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Research Article
Efficiency of Nano-Zinc Foliar Spray on Growth, Yield and Fruit Quality of Flame Seedless Grape

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Abstract
Background and Objective: Nano-fertilizers are effective in improving solubility, bioavailability, efficiency and uptake ratio of soil nutrients. This study was investigated the effect of different concentrations of nano-zinc on growth, leaf mineral content and yield also fruit quality of flame seedless grape vine. **Materials and Methods:** This investigation was carried out through two successive seasons (2014 and 2015) to investigate the effect of nano-zinc on growth, leaf mineral content, yield and fruit quality of 5 years old flame seedless grapevines, cultivated in a private orchard at Sammond region, Gharbia Governorate, Egypt. Six treatments were applied as a randomized complete block design with three replications. Grapevines were sprayed with: T1 water only (control), T2 zinc sulphate at 565 ppm, T3 zinc EDTA at 140 ppm, T4 nano-zinc at 0.4 ppm, T5 nano-zinc at 0.8 ppm and T6 nano-zinc at 1.2 ppm. The used nano-zinc was 25 nm. **Results:** The result indicated that spraying grape vines with 0.4 ppm nano-zinc increased significantly leaf area and fresh weight compared with the control, while1.2 ppm nano-zinc increased significantly total carbohydrate, leaf concentration of Fe, No. of clusters, cluster weight and yield. Also results showed that 0.4, 0.8 and 1.2 ppm of nano-zinc had a significant increase on yield compared with conventional fertilizer. The utilization rate of nano-zinc fertilizer was decreased, which means saving the amounts of zinc fertilizer in production practice. **Conclusion:** So it was concluded that spraying vines with 0.4 ppm as nano-zinc was the best treatment for increasing some vegetative parameters.

Key words: Nano-zinc, flame seedless grape, leaf mineral content, nano-fertilizers, seedless grapevine, soil nutrients, zinc fertilizer

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Grape is considered as one of the most popular and favorite fruit crops in the world, for being of nice taste and high nutritional value. In Egypt, it ranks the second fruit crops and is consumed mainly as fresh fruits. The cultivated area has grown rapidly in the last two decades and reached 196900 feddans[1]. Zinc has been considered as an essential micronutrient for metabolic activities in plants. It regulates the various enzyme activities and required in biochemical reactions leading to formations of chlorophyll and carbohydrates[2,3]. The crop yield and quality that produced are affected by the deficiency[4] of Zn.

Zinc nano-particle is used in various agricultural experiments to understand its effect on growth, germination and various other properties. Most of the farmers are using either zinc sulfate or EDTA-Zn chelate for soil and foliar applications, however the low efficiency have demonstrated the essentiality and role of zinc in plant growth, reproduction and yield[5]. It has been indicated that the retention time of Zn in the plant system is low and hence, the bioavailability of Zn for long period is not sure with the use of $ZnSO_4$ fertilizer. Under high temperatures conditions $ZnSO_4$ has a large salt index and it may show burning injury if the plants are soft or sensitive[6]. Nano-particles with smaller particle size and large surface area are expected to be the ideal material for use as Zn fertilizer in plants.

A number of researchers have reported the essentiality and role of zinc for plant growth and yield[5,6]. The use of micronutrients in the form of nano-particles can be used in crop production to increase yield[7]. It has been postulated that nano-particles are more effective, can be utilized in agriculture for the precision farming and enhance productivity crop yields[8].

Spraying grapevines with zinc nano-particles (NPs) help to release the required nutrients gradually in small amounts and improve the spraying efficiency of zinc than the sulphate or chelated forms, also using nano form reduces the problems of soil pollution caused by the excess use of chemical fertilizers. This investigation was carried out to study the effect of different concentrations of nano-zinc on growth, leaf mineral content and yield also fruit quality of flame seedless grapevine.

MATERIALS AND METHODS

The present study was carried out during two successive seasons (from February-July, 2014 and from February-July, 2015) on flame seedless grapes (*Vitisvini fera* L.). Grapevines were about 5 years old and trained by bilateral horizontal cordon system, spur pruning (each with 2-3 eyes) and planted at 1.5×3.5 meter apart under flood irrigation system on loamy soil in a private orchard, at Sammond region, Gharbia Governorate, Egypt. Full description of the tested soil is given in Table 1.

In this study, grapevines were sprayed with: T1: Water only (control), T2: Zinc sulphate at 565 ppm, T3: Zinc EDTA at 140 ppm, T4: Nano zinc at 0.4 ppm, T5: Nano zinc at 0.8 ppm and T6: Nano zinc at 1.2 ppm. The used Nano zinc was 25 nm. The vines were sprayed three times (the first at full opening stage of the eyes, while the second was one month later and the third was one month after the second spray).

