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# Effect of Foliar Spraying with Zinc Oxide Nanoparticles on Vegetative Growth and Cluster Development of Flame Seedless Grapevine

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## ABSTRACT

This study was conducted during 2018 and 2019 seasons to determine the effect of foliar applications of both zinc oxide nanoparticles (ZnONPs) at concentrations of 60, 120, 240 and 480mgL$^{-1}$, and zinc chelated at 1.5 gL$^{-1}$ as well as without zinc application (control) for seven-years old Flame Seedless grapevines. The chosen vines were trellised by Spanish Parron supporting system, grown in a clay loam soil, spaced at 2 x 3 meters apart, irrigated under surface irrigation system, and spur-pruned at the third week of Dec. According to the results which appear that foliar applications of ZnONPs or chelated zinc enhanced vegetative growth characteristics and quality of berries. On the other hand, the increasing ZnONPs up to 480mgL$^{-1}$ reducing the most mentioned characters compared with control or convential zinc source in both seasons. Eventually, foliar spraying of ZnONPs, at rate of 240mgL$^{-1}$ gave the highest values of total gross return, net return and beneficial cost ratio. From results of the present study, it could be recommended the foliar application of ZnONPs for Flame Seedless grapevines to improving yield quantitatively and qualitatively, thus economic return.

***Keywords*:** Grapevines, Flame Seedless, zinc oxide nanoparticles, foliar application, chelated zinc.

## INTRODUCTION

Nanotechnology has shown promising role in various fields like medicine, pharmaceuticals, electronics, agriculture and other research areas (Goswami *et al*., 2019). In addition, Moghaddasi *et al*. (2013) reported that nanomaterials considered as the most important tools of technology with special physical and chemical properties, compared to mass materials. Nanomaterials have unique physicochemical properties such as, magnetic, plasmonic, electronic, catalytic properties, size-dependent optical and potential applications (Zahedi *et al*., 2019).

Nanotechnology is the creation of functional materials through control of matter at dimensions approximately between 1 and100 nm. Consequently, the potential benefits and application of nanotechnology to agriculture is also getting attention nowadays (Resham *et al*., 2015). Nanomaterials lead to better understanding of physiological, biochemical, and molecular mechanisms, which are effective on improvement the growth of the plants (Siddiqui *et al*., 2015). As a result of their high specific surface area, these nanomaterials have more solubility and can be absorbed quickly (Sabir *et al*., 2014). After foliar application, the nanoparticles are placed on the leaf surface near the pores of the stomata (Wang *et al*., 2013). Furthermore, they showed that the methods of application and leaf structure can play a vital role in nanoparticle absorption in the form of foliar uptake, their translocation into the plant, and their effects. Due to these nanoparticles create a useful biosynthetic pathway and then constantly increases store photosynthetic materials in the meristem cells (Venkatachalam *et al*., 2017).

Nanomaterials called as magic bullets which play role particular cellular organelles in plant to pass their content and after penetrating the cell, nanomaterials can move only by two ways such as the apoplast and the symplast, and this represents efficient method of nutrient delivery to improve plant performance as described by Goswami *et al.* (2019). However, nanoparticles may effect on the plant growth species either positively or negatively depending on its concentration used. In this experiment, using Zn in form nanoparticles and it is improving plant growth and development comparing with conventional form such as chelated bulk zinc as described by Rossi *et al*. (2019). Many studies reported that chemical fertilizers are less efficient compare to nano fertilizers (Tombuloglu *et al*. 2019). On the other hand, application of nano zinc in high concentration may be affect negatively on plant growth.

Zinc (Zn) is known as an essential trace element for the normal healthy growth and reproduction of plants, animals and humans. Zn is now considered the fourth most important yield-limiting nutrient after nitrogen, phosphorus, and potassium, especially under alkaline soil (Prasad *et al*., 2012). In plants, Zn absorbed in the form of divalent cations, and it plays as catalytic and/or structural constituent roles in many cell physiological processes. Also, Zn activates some enzymes as phosphor hydrolases, proteases, dehydrogenases, peptidases. Many enzymes include zinc as a cofactor, such as, carbonic anhydrase, carboxyl peptidase, superoxide dismutase, RNA polymerase (Amirjani *et al*., 2016).

Furthermore, Zn has many important physiological functions that could enhance vegetative growth and grape berry quality (Khan *et al*., 2019). The plants require zinc for the synthesis of tryptophan, where, there are two main roles of tryptophan as follow; 1st it direct effects on the plant growth; 2nd IAA synthesis (Castillo-Gonzalez *et al*., 2018). In this regard, Nicolas *et al.* (2013) reported that IAA induced (VvCEB1) gene that modifying the cell-wall network in grape and control cell expansion in grape. Importantly, Zn is a structural part of the ribosome and takes part in amino acid synthesis (Barker and Eaton, 2015), then synthesis proteins that required for cell

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division, cell differentiation and berry growth (Khan *et al*., 2019). Also, Zn may be improving the berry firmness by the inhibitory effect of Zn on various oxidative reactions (Zhao *et al*., 2013).

