season it was gradually decreased in tannins content by increasing the rate of spraying Zn irrespective the source expect with ZnSO4. So, the maximum values of tannins content was recorded by T2 & T10 in the first season and by T5, T6, T7 & T9 in the second season.

Concerning total phenols, T5 (4000 ppm ZnSO4) gave the maximum significant values in the two seasons followed closely by T15 (200 ppm ZnO NPs) in the second season only.

## Screening for bacteria synthesizing zinc nanoparticles

The abilities of 32 bacteria isolates obtained from arid soils were investigated for their abilities to synthesize zinc nanoparticles. Isolates were grown on MSM broth, and 5g wet biomass for each isolate was exposed to sterilized aqueous solution of zinc sulfate at dilution of 0.0001 g/l for 4 days. After addition of aqueous ZnSO4 for 4 days, the mycelia free medium of the 32 isolates showed a color change from colorless to yellow with varying degrees of intensities. Yellow color formation suggests the formation of Zn nanoparticles (Waghmare et al 2011).

Aqueous solutions of all isolates were subjected to spectral analysis using UV- spectrophotometer. Results of UV- measurements showed variation in optical densities between isolates ranging between 0.03 (isolates No. C1) to 0.59 (isolate No. D3) (Table, 8).

The reaction mixtures of 10 isolates showed relatively high optical densities of 0.4 or more, so they were selected for more confirmatory analyses to measure particle size using Zeta -seizer potential. The results of this test indicated a great variation in particle size between isolates ranging between

| Tanadanand                | TSS      | (%)      | T        | А        | TSS       | ТΑ       | m)nZ     | g/kg)    |
|---------------------------|----------|----------|----------|----------|-----------|----------|----------|----------|
| l reatment                | 2017     | 2018     | 2017     | 2018     | 2017      | 2018     | 2017     | 2018     |
| T1: Tap water (control)   | 13.7 h   | 12.2 g   | 1.22 ef  | 1.06 b-d | 11.16 b-d | 11.5 e-g | 1.37 gh  | 1.10 f   |
| T2: 1000 ppm -Znso4       | 14.9 d-h | 13.8 d-f | 1.36 b-f | 1.10 bc  | 10.93 b-d | 12.5 c-f | 1.98 c-f | 1.31 ef  |
| T3: 2000 ppm -Znso4       | 15.1 c-g | 13.8 d-f | 1.60 ab  | 1.04 b-d | 9.43 d    | 13.3 с-е | 2.02 с-е | 2.06 d-f |
| T4: 3000 ppm -Znso4       | 15.2 c-g | 14.1 d-f | 1.57 a-c | 1.06 b-d | 9.63 d    | 13.2 c-e | 2.34 bc  | 3.29 bc  |
| T5: 4000 ppm -Znso4       | 16.4 a-c | 14.6 b-f | 1.74 a   | 1.43 a   | 9.40 d    | 10.2 g   | 2.95 a   | 3.98 b   |
| T6: 1000 ppm-Mannitol-zn  | 13.9 gh  | 13.8 ef  | 1.44 b-e | 1.04 b-d | 9.60 d    | 13.2 c-e | 1.39 gh  | 1.86 d-f |
| T7: 2000 ppm-Mannitol-zn  | 15.3 b-f | 13.6 f   | 1.29 d-f | 1.07 b-d | 11.86 а-с | 12.7 c-f | 1.47 f-h | 2.79 cd  |
| T8: 3000 ppm-Mannitol-zn  | 16.7 a   | 14.0 d-f | 1.62 ab  | 1.12 b   | 10.40 b-d | 12.4 d-f | 2.27 b-d | 7.93 a   |
| T9: 4000 ppm-Mannitol-zn  | 16.0 a-d | 15.4 a-c | 1.72 a   | 1.36 a   | 9.26 d    | 11.2 e-g | 2.75 ab  | 8.85 a   |
| T10: 100 ppm - Bio- ZnNPs | 15.0 d-h | 13.9 d-f | 1.38 b-f | 0.94 de  | 10.86 b-d | 14.8 c   | 1.18 h   | 1.54 ef  |
| T11: 200 ppm - Bio- ZnNPs | 15.5 a-e | 15.6 ab  | 1.38 b-f | 0.74 f   | 11.20 b-d | 21.2 a   | 1.51 e-h | 1.84 d-f |
| T12: 300 ppm - Bio- ZnNPs | 16.4 a-c | 16.2 a   | 1.36 b-f | 0.74 f   | 12.10 ab  | 21.7 a   | 2.15 cd  | 2.26 c-f |
| T13: 400 ppm - Bio- ZnNPs | 16.6 ab  | 14.4 c-f | 1.53 a-d | 1.36 a   | 10.83 b-d | 10.5 fg  | 2.34 bc  | 2.39 c-e |
| T14: 100 ppm - ZnO NPs    | 15.4 a-e | 14.0 d-f | 1.17f    | 1.02 b-d | 13.23 a   | 14.3 cd  | 1.42 gh  | 1.53 ef  |
| T15: 200 ppm - ZnO NPs    | 14.3 e-h | 14.5 c-f | 1.32 c-f | 0.98 cd  | 10.93 b-d | 17.5 b   | 1.79 d-g | 1.81 d-f |
| T16: 300 ppm - ZnO NPs    | 14.8 d-h | 14.9 b-e | 1.57 a-c | 0.83 ef  | 9.40 d    | 20.1 a   | 1.81 c-g | 1.91 d-f |
| T17: 400 ppm - ZnO NPs    | 14.0 f-h | 15.0 b-d | 1.42 b-f | 0.75 f   | 9.86 cd   | 14.6 cd  | 2.16 cd  | 2.37 c-e |

