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Impact of active fresh yeast enriched with zinc on yield and fruit quality of Flame Seedless grapes

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Abstract

This investigation included two integrated experiments, the first one is microbiological experiment which carried out in Microbial Biotechnology Department, NRC, Cairo, Egypt, where multiple incorporation strategies were used for enrichment of yeast with zinc including; (1): In the first procedure, zinc was added to the liquid medium just after the yeast was inoculated (growth phase), (2): For integration, it was added after 24 h of incubation (non-growth phase) in the second procedure. The obtained results showed that the first method (growth phase) was adopted in order to reduce the chance of medium contamination. The concentration of zinc in yeast cells was raised as the concentration of zinc sulphate in the medium increased with 0.4 g/l zinc concentration in the medium, zinc incorporation in yeast cells was 81.25 % higher than with 0.0195 g/l zinc concentration in the medium. The zinc sulphate has no passive effect on yeast cell biomass, however the OD of yeast cell biomass was increased due to zinc concentration in the medium. The second experiment is horticultural one which accomplished over two consecutive seasons (2020 and 2021) on 9 years old Flame Seedless grapevines, cultivated at a private grove, in Sammnod region, Gharbiya Governorate, Egypt in order to study the impact of active fresh yeast extract enriched with Zn at two concentrations on yield, and fruit quality of Flame Seedless grapes grown on loamy soil under flood irrigation system. The experiment was established in a split-plot design with three replicates. The first factor was assigned in the main plot, which included four treatments as follows: (1) Zinc sulphate at 2.0 g/l; (2) Zinc chelate at 2.0 g/l; (3) Active fresh yeast extract enriched with Zn at 10 cm^3/l; (4) Active fresh yeast extract enriched with Zn at 20 cm^3/l. Each main plot was divided into two sub-plots, one of each was provided with soil application and the other sub-plot was assigned for foliar spray application. The treatments were applied twice a year (the first was carried out before fruit set and the second was done three weeks after fruit set). The results showed that different concentrations and addition methods of active fresh yeast extract enriched with Zn excessed fruit yield and promoted cluster physical and chemical properties as compared with the control during both studied seasons. Active fresh yeast extract enriched with Zn at 20 cm^3/l as spraying application was the best treatment for enhancing shoot length and diameter, number of leaves / shoot, leaf area surface, leaf chlorophyll content, cluster weight, yield per vine, weight of 100 berries, juice total soluble solids, leaf mineral content of Fe, Zn, and Mn during the two seasons of the study.

***Key words*:** *Saccharomyces cerevisiae*, Zinc, Bio fertilizer, Flame Seedless grape, Leaf mineral content, Yield, Fruit properties.

1. Introduction

Grape (*Vitis vinifera* L.) is one of the world's most important commercial fruit crops, growing successfully in both tropical and temperate climates. In Egypt, it ranked the second fruit crop and is primarily consumed as fresh fruit. The farmed area has developed fast in the last two decades due to its strong net return, and reached 172.533 feddan that produced 1586342 tones **[1]**.

Excessive chemical fertilizer use has a negative impact on groundwater and can lead to eutrophication in aquatic habitats. Recently, there is a lot of focus on additives which considered more natural, safe, with low-cost. Because of yeast is harmless, nutritious, and easy to use, it has become a popular issue in academic circles. It is a soluble paste or powder prepared from brewer's yeast or fresh yeast with a high biological activity level, low-molecular-weight organic materials, amino acids phosphorus, and trace elements are abundant in yeast extract. Furthermore, it is free of hormones that generated chemically and hazardous components **[2, 3]**. In this concern, the quantity and quality of needle mushrooms could be

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improved by using yeast extract **[4]**. The use of yeast increased vegetable vegetative growth, yield, and quality **[5-9]** as well as elemental content (N, P, K, Fe, and Zn) in vegetables **[5, 6, 10]**.

Climate, vineyard management, mineral nutrition, irrigation, and other factors all influence grapes output and quality. The importance of integrated nutrition management in enhancing productivity cannot be overstated. Micronutrients particularly play a critical role in vine development and production.

Zinc is an essential micronutrient for carbohydrate and protein metabolism, as well as the regulation of plant growth hormones like indole-3-acetic acid (IAA). Zn is also required for the activity of dehydrogenase, proteinase, and peptidase enzymes, as well as the creation and manufacture of starch. Zinc ions (Zn^{2+}) are necessary for the action of several enzymes, and zinc may be essential for chlorophyll production in some plants. In fact, it has been proven that zinc has a good influence on improving the yield **[11-14].** Zinc deficiency in cellular membranes makes them more vulnerable to oxidative damage and inhibits their function, however, maintaining the immune system's integrity necessitates enough zinc consumption **[15].**

The majority of Egyptian soils is calcareous that characteristic with high pH level. The availability of micronutrients is reduced in these soils, resulting in decreasing nutrient absorption. In addition, inconsistent use of phosphate fertilizers in poor soils induces micronutrient deficiencies, resulting in lower micronutrient concentrations in crop products and dry matter. Today's global policy is to limit the use of chemical fertilizers in order to reduce pollution and improve human health.

The present work aimed to evaluate the method of zinc incorporation into yeast cells and examine its effect on fruit physical and chemical properties of Flame Seedless grapes.

2. Material and Methods

2.1. The microbiological experiment

Microbial sample

The Egyptian sample of *Saccharomyces cerevisiae* was used in this study which obtained from microbial biotechnology laboratory, National Research Centre. Yeast extract, peptone, and glucose were used as a growth medium (YEPD).

Enrichment of yeast with zinc

For the enrichment of yeast with zinc, multiple incorporation strategies were used in this study including; (1): In the first procedure, zinc was added to the liquid medium just after the yeast was inoculated (growth phase). (2): For integration, it was added after 24 h of incubation (non-growth phase) in the second procedure **[16]**.