Measurements

Vegetative measurements: Average leaf area (cm^2) was measured using the 5th full expanded mature leaf from the shoot tip of each vine in mid-July by planimeter. Average leaf fresh and dry weight was determined as gram, average shoot length and diameter were determined as centimeter and number of leaves/shoot was counted.

Chemical measurements

Leaf total chlorophyll: Leaf total chlorophyll were determined in the fresh leaves was determined as spad units (spad = 100 mg chlorophyll/g fresh weight) by using Minolta chlorophyll meter (spad, 502) according to Wood *et al.*[9].

Total carbohydrates (%): Total carbohydrates were determined during the dormancy period (the last week of January), since the samples were taken from the basal part of shoots according to the method of DuBois *et al.*[10].

Table 1: Physical and chemical properties of the experimental soil

Soil depth	Sand (%)	Silt (%)	Clay (%)	Texture	pH	EC (ds m^{-1})	$CaCO_3$ (%)	Organic matter (%)
Physical analysis								
0-30 cm	10.8	44	45.2	Loamy	8.4	0.5	1.2	1.6
30-60 cm	12.8	44	43.2	Loamy	8.4	0.4	2	1.1
Soil depth	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Zn (ppm)	Mn (ppm)
Chemical analysis								
0-30 cm	0.13	0.6	0.9	4.2	1.1	7.8	3.4	3.2
30-60 cm	0.1	0.6	0.6	3.4	0.9	5.5	2.4	1.8

Leaf mineral content: Further in mid-July, 20 leaves sample include blade and petiole (6th leaf from the shoot tip) of each vine were collected to determine leaf mineral contents. Leaves were washed with distilled water then oven dried at 60-70°C until a constant weight. The dried samples were grind in a stainless steel knife mill and 0.2 g of the ground material of each sample was digested using a mixture of perchloric: Sulphuric acid 1:10 (v/v) according to the method of Jackson[11]. Nitrogen was determined as the method described by Pregl[12], while phosphorus was colorimetrically determined as the method of Truog and Meyer[13], potassium was determined using flame photometer according to the method of Mason[14] and also iron, zinc and manganese were determined using the atomic absorption apparatus according to the methods of Cottenie *et al*.[15].

Yield and fruit quality

Yield/vine (kg): Yield was harvested at the ripening stage when TSS% reached 16% and the color covered all bunch berries and the clusters number per vine were counted and weighted to estimate total yield per vine kilogram.

Fruit quality

Physical properties: Average cluster dimension (length and width as centimeter (cm)), average cluster weight (g), the weight of 100 berries (g) and juice weight/100 berries (g) were determined.

Chemical properties: Total soluble solids percentage in berry juice (TSS %) was determined using hand refractometer and total titratable acidity (%) was expressed as tartaric acid/100 mL juice[16]. Total anthocyanins of the berry skin (mg/100 g fresh weight) were determined according to Hsia *et al*.[17].

Statistical analysis: The experiment was set up in a randomized complete block design with three replicates and each replicate was one vine. All data obtained during this study were statistically analyzed and the differences between means at probability of 5% were differentiated using Duncan's multiple rang test[18].

RESULTS AND DISCUSSION

Effect on vegetative growth: Results in Table 2 indicated that T4 (0.4 ppm Nano zinc) affected significantly leaf area, leaf fresh weight comparing with all other treatments in the both seasons. As for leaf dry weight, in the first season T3, T4 and T6 enhanced it significantly. While in the second season, T4 gave the highest significant value.

On the other hand, the reverse was true with the vines which sprayed with T1 (Tap water) since they reflected the least values of leaf area, leaf fresh weight and leaf dry weight in two seasons of the study.

These results are matched with those of Sedghi *et al*.[19], who found that nano-zinc oxide increased significantly fresh and dry weight of soybean.

Results presented in Table 3 indicated that T4 (0.4 ppm Nano zinc) reflected the highest value in shoot length insignificantly with T2 and T3 in the first season while T5 reflected the highest value of shoot length insignificantly with T4 in the second season. Regarding shoot diameter and number of leaves/shoot, there was no significant difference between treatments in the two seasons. With respect to leaf total chlorophyll content, in the first season there was no significant difference between treatments. While in the second season T3, T4 and T6 scored the highest values. Cane total carbohydrates (%) was increased by T6 significantly compared with other treatments in the two seasons.