Means having the same letter(s) within a column are insignificantly different at 5% level.

Table 7a. Effect of spraying some sources and rates of Zinc on some fruit chemical properties of "wonderful" pomegranate trees in 2017 and 2018 seasons

| Treatment                 | Ascorb<br>(mg/10 | ic acid<br>00mL) | Anthoo<br>(mg/* | syanin<br>100g) | Tanı<br>(mg/1 | nins<br>100g) | Total p<br>(mg/1 | nenols<br>00g) |
|---------------------------|------------------|------------------|-----------------|-----------------|---------------|---------------|------------------|----------------|
|                           | 2017             | 2018             | 2017            | 2018            | 2017          | 2018          | 2017             | 2018           |
| T1: Tap water (control)   | 9.06 i           | 9.91 h           | 9.36 fg         | 7.79 ij         | 9.7 e         | 2.8 h         | 425.3 fg         | 446.6 f        |
| T2: 1000 ppm -Znso4       | 9.02 i           | 11.70 g          | 10.43 d-g       | 8.34 h-j        | 13.1 a        | 8.1 d-f       | 473.3 f          | 715.0 b-e      |
| T3: 2000 ppm -Znso4       | 9.77 hi          | 12.69 f-h        | 9.84 e-g        | 8.44 h-j        | 10.3 cd       | 9.1 c-e       | 545.0 e          | 826.6 bc       |
| T4: 3000 ppm -Znso4       | 11.14 f-h        | 16.53 e          | 10.53 d-g       | 13.05 c-e       | 4.5 i         | 8.8 c-e       | 682.0 bc         | 816.6 bc       |
| T5: 4000 ppm -Znso4       | 12.66 c-f        | 27.37 bc         | 15.57 a         | 14.78 a-c       | 4.5 i         | 9.9 a-c       | 850.6 a          | 863.3 ab       |
| T6: 1000 ppm-Mannitol-zn  | 13.35 cd         | 26.00 c          | 10.31 d-g       | 7.08 j          | 10.2 de       | 10.8 ab       | 307.6 h          | 773.3 b-d      |
| T7: 2000 ppm-Mannitol-zn  | 13.33 cd         | 28.27 bc         | 11.82 b-e       | 10.37 f-h       | 8.4 f         | 11.5 a        | 321.6 h          | 716.6 b-e      |
| T8: 3000 ppm-Mannitol-zn  | 15.03 ab         | 30.73 ab         | 12.76 bc        | 10.04 g-i       | 8.1 f         | 9.7 b-d       | 625.0 cd         | 676.6 b-f      |
| T9: 4000 ppm-Mannitol-zn  | 15.77 a          | 33.98 а          | 16.76 a         | 16.45 a         | 3.2 j         | 11.1 ab       | 474.0 f          | 683.3 b-f      |
| T10: 100 ppm - Bio- ZnNPs | 11.39 e-g        | 15.74 ef         | 8.61 gh         | 10.41 f-h       | 13.1 a        | 7.8 ef        | 683.0 bc         | 796.6 b-d      |
| T11: 200 ppm - Bio- ZnNPs | 12.90 c-e        | 21.04 d          | 12.20 b-d       | 11.93 d-g       | 11.1 b        | 5.6 g         | 607.0 d          | 556.6 d-f      |
| T12: 300 ppm - Bio- ZnNPs | 14.08 bc         | 20.80 d          | 12.96 b         | 12.74 c-f       | 10.8 bc       | 3.4 h         | 743.6 b          | 643.3 b-f      |
| T13: 400 ppm - Bio- ZnNPs | 15.26 ab         | 22.20 d          | 13.35 b         | 15.81 ab        | 5.3 h         | 2.4 h         | 365.6 gh         | 716.6 b-e      |
| T14: 100 ppm - ZnO NPs    | 11.05 gh         | 10.73 gh         | 10.63 d-g       | 11.50 d-g       | 11.2 b        | 3.1 h         | 621.3 cd         | 606.6 c-f      |
| T15: 200 ppm - ZnO NPs    | 12.23d-g         | 13.55 e-h        | 10.82 c-f       | 13.60 b-d       | 10.2 de       | 6.8 fg        | 477.6 f          | 1053.3 a       |
| T16: 300 ppm - ZnO NPs    | 12.59 c-g        | 14.21 e-g        | 11.28 b-f       | 12.81 c-f       | 6.0 g         | 7.1 fg        | 332.3 h          | 503.3 ef       |
| T17: 400 ppm - ZnO NPs    | 12.81 c-e        | 14.18 e-g        | 7.16 h          | 10.97 e-g       | 4.8 i         | 6.7 fg        | 365.3 gh         | 616.6 c-f      |