One liter of YEPD medium was sterilized, and 200 ml of it was divided among four conical flasks and inoculated under aseptic conditions. 500 mg of zinc sulphate powder was dissolved in 100 ml distilled water to make a zinc sulphate solution. In the four conical flasks, different amounts of zinc sulphate solution were added at concentrations of 0.0195 g/l, 0.143 g/l, 0.2 g/l, and 0.4 g/l, respectively, and kept in an incubator cum shaker at 120 rpm and 30ºC. The total zinc concentration, absorbance, and dry weight of yeast samples were measured after 24h.

Biomass concentration determination

The biomass concentration was measured at 620 nm using a UV-Spectrophotometer (Spectronic Instruments, USA). A 1.0 g of zinc metal was dissolved in 30 ml of 1:1 nitric acid solution (15 ml of concentrated nitric acid + 15 ml of Millipore water) for use in an Atomic Absorption Spectrophotometer. This solution was heated to 60 °C for 10 min to completely dissolve the zinc metal. Millipore water was used to chill the solution and bring it up to volume in a 1000 ml volumetric flask.

Zinc level in yeast cells determination

The zinc level in yeast cells was determined using an Atomic Absorption Spectrophotometer (AAS) (Shimadzu Scientific Instrument Inc., USA).

A modified Demirci approach **[17]** was employed for sample preparation using Atomic Absorption Spectroscopy (AAS). The supernatant was removed after centrifuging the yeast sample for 15 min at 10,000 rpm and 4 ºC. To remove all of the culture medium that was adsorbed on the cell surface, cells were washed three times with 0.9 % saline water. A 0.1 g of dried yeast sample was placed in a 300 ml Kjeldahl flask. A 5.0 ml of concentrated nitric acid was added to the dried sample and heated to 160 °C until the initial intense reaction stopped. A 2.0 ml of concentrated sulfuric acid was added, and the heating was maintained, with adding concentrated nitric acid in small increments until the solution was colorless. The heating was continued until a dense sulfuric acid fume was produced. After cooling, the contents were filtered into 50 ml volumetric flask and diluted to the volume with distilled water. The absorbance of the sample was measured at 213.9 nm using a Flame Atomic Absorption Spectrophotometer.

2.2. The horticultural experiment

This experiment was implemented during two sequential seasons (2020 and 2021) on Flame Seedless grapevines (*Vitis vinifera* L.), at a private grove in Sammnod region, Gharbiya Governorate, Egypt. Nine years old grapevines were 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.

To determine physical and chemical properties of the soil, samples were obtained at two depths 0-30 and 30-60 cm below the soil surface.

Table 1. Physical and chemical properties of the experimental soil.

Soil depth	Sand %	Silt %	Clay %	Texture
0–30cm	10.8	44	45.2	loamy
30–60cm	12.8	44	43.2	loamy
Soil depth	pH	EC (dSm^{-1})	$CaCO_3$ %	Organic matter%
0–30 cm	8.4	0.5	1.2	1.6
30–60cm	8.4	0.4	2	1.1
Soil depth	N %	P %	K %	Ca %
0–30cm	0.13	0.6	0.9	4.2
30–60cm	0.10	0.6	0.6	3.4
Soil depth	Mg %	Fe ppm	Zn ppm	Mn ppm
0–30cm	1.1	7.8	3.4	3.2
30–60cm	0.9	5.5	2.4	1.8

As the data shown in Table (1), the texture of the soil was loamy, the electrical conductivity (EC) ranged from 0.4 to 0.5 ds/m, the pH was 8.4, and the $CaCO_3$ ranged between 1.2 and 2%. So, the soil is loamy, non-saline, and non-calcareous.

The experiment was established in a split-plot design with three replicates. Each replicate contained four main plots. Each vine was considered as an experimental unit. The first factor assigned in the main plot, which included four treatments as follows:

T1) Zinc sulphate at 2.0 g/l as foliar spray.
T2) Zinc sulphate at 2.0 g/l as soil application.
T3) Zinc chelate at 2.0 g/l as foliar spray.
T4) Zinc chelate at 2.0 g/l as soil application.
T5) Active fresh yeast extract enriched with zinc at 10 cm^3/l as foliar spray.
T6) Active fresh yeast extract enriched with zinc at 10 cm^3/l as soil application.
T7) Active fresh yeast extract enriched with zinc at 20 cm^3/l as foliar spray.
T8) Active fresh yeast extract enriched with zinc at 20 cm^3/l as soil application.

Treatments were applied twice in each season (the first was carried out before fruit set and the second was done three weeks after fruit set).

As a result of the unsuitable environmental conditions in the first season, all fruit crops including grapes were negatively affected. These effects were noticed in the results of the first season.

2.3. Measurements

2.3.1. Vegetative measurements

In mid-July, the fifth fully developed mature leaf from each vine's shoot tip was used to measure the average leaf area surface (cm^2) using the planimeter. The average length and diameter of the shoots were measured in centimeters, and the number of leaves per shoot was recorded.

2.3.2. Chemical determinations

2.3.2.1. Leaf total chlorophyll: Using a Minolta chlorophyll meter, total chlorophyll in fresh leaf samples was measured as spad units (spad = 100 mg chlorophyll/g fresh weight).

2.3.2.2. Leaf mineral content: In mid-July, 20 leaf samples were collected from each vine, including the blade and petiole (6^{th} leaf from the shoot tip) to measure leaf mineral content. The leaves were rinsed in distilled water and dried in the oven at 60-70°C until they reached a consistent weight. According to the method of Jackson **[18]**, the dried samples were grind in a stainless steel knife mill, and 0.2 gm of the powdered material of each sample was digested with a 1:10(v/v) mixture of perchloric acid and sulphoric acid. As the described method of Pregl **[19]**, nitrogen was determined, whereas Truog and Meyer **[20]** colorimetric method was used for determining phosphorus, while as potassium was measured with the flame photometer according to Mason's method **[21]**. Iron, zinc, and manganese were measured with an atomic absorption apparatus according to the methods of Cotteine **[22]**.