Zn is a structural part of the ribosome and takes part in amino acid synthesis, nitrogen metabolism, and responsible for its structural integrity (Barker and Eaton, 2015). Also, the positive role of Zn in enhancement K and Mn contents in leaves petioles which described as synergic effect between Zn, K and Mn (Amirjani *et al*., 2016). On the other hand, they reported that increasing Zn levels reduced P and Fe levels which could be due to antagonistic effect among Zn, Fe and P. In addition to the positive effects of Zn on chlorophyll content in grape leaves has been proposed as follows: 1st role of Zn in activating the protein synthetase in chlorophyll biosynthesis pathway; 2nd Zn consider as cofactor of carbonic anhydrase and helps to elevate $CO_2$ concentration and enables $CO_2$ distribution in the chloroplast; 3th Zn enhance an indirect chlorophyll biosynthesis by protect it from free radicals by activate some antioxidant enzymes like glutathione reductase and ascorbate peroxidase (Amirjani *et al*., 2016). Eventually, the mechanism of effects Zn on antioxidants activity has been illustrated as follows: 1st Zn can be complexes with sulfhydryl groups and phospholipids, where it protects the proteins and lipids of membranes against oxidative damage (Broadley *et al*., 2012); 2nd Zn can regulate the synthesis of antioxidants enzymes such as; superoxide dismutase, peroxidase, catalase and ascorbate (Noreen *et al*., 2021).

Using ZnONPs could impact the mammals health. Another study will be carry out to explore the impact of ZnONPs on rabbits health as indicator for health concern.

The purpose of the present study is to give some additional information on the effect of ZnONPs at different doses on vegetative growth characteristic, yield and berry quality of Flame Seedless grapevines.

## MATERIALS AND METHODS

This study was conducted during the two successive seasons of 2018 and 2019 in vineyard grown at the Experimental Farm of Sids Agricultural Research Station, Beni-Suef Governorate. The chosen vines were seven-years old Flame Seedless grapevines, grown in a clay loam soil, spaced at 2 x 3 meters apart, irrigated under surface irrigation system, and spur-pruned and trellised by Spanish Parron supporting system. For each experimental season, the pruning was done at last week of December, and 80 buds were left on each vine. The selected vines received the common horticultural practices as recommended by the Ministry of Agriculture.

**Soil analysis**

Soil samples were collected from a depth of 0 -30 cm taken before beginning of the study to determine some chemical and physical properties of soil according to A.O.A.C. (1995), and listed in Table 1.

**Table 1. Some physical and chemical properties of the experimental soil**

| Soil properties | Values |
|---|---|
| Physical properties: | |
| Particle size distribution: | |
| Clay (%) | 40.26 |
| Silt (%) | 34.51 |
| Sand (%) | 25.23 |
| Texture grade | Clay loam |
| Chemical properties: | |
| pH (1:2.5 soil-water suspension) | 8.08 |
| EC, soil paste (dS $m^{-1}$) | 1.23 |
| Organic matter (%) | 1.42 |
| $CaCO_3$ (%) | 1.15 |
| Available N ($ugg^{-1}$) | 24.5 |
| Available P ($ugg^{-1}$) | 16.7 |
| Available K ($ugg^{-1}$) | 186 |
| Available Zn ($ugg^{-1}$) | 0.7 |
| Available Fe ($ugg^{-1}$) | 1.8 |
| Available Mn ($ugg^{-1}$) | 1.2 |

**Zinc oxide nanoparticles preparation:**

ZnONPs were prepared in Labs of Sids Agricultural Research Station, where synthesized by the precipitation method using precursors $Zn(NO_3)_2.6H_2O$ and KOH. (0. 4 M), then added to watery (0. 2 M) zinc nitrate under strong attractive mixing until shaping KOH a white suspension. Centrifuge the suspension at 5000 rpm for 20 minutes, and then washed three times with refined water, calcined for 3hrs at 500 °C (Pandey and Mishra, 2012).

**Zinc oxide nanoparticles characterizations:**

The methods for characterizing ZnONPs were characterized with two techniques. 1st X-ray diffraction (XRD) investigation was performed at room temperature (29 °C) and (20- 80 theta degree) with CuK radiation (Fig. 1). 2nd FT-IR absorption spectrum was worked out by using OPUS, Spectroscopy, V.7.2 that used to characterize the size of the ZnONPs (Fig. 2). ZnONPs_in this study_with mean particle diameter of 34.6 nm.