Means having the same letter(s) within a column are insignificantly different at 5% level.

## Efficacy of Zinc Foliar Application from Different Sources on Productivity and Fruit Quality of Wonderful Pomegranate trees

34-922 nm **(Table, 9)**. The isolate D3 was selected for further studies, since it synthesized the smallest particle size of 34nm.

**Table 8.** Screening for *bacteria* isolates synthesizing zinc nanoparticles

| Isolate       | Optical | Isolate | Optical |
|---------------|---------|---------|---------|
| No.           | density | No.     | density |
| C1            | 0.03    | N1      | 0.31    |
| C2            | 0.37    | N2      | 0.08    |
| C3            | 0.08    | N3      | 0.32    |
| C4            | 0.04    | N4      | 0.53    |
| C5            | 0.32    | N5      | 0.38    |
| C6            | 0.067   | N6      | 0.06    |
| C7            | 0.41    | D3      | 0.59    |
| C8            | 0.47    | F9      | 0.37    |
| Z1            | 0.52    | Y12     | 0.05    |
| Z2            | 0.57    | Y1      | 0.08    |
| Z3            | 0.07    | Y3      | 0.02    |
| Z4            | 0.12    | Y4      | 0.09    |
| Z5            | 0.52    | Y7      | 0.32    |
| 5S1           | 0.41    | K2      | 0.50    |
| BLUE          | 0.08    | K3      | 0.46    |
| R2            | 0.11    | E1      | 0.56    |
| Total isolate | es 32   |         |         |

**Table 9.** Particles size measurements by zeta seizer potential for selected bacteria isolates (arranged in ascending order).

| Isolate     | diameter | Isolate | diameter |
|-------------|----------|---------|----------|
| No.         | (nm)     | No.     | (nm)     |
| D3          | 34       | Z5      | 456      |
| Z2          | 70       | K2      | 453      |
| E1          | 123      | C8      | 683      |
| N4          | 165      | K3      | 835      |
| Z1          | 386      | C7      | 922      |
| Total isola | tes 10   |         |          |

-Identification of Achromobacter isolate D3 up to species

### Identification of D3 isolate

Based on gram reaction D3 isolate was a gram negative bacilli non spore former bacterium. Furthermore, sequence obtained from D3 isolate was identified as *Achromobacter deleyi* with 99.56% similarity as showed in phylogenetic tree (**Fig. 3**). The strain was deposited in the GenBank under accession number MN160632.