2.3.3. Yield and cluster quality

2.3.3.1. Yield/vine (kg): When the total soluble solids (TSS) reached 16.0 % and the color covered all bunch berries, the yield was harvested. Clusters per vine were counted and weighed to determine the total yield per vine as kg.

2.3.3.2. Fruit properties:

Physical properties

Average cluster dimensions (length and width in cm), average cluster weight (g), the weight of 100 berries (g), and juice weight / 100 berries (g) were measured.

Chemical properties

Total soluble solids percentage (TSS%) in berry juice was determined using a hand refractometer, also the total titratable acidity (%) was determined and expressed as ml tartaric acid /100 ml juice as the methods of AOAC **[23].** Total anthocyanins of the berry skin (mg/100g fresh weight) were determined according to the method of Husia *et al.* **[24].**

2.4. Statistical analysis

After testing for the homogeneity of error variances using the Levene test **[25]** and testing for normality distribution according to Shapiro and Wilk **[26],** all data were subjected to analysis of variance for a split-plot design **[27].** Significant differences in means were compared statistically at $p \leq 0.05$ using the least significant difference (LSD) tests. The CO-STAT program was used for the statistical analysis.

3. Results

3.1. The microbiological experiment

In this work, two distinct strategies for enriching yeast with zinc as a trace element were used. Zinc was supplied to the liquid medium immediately after the yeast was inoculated (growth phase) in the first approach, and after 24h of incubation (non-growth phase) in the second method for integration. Figure (1) illustrates Zn content in yeast cells during growth and static phases with varying zinc sulphate concentrations in the culture medium. The results show that the concentration of zinc in yeast cells was raised as the concentration of zinc sulphate in the medium increased. With 0.4 g/l of zinc concentration in the medium, zinc incorporation in yeast cells was 81.25 % higher than with 0.0195 g/l of zinc concentration in the medium (Figure 1).

3.2. Effect of zinc on the yeast cell growth

Biomass was calculated using a UV spectrophotometer to measure zinc absorbance. Figure (2) depicts the influence of zinc on yeast cell development. As demonstrated in the figure, the OD of zinc sulphate alone was not changed, however, the OD of yeast cell biomass was increased due to zinc concentration in the medium.

3.2. Horticultural experiment

3.2.1. Soil mineral content

This search displayed four types of Zn that applied to the soil such as Zn sulphate, Zn chelate, active fresh yeast extract enriched with Zn at 10 cm^3/l and active fresh yeast extract enriched with Zn at 20 cm^3/l. It is clear from Table (2) that the highest rate of nitrogen (27.65 ppm), phosphorous (13.10 ppm), potassium (144 ppm), iron (0.88 ppm), Zn (0.77 ppm) and manganese (0.64 ppm) were obtained due to applying active fresh yeast enriched with Zn at 20 cm^3/l followed by active fresh yeast enriched with Zn at 10cm^3/l comparing with the other treatments. A decrease in pH was noticed with applying active fresh yeast treatments comparing with mineral or chelate forms. This decrease in pH may be responsible for more availability of the other minerals.

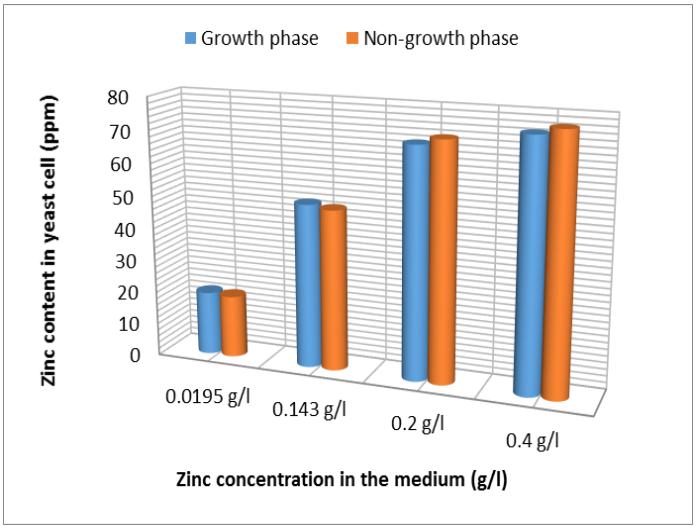

Fig. 1. Effect of different methods for enrichment yeast with Zinc.

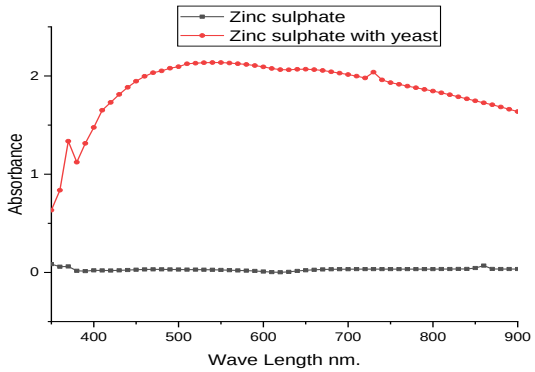

Fig. 2. Effect of zinc on the yeast cell growth.

3.2.2. Leaf mineral content

Results presented in Table (3) indicate that the highest significant values of nitrogen and potassium in the leaves were reflected due to active fresh yeast enriched with Zn at 20 cm^3/l as soil application followed by the same concentration as foliar spray in both seasons, while, the lowest values of nitrogen and potassium were recorded from zinc sulphate as soil application then as foliar spray in the two seasons.