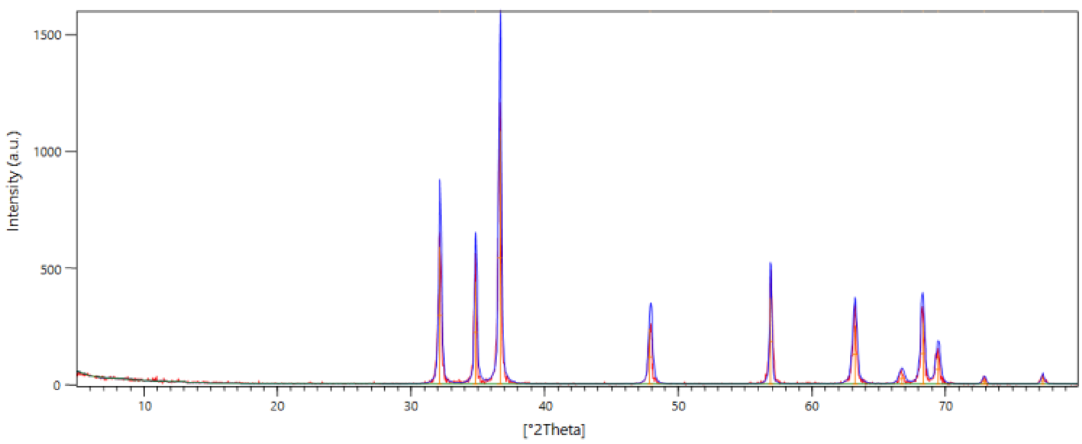


**Fig. 1. X-ray diffraction (XRD) of ZnONPs.**

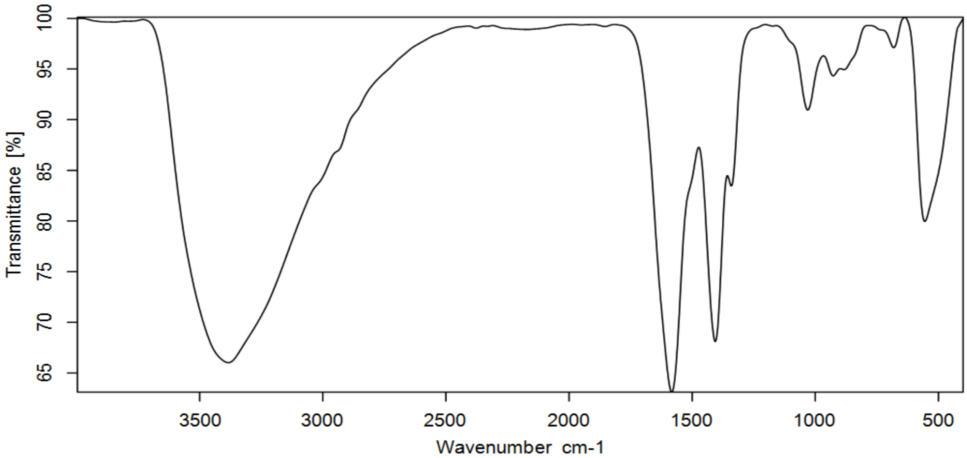


**Fig. 2. FT-IR spectrum (IR) of ZnONPs.**

This experiment included six treatments. Each treatment was replicated three times, two vine per each. All treatments were sprayed four times at growth start (4-to 8-inch shoots), just before bloom, two weeks after berry setting and just before veraison stage as follow:

1 - Control (without zinc application).
2 - Convential zinc source [chelated zinc (12.5%) at 1.5gL$^{-1}$].
3 - ZnONPs at 60mgL$^{-1}$.
4 - ZnONPs at 120mgL$^{-1}$.
5 - ZnONPs at 240mgL$^{-1}$.
6 - ZnONPs at 480mgL$^{-1}$.

**The following parameters were assessed:**

**Vegetative growth parameters:**

Five vegetative shoots were randomly chosen per vine to measure the following parameters at the growing two seasons:

- Shoot length (cm).
- Average leaf area (cm$^2$): ten leaves were taken from the apical vegetative shoots for each replicate to measure average leaf area (cm$^2$) according to Ahmed and Morsy (1999).
- Average internodes length was determined by dividing shoot length by the number of internodes.
- Cane thickness (cm) was measured in the five basal internodes were selected and labeled five fruiting canes per vine before harvest and determined at mid of December by using a vernier caliper.

**Leaf chemical analysis:**

At harvest date, leaves were collected from 5-7$^{th}$ mature leaves from shoot top to measure the following parameters at the growing season:

- Total leaf Chlorophyll: total chlorophyll content was measured by using the nondestructive Minolta chlorophyll meter model SPAD 502 (Wood, 1993).
- N, P, K, Zn, Fe and Mn percentage in petioles samples of leaves were determined according to A.O.A.C. (1995).

**Yield and physical cluster and berries characteristics:**

At harvest date (1$^{st}$ week of June) in both seasons, five clusters per vine were harvested at the ripening stage when juice TSS% reached about 16-17% in control treatment to measure the following parameters:

- Yield: yield/vine (kg) was calculated by multiplying number of clusters/vine by cluster weight and expressed in weight (kg).
- Average cluster length (cm)
- Average cluster width (cm)
- Average cluster weight (g).
- Average of number of clusters/ vine.
- Average berry weight (g).
- Average berry length and diameter by using a Vernier caliper.
- Average berry firmness (g/cm$^2$): was determined by using Penetrometer, Model FT 011, Italy.