# Characterization of zinc nanoparticles (ZnNPs) synthesized by Achromobacter deleyi D3

The characteristics of zinc nanoparticles (ZnNPs) synthesized by *Achromobacter deleyi* D3 was investigated by different analytical techniques.

## a- UV-visible spectrophotometer

UV-visible spectrophotometer of aqueous ZnSO4 treated by *Achromobacter deleyi* D3 was conducted after 4 days at different wavelengths (Fig. 4). The ZnSO4 treated *Achromobacter deleyi* showed maximum absorption at 209 nm corresponding to ZnSO4. Waghmare et al (2011) reported that zinc nanoparticles synthesized by *Streptomyces* showed its peak at 350 nm.

# a- Transmission Electron Microscopy (TEM) examination

TEM is a microscopy technique that uses a beam of electrons that transmits and interacts with a specimen forming an image. (Raliya et al 2014).

Representative TEM images showed different sizes of ZnNPs which arose from the biodegradation of ZnSO4 by Achromobacter deleyi (Fig. 5). The diameter of these nanoparticles fluctuated from 20-42nm. In addition, Waghmare et al (2011) recorded that TEM image of zinc nanoparticles indicated well dispersed polymorphic zinc nanoparticles with sizes ranged from c- Fourier transform infrared spectroscopy (FTIR)

Typically, we used infrared spectroscopic analysis to determine the sample's functional groups. It is the absorption measurement of different infrared frequencies by a sample fount in the path of an infrared beam (**Raliya et al 2014**). The wavelength of the absorbed light is a feature of the chemical bond. FTIR can be used for quantitative analyses as the strength of the absorption is proportional to the concentration.

Data presented in **Fig. (6)** show the absorption in the region 1000 to1200 cm<sup>-1</sup> confirming the presence of C-O or O-H and the absorption in the region 2800cm<sup>-1</sup> to 3200cm<sup>-1</sup> confirming the presence of O-H and CHO functional groups. The absorption in the region 1200 to 1500cm<sup>-1</sup> corresponds to C=O. The absorptions in the region 2300 – 2900 cm<sup>-1</sup>, confirmed the presence of carbonyl (-C=O) groups. The absorption in the region 1600 to1900 cm<sup>-1</sup> confirmed the presence of N-H. The presence of these chemical groups, i.e., C-O, O-H, CHO, C=O, - C=O and N-H indicate amide linkage of proteins of biological origin (**Duran et al 2005**).

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**Fig. 3.** Maximum-likelihood phylogenetic tree using 16SrRNA sequence (690 bp). It shows the tree with the highest log probability (-1029.37). The percentage of trees where the associated taxa are clustered next to the branches is shown. Bar, 0.001 substitutions per site.



Fig. (4). UV-visible spectrum of aqueous solution during the synthesis of zinc nanoparticles

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Fig. 5. Transmission electron microscopy image of Zinc nanoparticles synthesized by Achromobacter deleyi



**Fig. 6.** Fourier transform infrared spectroscopy (FTIR) functional groups of zinc nanoparticles synthesized by *Achromobacter deleyi* 

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The present results revealed that zinc nanoparticles could be successfully synthesized by *Achromobacter deleyi*. The U.V. visible spectroscopy and Transmission electron microscopy and Fourier transform infrared spectroscopy clearly show the polymorphic nanoparticles with 20 to 42 nm.

### **Conclusion and Recommendation**

According to the overall above data, it could be proved that control treatment (untreated trees) medium and high levels of Zinc Oxide nanoparticle (ZnO NPs) (200, 300 and 400 ppm ZnO NPs) gave the least significant values of productivity and fruit quality of "Wonderful" pomegranate trees. On the other hand, treatments of Zn mannitol complex and Bio Nano Zn especially (3000, 4000 ppm Zn mannitol complex) and (200, 300, 400 ppm Bio Nano zinc (Bio ZnNPs)) gave the maximum significant values of productivity and fruit quality of "Wonderful" pomegranate trees.