Regarding phosphorous content in the leaves, there was no significant difference among the treatments in the two experimental seasons.

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Table 2. Some physical and chemical soil properties as affected by soil application with different zinc forms and concentrations.

Treatments	EC (dSm^{-1})	pH	Available nutrients (ppm)					
			N	P	K	Fe	Zn	Mn
Zn sulphate as soil application at 2.0 g/l	0.62	7.92	23.46	13.09	101.0	0.698	0.621	0.601
Zn chelate as soil application at 2.0 g/l	0.64	8.04	21.09	11.01	89.19	0.619	0.598	0.543
Active fresh yeast enriched with Zn at 10 cm^3/l as soil application	0.60	7.99	25.98	11.98	127.0	0.786	0.645	0.567
Active fresh yeast enriched with Zn at 20 cm^3/l as soil application	0.63	7.98	27.65	13.10	144.0	0.887	0.775	0.645

The results tabulated in Table (4) indicate that the yeast extract enriched with zinc at the rate of 20 cm^3/l gave the highest values of Zn and Mn, where spraying grapevines recorded the highest significant values of Zn (52.90 & 54.90 ppm) and Mn (32.00 & 34.00 ppm) followed by soil application with the same concentration which scored 49.90 & 51.90 ppm for Zn and 30.16 & 32.16 ppm for Mn in both seasons. Meanwhile, the lowest values of Zn (29.50 & 31.50 ppm) and Mn (19.70 & 21.70 ppm) were obtained from zinc sulphate as soil treatment followed by the spraying application of the same material in the two seasons.

Regarding Fe content, yeast enriched with zinc at the rate of 10 cm^3/l was the best treatment especially as foliar spray which recorded 105.00 & 107.00 ppm without significant difference than yeast enriched with zinc at 20 cm^3/l (104.33 & 106.33 ppm) as foliar spray in both seasons. While the lowest values (99.70 & 101.70 ppm) were obtained from zinc sulphate treatment as soil application in the two experimental seasons.

3.3.1 Vegetative growth

The results presented in Table (5) and Figures (3, 4, 5, 6) show that active fresh yeast enriched with Zn at 10 or 20 cm^3/l as foliar spray or soil application was significantly responsible for motivating the growth parameters such as shoot length, shoot diameter, number of leaves/ shoot and leaf area surface of Flame Seedless grapevines in compared with the other treatments.

Increasing concentration of active fresh yeast enriched with Zn in the study was associated with the promotion. The maximum values of shoot length, number of leaves / shoot, leaf area and chlorophyll content were observed on the vines received the two spraying applications of active fresh yeast enriched with zinc at 20 cm^3/l followed by 10 cm^3/l in both experimental seasons, while the vines treated with Zn sulphate and Zn chelate as soil application recorded the minimum values in both seasons.

Regarding shoot diameter, there was no significant difference among the treatments.

3.3.2 Yield and fruit quality

The results presented in Table (6) and Figures (7, 8) illustrate that the active fresh yeast extract enriched with Zn at 10 or 20 cm^3/l as spraying or soil applications were significantly accompanied with improving cluster vine number, cluster weight and yield per vine comparing with the control.

The highest significant values of cluster number/vine, cluster weight and yield (kg) in the two seasons were obtained from active fresh yeast enriched with zinc at 20 cm^3/l as spraying application. Meanwhile, zinc sulphate as soil application recorded the lowest number of clusters/ vine, cluster weight and yield /vine in the first and the second seasons.

The results in Table (7) and Figures (9, 10) show that treating Flame Seedless grapevines with active fresh yeast extract enriched with Zn at 10 or 20 cm^3/l as foliar spray or soil application significantly had a positive effect on improving cluster length, cluster width, weight of 100 berries and juice of 100 berries compared with the other treatments.

Spraying active fresh yeast extract enriched with zinc at 20cm^3/l gave the highest values of cluster length, cluster width, weight of 100 berries and juice weight of 100 berries in both experimental seasons.

Spraying vines with zinc sulphate recorded the lowest values of cluster length while zinc sulfate as soil application gave the lowest values of cluster width and weight of 100 berries, however, zinc chelate which applied to the soil showed the least value of juice weight of 100 berries in both seasons.

As shown in Table (8) and Figures (11, 12), spraying active yeast extract enriched with Zn at 20 cm^3/l scored the highest values of TSS and anthocyanin with no significant difference than spraying active yeast enriched with Zn at 10 cm^3/l in the two experimental seasons.

Table 3. Effect of active fresh yeast enriched with zinc on leaf micronutrients of flame seedless grapes.

Year	Measurements	Micronutrients (ppm)								
		Fe			Zn			Mn		
		Foliar spray	Soil addition	Mean	Foliar spray	Soil addition	Mean	Foliar spray	Soil addition	Mean
2020	**Zn sulphate (2.0 g/l)**	104.00a	99.70c	101.85b	31.33f	29.50h	30.41d	21.70e	19.70g	20.71d
	Zn chelate (2.0 g/l)	102.00b	99.86c	100.93b	32.73e	30.70g	31.71c	22.10e	20.93f	21.51c
	Act. Y. + Zn (10 cm^3/l)	105.00a	101.00bc	103.00a	49.50c	48.50d	49.00b	29.34c	28.00d	28.67b
	Act. Y. + Zn (20 cm^3/l)	104.33a	102.00b	103.16a	52.90a	49.90b	51.40a	32.00a	30.16b	31.08a
	Mean	103.83a	100.64b	-	41.61a	39.65b	-	26.28a	24.69b	-
2021	**Zn sulphate (2.0 g/l)**	106.00a	101.70c	103.85b	33.33f	31.50h	32.41d	23.70e	21.70g	22.70d
	Zn chelate (2.0 g/l)	104.00b	101.86c	102.93b	34.73e	32.70g	33.71c	24.10e	22.93f	23.51c
	Act. Y. + Zn (10 cm^3/l)	107.00a	103.00bc	105.00a	51.50c	50.50d	51.00b	31.34c	30.00d	30.67b
	Act. Y. + Zn (20 cm^3/l)	106.33a	104.00b	105.16a	54.90a	51.90b	53.40a	34.00a	32.16b	33.08a
	Mean	105.83a	102.64b	-	43.61a	41.65b	-	28.28a	26.69b	-

Table 4. Effect of active fresh yeast enriched with zinc on leaf macronutrients Fe, Zn and Mn of flame seedless grapes.