**Berry chemical analysis:**

- Total soluble solids (T.S.S %) in juice was determined by using a hand refractometer.
- Acidity: titratable acidity (as gram tartaric acid/100mL juice) was estimated by the method of A.O.A.C. (1995).
- T.S.S. / acid ratio.
- Total anthocyanin (mg/100g berries juice) was measured according to Ranganna (1979).
- Antioxidant activities in juice (DPPH%): the antioxidant activities was evaluated by (2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method according to the procedure of Chen *et al*. (2008).

**Total carbohydrates in the canes and pruning weight:**

- Total carbohydrates were taken from fruiting canes for next season and determined at winter pruning according the methods of Du-Bois *et al.* (1956)
- Pruning weight /vine: was measured at dormancy period (winter pruning).

**Economic analysis:**

The economic analysis was performed to estimate the net return and beneficial cost ratio. The cultivation cost was determined as sum of land rent, vine maintenance, pruning, inorganic and organic fertilizers, hoeing, irrigations, dormex and growth regulators as well as insect, fungal diseases and weed control. Also, the costs of zinc treatments were estimated (Table 2). The two economic parameters were calculated as follow:

Gross return (L.E/fed) = total vine yield (kg) X [total No. of vines/fed x price of grapes (L.E)].

Net return (L.E/fed) = gross return – total cultivation cost.

$$\text{Beneficial cost ratio} = \frac{\text{gross return}}{\text{total cultivation cost}}.$$

**Experimental design and statistical analysis:**

The experiment design was arranged in randomized complete block design (RCBD) in three replicates, each replicate equal two vine. The statistical analysis of the present data was carried out according to Snedecor and Cochran, (1980). Averages were compared using L.S.D. values at 5 % level (Mead *et al*., 1993).

**Table 2. Cultivation cost (L.E/ fed$^{-1}$)**

| cultivation cost | Average two seasons |
|---|---|
| **Common cost** | |
| Land rent | 12000 |
| Vineyard maintenance | 500 |
| Winter pruning | 3360 |
| Farmyard manure | 2210 |
| Hoeing | 500 |
| Irrigations | 1875 |
| Fertilizers | 4495 |
| Foliar application of dormex | 1140 |
| Insect control | 1785 |
| Fungal diseases control | 2590 |
| Weed control | 600 |
| Foliar application of growth regulators | 1120 |
| Foliar application of nutrients | 990 |
| **Total common cost** | **33165** |
| **Variable cost** | |
| Control (without zinc application) | 0 |
| Chelated zinc | 840 |
| ZnONPs at 60 mgL$^{-1}$ | 654 |
| ZnONPs at 120 mgL$^{-1}$ | 708 |
| ZnONPs at 240 mgL$^{-1}$ | 816 |
| ZnONPs at 480 mgL$^{-1}$ | 1032 |

# RESULTS AND DISCUSSION

## Results

### Vegetative growth parameters

The effects of Zn treatments on the vegetative growth parameters such as, average of shoot length, average internodes length, leaf area, cane thickness and pruning weight are presented in Table 3. A significant effects between the different sources and concentrations of zinc were observed in 2018 and 2019 seasons. The best results obtained under foliar application of ZnONPs at 240mgL$^{-1}$ compared with other treatments in 2018 and 2019 seasons, while the treatment of without zinc recorded the lowest one.

### Chemical constitutes of grape leaves and canes

The highest values of N and K in leaves petioles were found due to the foliar application of ZnONPs at 240mgL$^{-1}$ treatment (Table 4) compared with other treatments in two seasons. On contrary, there are negative relationship between Zn and P, and the highest values were obtained from the foliar application of ZnONPs at 120mgL$^{-1}$ in this concern; whereas no significant differences were found between ZnONPs 120 and 240mgL$^{-1}$ treatments in both seasons. Also, the lowest values of P were found in the high dose of ZnONPs treatment compared with other treatments during 2018 and 2019 seasons.

The total chlorophyll in grape leaves and total carbohydrates in fruiting canes were affected by different treatments. The best values of these characters were obtained from the foliar application o ZnONPs at 240mgL$^{-1}$, while the lowest values were found in control during two seasons.