It could be recommended by spraying "Wonderful" pomegranate trees by 3000 ppm zinc mannitol complex or 300 ppm Bio Nano zinc (Bio ZnNPs) twice (the first before one week from full bloom and the second after month from the first), it promoted and increased productivity, marketable fruit and fruit quality while Zinc Oxide nanoparticle (ZnO NPs) treatments especially (200, 300 and 400 ppm), it gave negative effects on productivity and fruit quality of "Wonderful" pomegranate trees .Therefore it seems that the Zinc Oxide nanoparticle (ZnO NPs) effect depends on the concentration and composition of the NPs.

### Significance Statement

This conclusion discovered that Bio Nano zinc (Bio ZnNPs) could be beneficial for spraying "Wonderful" pomegranate trees, reduced the amounts of zinc needed for pomegranate fertilizer. This investigation may help the researchers and growers to uncover the critical areas of using Bio Nano zinc (Bio ZnNPs) as a fertilizer in pomegranate trees. Further more researches are important to study another low levels of Zinc Oxide nanoparticle (ZnO NPs) below (100 ppm ZnO NPs) which may improve yield and quality.

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مقارنة كفاءة الرش الورقى للزنك من مصادر مختلفة على انتاجية وجودة ثمار أشجار الرمان وندرفول

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التجرية سبعة عشر معاملة فى تجرية بسيطة ووزعت فى قطاعات تامة العشوائية ومثلت كل معاملة بخمسة مكررات. وقد أوضحت النتائج:

تم التحصل على أعلى القيم للعقد والثمار المتبقية وأقل القيم للتساقط عند الرش بمعاملات النانو زنك الحيوى وبالأخص المعاملة (النانو زنك الحيوى 400 جزء فى المليون). فى حين أعطت المعاملة (معقد مانيتول الزنك 3000 و 4000 جزء فى المليون) أعلى القيم زنك الحيوى 300 و 400 جزء فى المليون) أعلى القيم للانتاجية وأددت الى زيادة نسبة الثمار القابلة للتسويق وجودة الثمار (الطبيعية–الكيميائية) لثمار أشجار الرمان صنف وندرفول. وعلى ذلك يمكن التوصية برش أشجار الرمان صنف وندرفول بى (3000 جزء فى المليون بالنانو بمعقد مانيتول الزنك أو 300 جزء فى المليون بالنانو ناك الحيوى). علاوه على ذلك 200 جزء فى المليون من النانو المعدنى والتى من المحتمل أن تؤدى الى زيادة فى المحصول وتحسين جودة الثمار .

الكلمات المفتاحية: الرش الورقى، الرمان، محصول، سلفات زنك، معقد مانيتول الزنك، جزيئات نانو زنك حيوي، جزيئات نانو اوكسيد زنك الموجـــــز

ف\_\_\_ الأون\_ة الأخيرة أصربحت الجزيئات النانومتريــــة وبخاصــــة للعناصــر الغذائيـــة الصغرى هم البديل الشائع لنظائرها من الأسمدة المعدنية ولتقيم كفاءة استخدامها تم اجراء هذه التجربة لدراسة تأثير الرش الورقي للزنك مــن مصــادر مختلفــة ودراســة تأثيرهــا علمي الانتاجية ، جودة الثمار وزبادة نسبة الثمار القابلة للتسويق لأشجار الرمان صنف وندرفول. تم اجراء تجربة حقلية في موسمين متتــــالين 2018 – 2017 علـــــي أشــــجار الرمان صنف وندرفول البالغة من العمر 7 سينوات والمزروعية بمزرعية حجيازي عنيد الكيا\_\_و 57 طرب\_\_ق الق\_\_اهرة – الأس\_\_كنربـة الصـــحراوي. حيــث اســـتخدم أربـــع مصـــادر للزنك (سلفات الزنك – معقد مانيتول الزنك-النانو زنك الحيوي – النانو زنك المعدني) حيث تضمنت كل مادة الرش بأربعة تركيــزات وكــان عــدد الرشــات رشــتين الاولـــي قبل اسبوع من التفتح الكامل والثانية بعد شهر من الرشة الأولى. وعلى ذلك تضمنت

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