Year	Measurements	Macronutrients (%)								
		N			P			K		
		Foliar spray	Soil addition	Mean	Foliar spray	Soil addition	Mean	Foliar spray	Soil addition	Mean
2020	**Zn sulphate (2.0 g/l)**	1.33e	1.23f	1.28d	0.10a	0.10a	0.10a	1.03ef	1.01f	1.02d
	Zn chelate (2.0 g/l)	1.47d	1.32e	1.39c	0.48a	0.11a	0.30a	1.13d	1.05e	1.09c
	Act. Y. + Zn (10 cm^3/l)	1.65b	1.61c	1.63b	0.23a	0.21a	0.22a	1.55c	1.53c	1.54b
	Act. Y. + Zn (20 cm^3/l)	1.66b	1.81a	1.73a	0.26a	0.28a	0.27a	1.61b	1.65a	1.63a
	Mean	1.53a	1.49b	-	0.27a	0.18a	-	1.33a	1.31b	-
2021	**Zn sulphate (2.0 g/l)**	1.43e	1.33f	1.38d	0.15a	0.15a	0.15a	1.05ef	1.03f	1.04d
	Zn chelate (2.0 g/l)	1.57d	1.42e	1.49c	0.53a	0.16a	0.34a	1.15d	1.07e	1.11c
	Act. Y. + Zn (10 cm^3/l)	1.75b	1.71c	1.73b	0.28a	0.26a	0.27a	1.57c	1.55c	1.56b
	Act. Y. + Zn (20 cm^3/l)	1.76b	1.91a	1.83a	0.31a	0.33a	0.32a	1.63b	1.67a	1.65a
	Mean	1.63a	1.59b	-	0.31a	0.22b	-	1.35a	1.33b	-

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Table 5. Effect of active fresh yeast enriched with zinc on shoot length, shoot diameter and No. of leaves/shoot of flame seedless grapes.

Year	Measurements	Shoot length (cm)			Shoot diameter (mm)			No. of leaves/shoot		
		Foliar spray	Soil addition	Mean	Foliar spray	Soil addition	Mean	Foliar spray	Soil addition	Mean
2020	**Zn sulphate (2.0 g/l)**	87.00b-d	81.00cd	84.00b	0.46ab	0.33b	0.40a	14.66a	11.00c	12.83b
	Zn chelate (2.0 g/l)	89.00bc	79.00d	84.00b	0.46ab	0.36ab	041a	14.00ab	12.00bc	13.00b
	Act. Y. + Zn (10 cm^3/l)	94.33ab	93.33ab	93.83a	0.46ab	0.36ab	0.41a	14.00ab	14.33ab	14.16ab
	Act. Y. + Zn (20 cm^3/l)	100.66a	94.33ab	97.50a	0.50a	0.36ab	0.43a	15.66a	15.00a	15.33a
	Mean	92.75a	86.91b	-	0.47a	0.35b	-	14.58a	13.08b	-
2021	**Zn sulphate (2.0 g/l)**	89.00b-d	83.00cd	86.00b	0.48ab	0.35b	0.41a	16.66a	13.00c	14.83b
	Zn chelate (2.0 g/l)	91.00bc	81.00d	86.00b	0.48ab	0.38ab	0.43a	16.00ab	14.00bc	15.00b
	Act. Y. + Zn (10 cm^3/l)	96.33ab	95.33ab	95.83a	0.48ab	0.38ab	0.43a	16.00ab	16.33ab	16.16ab
	Act. Y. + Zn (20 cm^3/l)	102.66a	96.33ab	99.50a	0.52a	0.38ab	0.45a	17.66a	17.00a	17.33a
	Mean	94.74a	88.91b	-	0.49a	0.37b	-	16.58a	15.08b	-

Table 6. Effect of active fresh yeast enriched with zinc on No. of clusters/vine and cluster weight of flame seedless grapes.

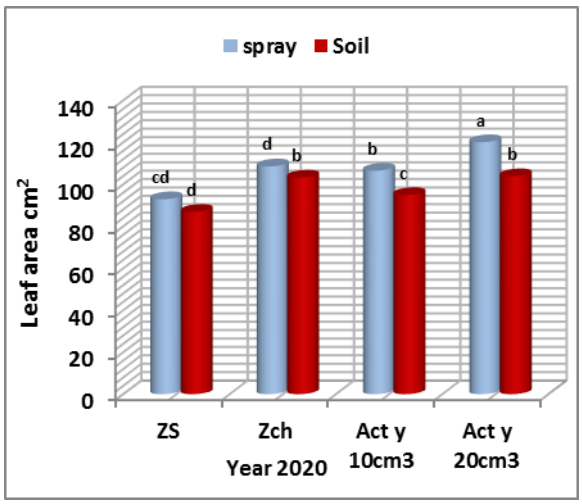
Year	Measurements	No. clusters/vine			Cluster weight (g)		
		Foliar spray	Soil addition	Mean	Foliar spray	Soil addition	Mean
2020	**Zn sulphate (2.0 g/l)**	11.00de	10.00e	10.50c	343.00bc	256.00c	299.50c
	Zn chelate (2.0 g/l)	13.33bc	12.00cd	12.66b	386.66ab	328.33bc	357.50bc
	Act. Y. + Zn (10 cm^3/l)	14.00ab	13.00bc	13.50ab	453.33a	396.00ab	424.66ab
	Act. Y. + Zn (20 cm^3/l)	15.00a	13.66ab	14.33a	474.00a	395.33ab	434.66a
	Mean	13.33a	12.16b	-	414.25a	343.91b	-
2021	**Zn sulphate (2.0 g/l)**	23.40de	21.60e	22.50c	322.88bc	241.46c	282.17c
	Zn chelate (2.0 g/l)	27.59bc	25.20cd	26.38ab	363.74ab	309.15bc	336.44bc
	Act. Y. + Zn (10 cm^3/l)	28.80ab	27.00bc	27.90b	426.14a	372.48ab	399.31ab
	Act. Y. + Zn (20 cm^3/l)	30.60a	28.18ab	29.39a	445.48a	371.86ab	408.67a
	Mean	27.59a	25.48b	-	389.56a	323.73b	-