**Table 3. Effect of the foliar application of different sources and levels of zinc on some vegetative growth parameters of Flame Seedless grapevines.**

| Treatments | Shoot length (cm) | | Internodes length (cm) | | Leaf area (cm$^2$) | | Cane thickness (cm) | | Pruning weight (kg) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| 1- Control | 135.7 | 135.2 | 10.01 | 10.00 | 103.3 | 103.5 | 0.90 | 0.91 | 2.63 | 2.62 |
| 2- Chelated zinc at 1.5gL$^{-1}$ | 177.3 | 182.7 | 12.58 | 12.69 | 143.6 | 148.7 | 1.21 | 1.30 | 3.74 | 3.80 |
| 3- ZnONPs at 60mgL$^{-1}$ | 145.7 | 149.7 | 10.99 | 11.15 | 111.9 | 120.9 | 0.93 | 0.97 | 2.86 | 2.97 |
| 4- ZnONPs at 120mgL$^{-1}$ | 166.3 | 169.3 | 11.37 | 11.49 | 127.5 | 131.2 | 1.13 | 1.20 | 3.55 | 3.60 |
| 5- ZnONPs at 240mgL$^{-1}$ | 191.7 | 211.0 | 13.15 | 13.27 | 159.1 | 164.4 | 1.32 | 1.40 | 3.81 | 3.94 |
| 6- ZnONPs at 480mgL$^{-1}$ | 173.7 | 175.1 | 11.77 | 11.85 | 133.9 | 137.3 | 1.27 | 1.33 | 3.62 | 3.64 |
| New L.S.D at 5 % | 3.4 | 5.2 | 0.38 | 0.34 | 6.2 | 6.1 | 0.03 | 0.03 | 0.06 | 0.04 |

**Table 4. Effect of the foliar application of different sources and levels of zinc on N, P, K in leaves petioles and some chemical constituents of leaves and canes in Flame Seedless grapevines.**

| Treatments | N (%) | | P (%) | | K (%) | | Total chlorophyll (SPAD) | | Total carbohydrates (g/100g) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| 1-Control | 1.96 | 1.95 | 0.21 | 0.20 | 1.57 | 1.55 | 30.2 | 30.8 | 20.26 | 20.25 |
| 2- Chelated zinc at 1.5gL$^{-1}$ | 2.27 | 2.28 | 0.29 | 0.28 | 1.85 | 1.87 | 37.0 | 38.7 | 26.63 | 27.83 |
| 3- ZnONPs at 60mgL$^{-1}$ | 2.02 | 2.05 | 0.25 | 0.24 | 1.63 | 1.68 | 33.7 | 35.2 | 21.41 | 22.71 |
| 4- ZnONPs at 120mgL$^{-1}$ | 2.21 | 2.22 | 0.31 | 0.32 | 1.92 | 1.93 | 35.5 | 37.4 | 23.46 | 24.76 |
| 5- ZnONPs at 240mgL$^{-1}$ | 2.33 | 2.36 | 0.30 | 0.29 | 1.98 | 2.01 | 42.0 | 44.1 | 30.50 | 34.70 |
| 6- ZnONPs at 480mgL$^{-1}$ | 2.23 | 2.24 | 0.22 | 0.19 | 1.80 | 1.78 | 35.9 | 36.7 | 25.30 | 26.40 |
| New L.S.D at 5 % | 0.02 | 0.02 | 0.02 | 0.04 | 0.05 | 0.06 | 0.4 | 0.6 | 1.15 | 2.00 |

### Microelements of leaves petioles

There are positive relationship between Zn treatments and Zn contents in leaves petioles in 2018 and 2019 seasons (Table 5). In this concern, the highest values were found under foliar application of high dose of ZnONPs, while the lowest values were found in control in two seasons. On the other hand, the highest dose of ZnONPs treatment was significantly decreased contents of Fe and Mn in leaves petioles when compared with other treatments in two seasons (Table 5). While, the highest values of Fe and Mn in leaves petioles were found due to added 120mgL$^{-1}$ ZnONPs treatment.

### Yield and physical cluster and berries parameters

It was evident from Tables 6 & 7 that yield and cluster parameters such as, cluster length, cluster width, cluster weight, number of clusters per vine, and berry parameters, namely; berry weight, berry length, berry width and berry firmness were affected due to different treatments during the two seasons. The best values in above mentioned characters were obtained from the foliar application of ZnONPs at 240mgL$^{-1}$ when compared with other treatments for 2018 and 2019 seasons.

**Table 5. Effect of the foliar application of different sources and levels of zinc on some microelements of leaves petioles in Flame Seedless grapevines.**

| Treatments | Fe (ppm) | | Zn (ppm) | | Mn (ppm) | |
|---|---|---|---|---|---|---|
| | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| 1-Control | 113.6 | 114.1 | 29.0 | 30.2 | 77.1 | 77.4 |
| 2- Chelated zinc at 1.5gL$^{-1}$ | 143.1 | 146.3 | 68.3 | 74.4 | 91.4 | 93.9 |
| 3- ZnONPs at 60mgL$^{-1}$ | 122.2 | 126.5 | 32.5 | 37.6 | 81.1 | 84.5 |
| 4- ZnONPs at 120mgL$^{-1}$ | 155.3 | 164.5 | 55.9 | 61.6 | 98.5 | 103.3 |
| 5- ZnONPs at 240mgL$^{-1}$ | 127.1 | 130.8 | 81.1 | 88.0 | 85.1 | 88.3 |
| 6- ZnONPs at 480mgL$^{-1}$ | 101.7 | 106.8 | 123.9 | 131.2 | 65.3 | 66.5 |
| New L.S.D at 5 % | 4.5 | 4.3 | 3.4 | 4.8 | 3.9 | 3.8 |