On the other hand, the reverse was true with the vines which sprayed with T1 since they reflected the least values of shoot length, shoot diameter, number of leaves/shoot, total chlorophyll and total carbohydrates contents in the two seasons of the study. These results are matched with those of Panwar *et al*.[20], as tomato plants sprayed with 20 mg L^{-1} ZnO showed improved growth and biomass production as compared to control plants.

Table 2: Effect of nano-zinc spraying on leaf area, leaf fresh and dry weight of flame seedless grapes in 2014 and 2015 seasons

	Leaf area (cm^2)		Leaf fresh weight (g)		Leaf dry weight (g)	
Treatments	2014	2015	2014	2015	2014	2015
T1 (Tap water)	135.77^{d}	129.36^{c}	1.70^{f}	1.5^{c}	0.63^{c}	0.7^{d}
T2 ($ZnSO_4$)	165.60^{c}	169.6^{b}	2.50^{d}	2.6^{b}	0.97^{b}	1.1^{bc}
T3 (Zn EDTA)	171.17^{b}	174.23^{b}	2.30^{e}	2.6^{b}	1.17^{a}	1.23^{b}
T4 (0.4 ppm N)	197.17^{a}	195.83^{a}	3.40^{a}	3.8^{a}	1.13^{a}	1.73^{a}
T5 (0.8 ppm N)	174.50^{b}	176.3^{b}	2.70^{c}	2.8^{b}	0.97^{b}	0.93^{cd}
T6 (1.2 ppm N)	174.10^{b}	175.46^{b}	2.93^{b}	2.9^{b}	1.17^{a}	1.26^{b}

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

Table 3: Effect of nano-zinc on shoot length and diameter, number of leaves/shoot, total chlorophyll and total carbohydrate of flame seedless grapes in 2014 and 2015 seasons

	Shoot length (cm)		Shoot diameter (mm)		No. of leaves/shoot		Total chlorophyll (SPAD) value		Cane total carbohydrate (%)	
Treatments	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
T1 (Tap water)	33.00^{c}	26.66^{d}	0.77^{ab}	0.73^{a}	25.66^{a}	20.66^{a}	28.57^{a}	29.00^{b}	3.05^{e}	3.5^{e}
T2 ($ZnSO_4$)	50.67^{ab}	43.33^{c}	0.77^{ab}	0.73^{a}	24.33^{a}	20.66^{a}	32.23^{a}	34.30^{ab}	3.20^{d}	3.6^{e}
T3 (Zn EDTA)	51.00^{ab}	42.66^{c}	0.73^{b}	0.73^{a}	25.66^{a}	22.00^{a}	34.93^{a}	37.26^{a}	3.22^{d}	4.2^{d}
T4 (0.4 ppm N)	54.67^{a}	52.00^{a}	0.80^{ab}	0.83^{a}	25.00^{a}	22.33^{a}	35.63^{a}	37.63^{a}	3.69^{c}	4.6^{c}
T5 (0.8 ppm N)	47.00^{b}	54.66^{a}	0.83^{a}	0.86^{a}	26.66^{a}	21.66^{a}	31.70^{a}	34.23^{ab}	4.31^{b}	5.6^{b}
T6 (1.2 ppm N)	47.00^{b}	48.66^{b}	0.83^{a}	0.80^{a}	26.33^{a}	24.00^{a}	32.50^{a}	35.23^{a}	6.89^{a}	6.8^{a}

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

Table 4: Effect of nano-zinc on macro-nutrients in the leaves of flame seedless grapes in 2014 and 2015 seasons

	N (%)		P (%)		K (%)		Ca (%)		Mg (%)	
Treatments	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
T1 (Tap water)	1.25^{bc}	1.33^{a}	0.19^{b}	0.18^{b}	2.03^{c}	1.70^{ab}	1.68^{a}	1.63^{a}	0.53^{a}	0.55^{a}
T2 ($ZnSO_4$)	1.25^{bc}	1.29^{a}	0.17^{c}	0.17^{b}	2.16^{a}	1.54^{b}	1.52^{c}	1.57^{abc}	0.56^{a}	0.57^{a}
T3 (Zn EDTA)	1.23^{c}	1.33^{a}	0.18^{bc}	0.18^{b}	1.82^{d}	1.63^{ab}	1.43^{d}	1.52^{c}	0.35^{c}	0.54^{a}
T4 (0.4 ppm N)	1.42^{ab}	1.35^{a}	0.16^{c}	0.17^{b}	1.38^{f}	1.68^{ab}	1.33^{e}	1.59^{ab}	0.34^{c}	0.57^{a}
T5 (0.8 ppm N)	1.44^{a}	1.34^{a}	0.20^{a}	0.21^{a}	2.12^{b}	1.59^{ab}	1.63^{b}	1.62^{a}	0.37^{c}	0.55^{a}
T6 (1.2 ppm N)	1.19^{c}	1.29^{a}	0.16^{c}	0.18^{b}	1.74^{e}	1.72^{a}	1.53^{c}	1.54^{bc}	0.42^{b}	0.55^{a}