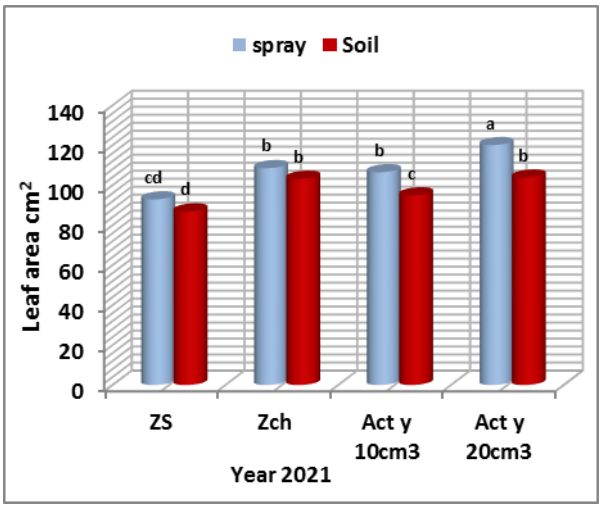
Table 7. Effect of active fresh yeast enriched with zinc on cluster length, cluster width and juice weight of 100 berries of flame seedless grapes.

Year	Measurements	Cluster length (cm)			Cluster width (cm)			Juice weight /100 berries (g)		
		Foliar spray	Soil addition	Mean	Foliar spray	Soil addition	Mean	Foliar spray	Soil addition	Mean
2020	**Zn sulphate (2.0 g/l)**	17.33c	18.00bc	17.66c	14.50bc	12.50d	13.50b	208.14c	210.35c	209.24b
	Zn chelate (2.0 g/l)	21.33a	20.00a-c	20.66ab	16.16ab	13.16cd	14.66ab	217.55bc	204.18c	210.86b
	Act. Y. + Zn (10 cm^3/l)	20.66ab	18.00bc	19.33bc	14.33c	12.83cd	13.58b	230.02bc	247.54ab	238.78a
	Act. Y. + Zn (20 cm^3/l)	22.00a	20.50ab	21.25a	16.33a	14.33c	15.33a	269.91a	225.96bc	247.94a
	Mean	20.33a	19.12a	-	15.33a	13.20b	-	231.40a	222.01a	-
2021	**Zn sulphate (2.0 g/l)**	19.33c	20.00bc	19.66c	16.50bc	14.50d	15.25b	198.38c	200.27c	199.32b
	Zn chelate (2.0 g/l)	23.33a	22.00a-c	22.66ab	18.16ab	15.16cd	16.66ab	207.30bc	194.62c	200.96b
	Act. Y. + Zn (10 cm^3/l)	22.66ab	20.00bc	21.33bc	16.33c	14.83cd	15.58b	219.20bc	236.19ab	227.69a
	Act. Y. + Zn (20 cm^3/l)	24.00a	22.50ab	23.25a	18.33a	16.33c	17.33a	255.88a	215.05c	235.46a
	Mean	22.33a	21.12a	-	17.33a	15.20b	-	220.19a	211.53a	-

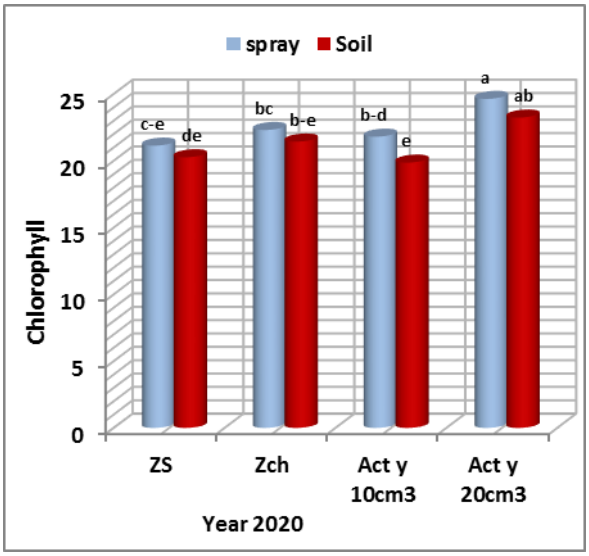
Table 8. Effect of active fresh yeast enriched with zinc on TSS and acidity of flame seedless grapes.