**Table 6. Effect of foliar application of different sources and levels of zinc on yield/vine and physical characteristics of cluster in Flame Seedless grapevines.**

| Treatments | Yield/ vine (kg) | | cluster length (cm) | | cluster width (cm) | | cluster weight (g) | | No. of clusters /vine | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| 1- Control | 12.18 | 12.35 | 16.25 | 17.19 | 13.01 | 13.18 | 553.5 | 556.2 | 22.0 | 22.2 |
| 2- Chelated zinc at $1.5gL^{-1}$ | 13.47 | 15.86 | 23.06 | 23.91 | 14.94 | 15.34 | 623.7 | 634.2 | 21.6 | 25.0 |
| 3- ZnONPs at $60mgL^{-1}$ | 12.67 | 13.40 | 18.61 | 19.30 | 13.25 | 13.55 | 573.2 | 580.3 | 22.1 | 23.1 |
| 4- ZnONPs at $120mgL^{-1}$ | 12.93 | 14.52 | 21.18 | 22.01 | 13.42 | 13.92 | 598.4 | 610.1 | 21.6 | 23.8 |
| 5- ZnONPs at $240mgL^{-1}$ | 14.16 | 17.04 | 24.80 | 25.70 | 16.84 | 17.46 | 649.5 | 665.8 | 21.8 | 25.6 |
| 6- ZnONPs at $480mgL^{-1}$ | 13.63 | 15.09 | 21.70 | 22.50 | 13.98 | 14.29 | 611.1 | 620.9 | 22.3 | 24.3 |
| New L.S.D at 5 % | 0.15 | 0.68 | 0.48 | 0.49 | 0.15 | 0.34 | 12.6 | 13.3 | N.S. | 0.5 |

**Table 7. Effect of foliar application of different sources and levels of zinc on some physical characteristics of the berry in Flame Seedless grapevines**

| Treatments | Berry weight (g) | | Berry length (cm) | | Berry width (cm) | | Berry firmness (g/cm²) | |
|---|---|---|---|---|---|---|---|---|
| | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| 1-Control | 3.38 | 3.40 | 1.51 | 1.52 | 1.47 | 1.48 | 217.7 | 222.5 |
| 2- Chelated zinc at $1.5gL^{-1}$ | 3.67 | 3.84 | 1.62 | 1.65 | 1.61 | 1.64 | 267.1 | 274.3 |
| 3- ZnONPs at $60mgL^{-1}$ | 3.41 | 3.69 | 1.56 | 1.58 | 1.54 | 1.55 | 225.9 | 231.2 |
| 4- ZnONPs at $120mgL^{-1}$ | 3.57 | 3.73 | 1.59 | 1.61 | 1.57 | 1.60 | 249.5 | 254.8 |
| 5- ZnONPs at $240mgL^{-1}$ | 3.58 | 3.92 | 1.63 | 1.67 | 1.62 | 1.66 | 290.3 | 301.5 |
| 6- ZnONPs at $480mgL^{-1}$ | 3.69 | 3.72 | 1.60 | 1.63 | 1.58 | 1.57 | 258.1 | 263.0 |
| New L.S.D at 5 % | 0.01 | 0.03 | 0.01 | 0.02 | 0.01 | 0.03 | 8.2 | 8.4 |

**Chemical characteristics of berries**

The chemical characteristics of berries namely, T.S.S%, total acidity, T.S.S/acid ratio, total anthocyanin and antioxidants activity (DPPH %) are shown in Table 8. The foliar application with ZnONPs at $120mgL^{-1}$ gave the highest values of T.S.S. %, T.S.S./acid ratio and the lowest values of total acidity, whereas no significant differences were found between ZnONPs 120 or $240mgL^{-1}$ treatments in both seasons in this respect.

On the other hand, the foliar application of ZnONPs at $240mgL^{-1}$ gave the highest values of total anthocyanin and antioxidants activity (DPPH%) compared with other treatments during 2018 and 2019 seasons.

**Table 8. Effect of the foliar application of different sources and levels of zinc on some chemical characteristics of the berry in Flame Seedless grapevines.**