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

Table 5: Effect of nano-zinc on micro nutrients in leaves of flame seedless grapes in 2014 and 2015 seasons

	Fe (ppm)		Zn (ppm)		Mn (ppm)	
Treatments	2014	2015	2014	2015	2014	2015
T1 (Tap water)	135.33^{b}	136.66^{a}	19.83^{d}	22.56^{c}	100.63^{ab}	112.13^{b}
T2 ($ZnSO_4$)	125.30^{d}	138.63^{a}	55.17^{c}	55.93^{b}	98.67^{b}	113.46^{ab}
T3 (Zn EDTA)	122.50^{e}	135.83^{a}	54.60^{c}	56.26^{b}	101.53^{ab}	113.53^{ab}
T4 (0.4 ppm N.)	131.63^{c}	137.63^{a}	62.67^{b}	66.0^{a}	99.67^{ab}	118.00^{a}
T5 (0.8 ppm N.)	134.23^{b}	136.23^{a}	67.63^{a}	69.3^{a}	108.67^{ab}	110.33^{b}
T6 (1.2 ppm N.)	137.83^{a}	138.5^{a}	69.27^{a}	69.3^{a}	111.00^{a}	115.33^{ab}

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

Effect on leaf mineral content

Macro-elements: In Table 4, it is observed that nitrogen, in the 1st season T5 gave the highest value with no significant difference with T4. While in the second season there was no significant difference between treatments. T5 had a significant increase in leaves content of P compared with the other treatments in the two seasons. In constant no specific trend could be noticed on leaf K, Ca, Mg content between treatments during the two studied seasons.

These results are in harmony with those found by Kisan *et al.*[21], who studied the effect of nano-zinc on the leaf physical and nutritional status of spinach.

Micro-elements: It is observed from Table 5 that T6 increased significantly iron concentration in the first season only while, in the second season there was no significance between treatments.

Regarding zinc concentration, in the first season T5 and T6 recorded the highest values leaves compared with the other treatments. In the second season T4, T5 and T6 scored the highest values. As for manganese concentration, differences were not so acute and did not reach level of significance in most cases during two seasons.

Effect on yield: Concerning yield of flame seedless grape, results presented in Table 6 revealed that, the heaviest cluster weight associated with the greatest yield (kg/vine) were coupled with three nano-zinc treatments (T4, T5 and T6). Differences were significant during both seasons as compared to other treatments from one level and these superior 3 treatments were equally the same from statistical point of view except with T4 during the second season regarding average cluster weight.

On the other hand, the reverse was true when the vines received T1 spraying with tap water such treatment gave the least number of clusters/vine, cluster weight and yield in the two seasons.

These results are matched with those of Afshar *et al.*[22], who worked on wheat using 5 levels of nano-zinc oxide (0, 24, 36, 48 and 60 g ha^{-1}) maximum yield rate of treated

Table 6: Effect of nano-zinc on yield, number and weight of cluster of flame seedless grapes in 2014 and 2015 seasons

	No. of clusters/vine		Cluster weight (g)		Yield (kg)	
Treatments	2014	2015	2014	2015	2014	2015
T1 (Tap water)	10.2^{f}	14.0^{b}	211.66^{c}	261.66^{d}	2.11^{d}	3.60^{d}
T2 ($ZnSO_4$)	22.6^{ab}	27.0^{b}	279.67^{b}	361.33^{c}	6.30^{c}	9.79^{c}
T3 (Zn EDTA)	27.0^{d}	32.0^{a}	304.67^{b}	374.66^{c}	8.21^{b}	11.99^{b}
T4 (0.4 ppm N)	30.33^{c}	33.6^{a}	426.00^{a}	426.66^{b}	12.87^{a}	14.34^{a}
T5 (0.8 ppm N)	33.6^{b}	33.3^{abc}	398.00^{a}	455.33^{a}	13.37^{a}	15.18^{a}
T6 (1.2 ppm N)	37.3^{a}	31.6^{a}	390.33^{a}	465.00^{a}	14.57^{a}	14.72^{a}

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

Table 7: Effect of nano-zinc on length and width of cluster, weight of 100 berries and juice weight of 100 berries of flame seedless grapes in 2014 and 2015 seasons