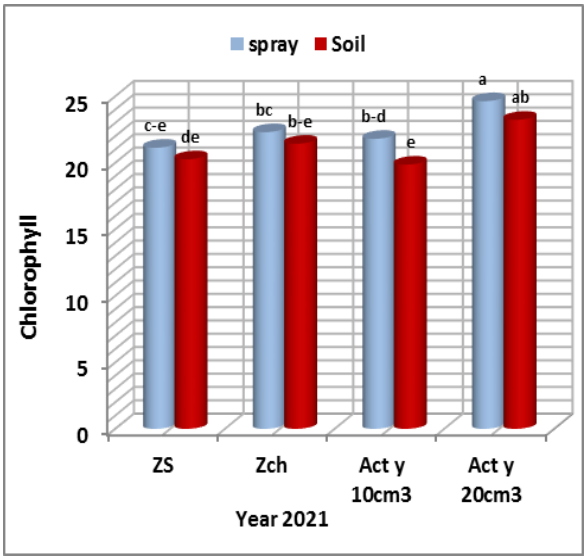
Year	Measurements	TSS %			Acidity %		
		Foliar spray	Soil addition	Mean	Foliar spray	Soil addition	Mean
2020	**Zn sulphate (2.0 g/l)**	16.80bc	16.30c	16.55b	0.67a	0.62a-c	0.64a
	Zn chelate (2.0 g/l)	17.26bc	16.46c	16.86b	0.64ab	0.63a-c	0.63a
	Act. Y. + Zn (10 cm^3/l)	19.60a	18.00b	18.80a	0.64ab	0.59bc	0.61ab
	Act. Y. + Zn (20 cm^3/l)	19.80a	19.26a	19.53a	0.58c	0.60bc	0.59b
	Mean	18.36a	17.50b	-	0.63a	0.61a	-
2021	**Zn sulphate (2.0 g/l)**	16.85bc	16.35c	16.60b	0.63a	0.59a-c	0.61a
	Zn chelate (2.0 g/l)	17.32bc	16.51c	16.91b	0.61ab	0.60a-c	0.60a
	Act. Y. + Zn (10 cm^3/l)	19.65a	18.05b	18.85a	0.61ab	0.56bc	0.58ab
	Act. Y. + Zn (20 cm^3/l)	19.85a	19.31a	19.58a	0.55c	0.57bc	0.56b
	Mean	18.41a	17.55b	-	0.60a	0.58a	-



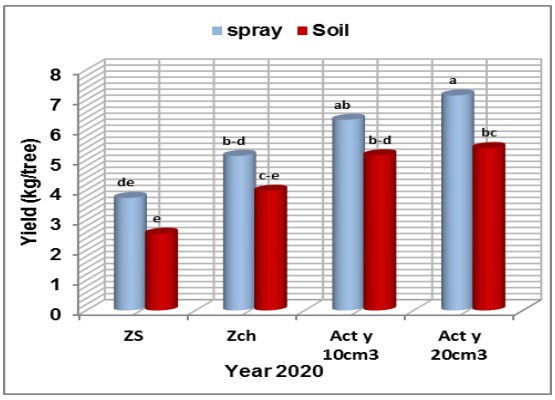


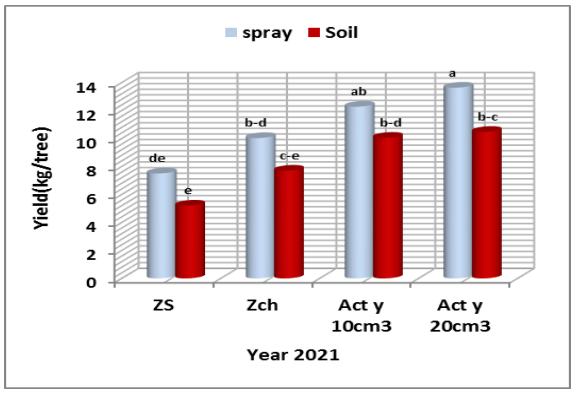
Figs. (3&4). Effect of different treatments on leaf area in 2020 and 2021 seasons.



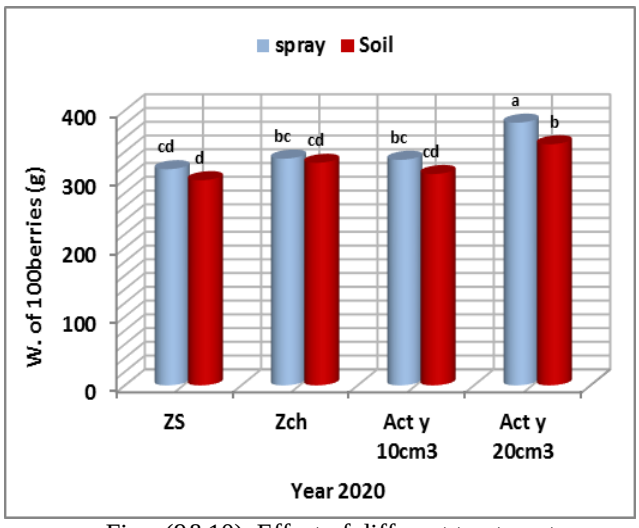


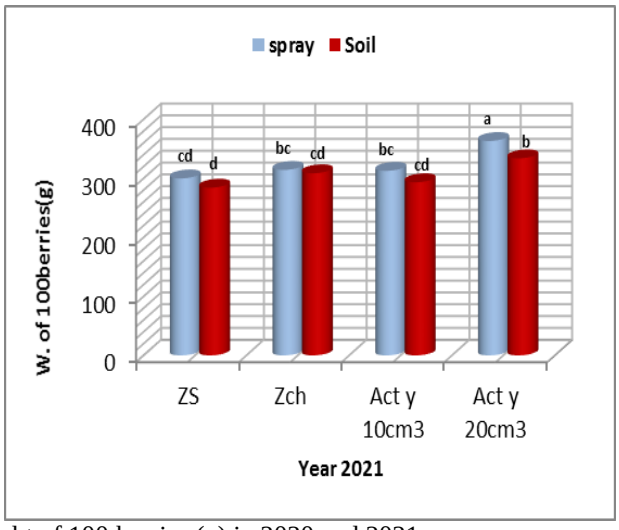
Figs. (5&6). Effect of different treatments on chlorophyll content in 2020 and 2021 seasons.