| Treatments | T.S.S (%) | | Acidity (%) | | T.S.S/ acidity ratio | | Total anthocyanin (g/100g) | | Antioxidants (DPPH %) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| 1-Control | 16.01 | 16.02 | 0.711 | 0.705 | 22.52 | 22.72 | 19.6 | 20.0 | 50.60 | 50.90 |
| 2- Chelated zinc at $1.5gL^{-1}$ | 17.90 | 18.21 | 0.578 | 0.562 | 30.97 | 32.40 | 24.7 | 24.9 | 71.80 | 72.90 |
| 3- ZnONPs at $60mgL^{-1}$ | 16.51 | 16.52 | 0.662 | 0.651 | 25.54 | 26.31 | 21.1 | 21.4 | 55.42 | 56.22 |
| 4- ZnONPs at $120mgL^{-1}$ | 18.65 | 18.92 | 0.545 | 0.528 | 34.22 | 35.83 | 22.9 | 23.0 | 67.69 | 68.65 |
| 5- ZnONPs at $240mgL^{-1}$ | 18.40 | 18.63 | 0.559 | 0.540 | 32.92 | 34.50 | 26.3 | 26.4 | 73.31 | 74.81 |
| 6- ZnONPs at $480mgL^{-1}$ | 17.23 | 17.74 | 0.611 | 0.596 | 28.20 | 29.77 | 23.3 | 23.5 | 70.12 | 70.42 |
| New L.S.D at 5 % | 0.40 | 0.40 | 0.030 | 0.033 | 2.60 | 2.63 | 0.4 | 0.5 | 1.69 | 1.75 |

**Economic analysis:**

The economic analysis (Table 9) showed that the highest gross return of 49560 and 59640 (L.E/fed), net return of 15579 and 25659 (L.E/fed), and cost beneficial ratio of 1.46 and 1.76; respectively in both growing seasons were obtained at the treatment of the foliar spraying of ZnONPs at rate of $240mgL^{-1}$ followed by the treatment of foliar or spraying of chelated zinc at rate of $1.5gL^{-1}$ or foliar spraying of nanoparticles zinc at rate of $480mgL^{-1}$. This may be attributed to its role in increasing total vine yield (Table 5). On the other hand, the treatment of no zinc application exhibited the lowest economic parameters.

**Table 9. Effect of the foliar application of different sources and levels of zinc on gross return, net return and beneficial cost ratio of Flame Seedless grapevines**

| Treatments | Gross return (L.E./ fed) | | Net return (L.E./ fed) | | Beneficial cost ratio | |
|---|---|---|---|---|---|---|
| | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| 1-Control | 42630 | 43225 | 9465 | 10060 | 1.29 | 1.30 |
| 2- Chelated zinc at $1.5gL^{-1}$ | 47145 | 55510 | 13140 | 21505 | 1.39 | 1.63 |
| 3- ZnONPs at $60mgL^{-1}$ | 44345 | 46900 | 10526 | 13081 | 1.31 | 1.39 |
| 4- ZnONPs at $120mgL^{-1}$ | 45255 | 50820 | 11382 | 16947 | 1.34 | 1.50 |
| 5- ZnONPs at $240mgL^{-1}$ | 49560 | 59640 | 15579 | 25659 | 1.46 | 1.76 |
| 6- ZnONPs at $480mgL^{-1}$ | 47705 | 52815 | 13508 | 18618 | 1.40 | 1.54 |
| New L.S.D at 5 % | 557 | 660 | 367 | 840 | 0.02 | 0.04 |

**Discussion**

ZnONPs are much smaller than conventional materials and due to a greater surface area to weight ratio, different shapes and higher penetrability, therefore foliar application of ZnONPs may have a significant effects on vegetative growth parameters, total chlorophyll in leaves and total carbohydrates in canes, and then positively reflect on the yield/vine, physical and chemical characteristics of the berries of Flame Seedless grapevines.

**Vegetative growth parameters**

The beneficial effects of Zn on the vegetative growth characters may be attributed to synthesis of auxins, whereas; plants require zinc for the synthesis of tryptophan, a key amino acid in the synthesis of auxin indole acetic acid

(Castillo-Gonzalez *et al*., 2018). Interestingly, Ojeda-Barrios *et al*. (2014) stated that Zn has improved leaf area, because zinc is helpful for membrane function, protein synthesis, and cell elongation.

**Chemical constitutes of leaves petioles and canes**

The detected enhancements of N content in leaves petioles due to Zn is a structural part of the ribosome and takes part in amino acid synthesis, nitrogen metabolism, and responsible for its structural integrity (Barker and Eaton, 2015). Also, the positive role of Zn enhancement K and Mn contents in leaves petioles was by synergic effects between Zn, K and Mn as described by Amirjani *et al*. (2016). On the other hand, they reported that increasing Zn levels reduced P and Fe levels, which could be due to antagonistic effect among Zn, Fe and P (Amirjani *et al*., 2016).

Furthermore, the foliar application of ZnONPs improvement carbohydrates contents of vegetative canes, which may be due to Zn involve in carbon metabolism through its direct action on activity on enzyme ribulose-bisphosphate carboxylase oxygenase, and this enzyme has a vital role in carbon metabolism in the Calvin cycle (Farooq *et al*., 2009). Meanwhile, Zn considers an important component of carbonic anhydrase enzyme as a stimulator of aldolase, which is involved in carbon metabolism. (Tsonev and Lidon, 2012).