	Cluster length (cm)		Cluster diameter (cm)		W.100 berry (g)		Juice weight (g)	
Treatments	2014	2015	2014	2015	2014	2015	2014	2015
T1 (Tap water)	19.50^{c}	19.00^{c}	12.00^{a}	12.66^{b}	230.33^{e}	235.00^{d}	42.20^{f}	77.00^{c}
T2 ($ZnSO_4$)	20.00^{bc}	22.00^{b}	13.33^{a}	14.33^{ab}	235.00^{de}	253.00^{d}	74.10^{d}	83.76^{c}
T3 (Zn EDTA)	20.00^{bc}	22.33^{ab}	13.67^{a}	14.33^{ab}	243.00^{d}	284.00^{c}	69.27^{e}	83.93^{c}
T4 (0.4 ppm N)	22.50^{ab}	23.5^{ab}	13.33^{a}	14.66^{ab}	303.00^{b}	330.00^{b}	99.53^{b}	113.83^{b}
T5 (0.8 ppm N)	23.67^{a}	24.66^{a}	14.67^{a}	15.66^{a}	345.00^{a}	365.33^{a}	160.83^{a}	153.63^{a}
T6 (1.2 ppm N)	21.67^{abc}	22.66^{ab}	14.33^{a}	14.33^{ab}	281.67^{c}	278.66^{c}	97.20^{c}	115.90^{b}

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

Table 8: Effect of nano-zinc on TSS, acidity and anthocyanin of flame seedless grapes in 2014 and 2015 seasons

	TSS (%)		Acidity (%)		Anthocyanin (%)	
Treatments	2014	2015	2014	2015	2014	2015
T1 (Tap water)	16.33^{a}	16.33^{ab}	0.80^{b}	0.73^{a}	28.95^{b}	29.74^{bc}
T2 ($ZnSO_4$)	16.67^{a}	17.33^{ab}	0.90^{a}	0.70^{a}	27.12^{e}	28.13^{c}
T3 (Zn EDTA)	17.00^{a}	17.66^{a}	0.70^{c}	0.66^{a}	27.39^{d}	28.05^{c}
T4 (0.4 ppm N.)	17.00^{a}	17.33^{ab}	0.70^{c}	0.66^{a}	28.80^{c}	30.13^{b}
T5 (0.8 ppm N.)	17.00^{a}	17.33^{ab}	0.60^{d}	0.63^{a}	33.26^{a}	33.37^{a}
T6 (1.2 ppm N.)	16.67^{a}	17.33^{ab}	0.60^{d}	0.63^{a}	20.44^{f}	28.44^{c}

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

nano-zinc oxide 60 g ha^{-1} and the lowest yield rate to the control treatments without foliar zinc oxide was obtained[23]. Working on peanut studied the effect of nano-ZnO (25 nm size). Pod yield per plant was 34% higher compared to chelated $ZnSO_4$.

Effect on physical properties: Results tabulated in Table 7 clearly indicated that T5 scored the best effect among all treatments in cluster length with no significant difference with T4 and T6 in the two seasons. As for weight of 100 berries and juice weight/100 berries, T5 increased significantly both berries parameters as compared with all other treatments in the two seasons. On the other hand, the least value of cluster length and diameter, weight of 100 berries and juice weight/100 berries was detected with vines sprayed with tap water T1. Such trend was true in the two seasons.

These results are matched with those of Laware and Raskar[8] who found that nano-zinc on onion showed significantly higher values for seeded fruit per umbel, seed weight per umbel and 1000 seed weight over control plants.

Effect on chemical properties: As shown in Table 8, results showed that TSS was not affected significantly by the different treatments in the two seasons. Regarding to acidity, T2 ($ZnSO_4$) recorded the highest significant value in the first season and no significant difference between treatments in the second season. On the other hand, the lowest values were obtained by spraying with nano-zinc in the two seasons. About anthocyanin, T5 gave the highest value with significant difference among treatments in the 2 seasons.

CONCLUSION

It could be concluded that spraying vines with 0.4 ppm as nano-zinc was the most effective treatment for increasing some vegetative parameters (leaf area, leaf fresh weight and leaf dry weight), leaf mineral content (Fe, Zn), total carbohydrate, fruit quality (No. clusters/vine, cluster weight and yield as kg and fruit skin anthocyanin content) of flame seedless grapes.

SIGNIFICANCE STATEMENT

This study discovered that Nano Zn particles could be beneficial for spraying grapevines, reduced the amounts of zinc needed for grape fertilizer and mitigated the problems of soil pollution caused by the excess use of chemical fertilizers. This study will help the researchers to uncover the critical areas of using Nano as a fertilizer in grapes that many researchers were not able to explore.

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