Additionally, the positive effects of Zn on chlorophyll content in grape leaves has been proposed as follows: 1st role of Zn in activating the protein synthetases in chlorophyll biosynthesis pathway; 2nd Zn consider as cofactor of Carbonic anhydrase and helps to elevate $CO_2$ concentration and enables $CO_2$ distribution in the chloroplast; 3th Zn enhance an indirect chlorophyll biosynthesis by protect it from free radicals by activate some antioxidant enzymes like glutathione reductase and ascorbate peroxidase (Amirjani *et al*., 2016).

**Physical of cluster and berries parameters**

Zn has many important physiological functions that could enhance berry quality (Khan *et al*., 2019). The plants require zinc for the synthesis of tryptophan, there are two main roles of tryptophan as follow; 1st it effects on the plant growth; 2nd IAA synthesis (Castillo-Gonzalez *et al*., 2018). In this context, Nicolas *et al*. (2013) reported that IAA induced (VvCEB1) gene that modifying the cell-wall network in grape and control cell expansion in grape. Furthermore, Zn is a structural part of the ribosome and takes part in amino acid synthesis (Barker and Eaton, 2015), then synthesis proteins that required for cell division, cell differentiation and berry growth (Khan *et al*., 2019). Also, Zn may be improving the berry firmness by the inhibitory effect of Zn on various oxidative reactions (Zhao *et al*., 2013). For this reasons, Zn may be enhanced the berry quality, and that reflect on cluster quality and increasing yield of Flame Seedless grapevines.

**Chemical characteristics of berries**

Chemical characteristics of the berries have positive effects by foliar application of ZnONPs treatments. Zn increasing T.S.S. % and reducing acidity % by increasing K element in leaves petioles. Additionally, the mechanism of effects Zn on antioxidant activity has been illustrated as follows: 1st Zn can be complexes with sulfhydryl groups and phospholipids, where it protects the proteins and lipids of membranes against oxidative damage (Broadley *et al*., 2012); 2nd Zn can regulate the synthesis of antioxidant enzymes such as, superoxide dismutase, peroxidase, catalase and ascorbate (Noreen *et al*., 2021). For this reasons, Zn improved antioxidants activity (DPPH %) in grape berry. Importantly, Chang-Zheng *et al*. (2015) reported that affecting application of zinc on anthocyanin content in the grape berry by the expression of related gene (VvMYBF1) with Zn in grape berry at stages of berry development.

## CONCLUSION

ZnONPs have been very useful in improving the vegetative growth characteristic, yield, and quality of grapevines. Meanwhile, foliar application with ZnONPs at $240 mgL^{-1}$ enhanced vegetative growth characteristic and berry quality of Flame Seedless grapevines. On the other hand, foliar application of ZnONPs at $480 mgL^{-1}$ were critical rate effect on grapevines growth, and most characteristic growth had low values compared with control during two seasons. Furthermore, the foliar spraying of ZnONPs at rate of $240 mgL^{-1}$ gave the highest values of total gross return, net return and beneficial cost ratio. Accordingly, from this study, it could be concluded that foliar application of ZnONPs at $240 mgL^{-1}$ had the best values in the most characters. Finally, nanomaterials need more studies to examine it effects, either positively or negatively on plants.

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## تأثير الرش الورقي بمركب النانو زنك على الصفات الخضرية والثمرية للعنب الفليم سيدليس

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أجريت هذه الدراسة خلال موسمي 2018 ، 2019 لدراسة تأثير الرش الورقي لكل من النانو زنك بتركيزات 60 ، 120 ، 240 و 480 ملليجرام /لتر و الزنك المخلبي بتركيز1.5 جم / لتر وأيضاً بدون معاملة زنك (الكنترول) لكرمات العنب الفليم سيدليس عمرها 7- سنوات . وكان نظام التدعيم للكرمات نظام التكعيبة البارون الاسبانية ، حيث كانت الكرمات نامية في تربة طينية طميية على مسافة زراعة 2 x 3 م ، حيث تم ري الكرمات بنظام الري السطحي ، وتم تقليم الكرمات تقليم دابري في الاسبوع الثالث من ديسمبر خلال الموسمين. وقد أظهرت النتائج أن الرش الورقي بالنانو زنك أو الزنك المخلبي قد أدى إلى تحسين خصائص النمو الخضرى وجودة الحبات. وعلى العكس من ذلك ، فإن زيادة تركيز النانو زنك الى 480 ملليجرام /لتر أدي الى انخفاض اغلب القيم للصفات السابقة مقارنة بالكنترول أو مصدر الزنك التقليدي في كلا الموسمين. وأخيراً ، أدى الرش الورقي بالنانو زنك بمعدل 240 ملليجرام/لتر الحصول على أعلى قيم للدخل من محصول العنب وكذلك أعلى قيم صافى الدخل وكذلك أعلى أربحية بالنسبة للتكلفة. ومن نتائج الدراسة الحالية يمكن التوصية برش العنب الفليم سيدليس بالنانو زنك لتحسين المحصول كماً ونوعاً وكذلك العائد الاقتصادي.