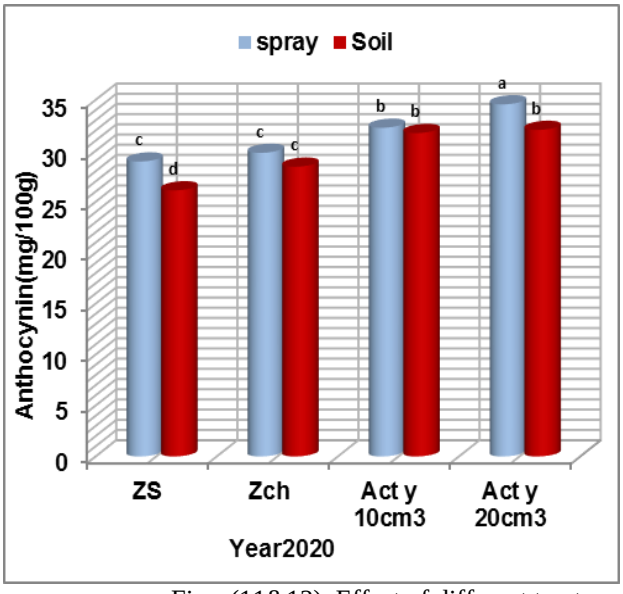


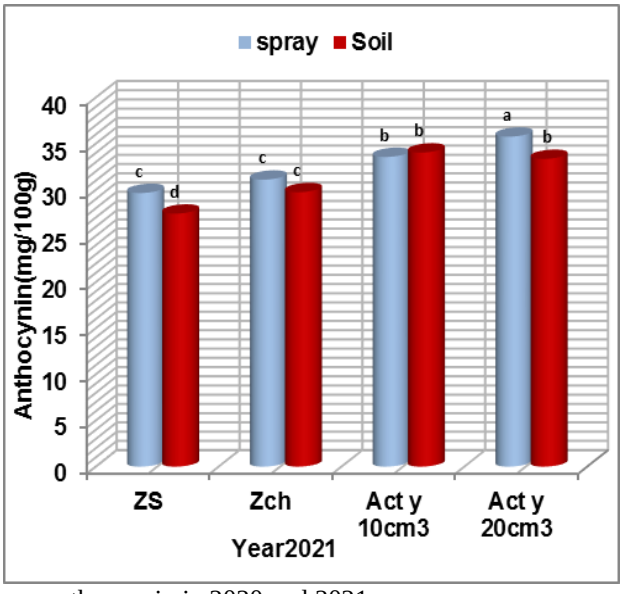
Figs. (7&8). Effect of different treatments on yield per vine in 2020 and 2021 seasons.





Figs. (9&10). Effect of different treatments on weight of 100 berries (g) in 2020 and 2021 seasons.





Figs. (11&12). Effect of different treatments on anthocyanin in 2020 and 2021 seasons.

On the other hand, the lowest values of TSS and anthocyanin were obtained from zinc sulphate treatment as soil application.

Regarding acidity, zinc sulphate treatment in the two addition forms gave the highest values, while active fresh yeast extract enriched with Zn at 20 cm^3/l as foliar spray scored the lowest values in the both seasons with no significant difference among the treatments.

4. Discussion

As for the microbiological experiment, yeasts have been used as a delivery vehicle for mineral supplements due to their ability to incorporate metals into their cells and their high concentration of protein. Yeasts are known for their ability to accumulate metal ions from aqueous solutions by different physicochemical interactions. The concentration of zinc in yeast cells was raised as increasing the concentration of zinc sulphate in the medium, since with 0.4 g/l of zinc concentration in the medium, zinc incorporation in yeast cells was 81.25% higher than with 0.0195 g/l of zinc concentration in the medium. Previous studies showed that, 3203 µg/g of zinc was incorporated in yeast cells in a medium containing 0.0287 g/l zinc sulfate **[28]**. There is no significant difference was found between the two methods for enrichment of yeast with zinc. Therefore, the first method (growth phase) was adopted in order to reduce the chance of medium contamination. As a result, the first approach

(growth phase) was chosen to minimize the risk of medium contamination.

According to our results, zinc sulphate has no passive effect on yeast cell biomass, however, the OD of yeast cell biomass was increased due to zinc concentration in the medium. In this concern, Anil *et al.* **[29]** showed that the yeast cell growth was influenced by increasing zinc concentration in the medium. There was a decrease of 10 % and 21 % in absorbance with the change in Zn concentration for the two different enrichment methods used. The more zinc added to the medium, the more the yeast growth is inhibited. This is probably due to less efficient cell division **[29]**. Previous studies which carried out with yeast reported that some elements such as copper showed rapid growth of yeast cells during 13–20 h, in which consumption of oxygen exceeded the supply of oxygen **[30]**. Thus, it could be conclude that the growth of yeast in the presence of trace elements depends mainly on the composition of the medium, which helps in continuous supply for the yeast cells with oxygen and increases the growth and the ability of the yeast to incorporate the trace elements from the medium in high concentrations.

Regarding the horticultural experiment, consumers are increasingly concerned about the link between food safety and human health. In this respect, natural sources (yeast, seaweed extracts, compost, etc.) play a crucial role **[31- 34]**. Farmers are more interested in using natural sources as bio-control agents in agriculture to provide safe food **[35, 36]**.

Comparing with the other treatments, spraying active fresh yeast enriched with Zn at 20cm^3/l has a positive effect on shoot length and diameter, number of leaves / shoot, leaf area, chlorophyll content, cluster weight, yield, weight of 100 berries, TSS, and leaf mineral content.

Similar findings were reported in many previous studies **[37- 40]**. Yeast has a beneficial influence on crop development **[41, 42]**. Spraying 'Keitte' mango trees with 0.2 percent yeast once at fully bloomed was very efficient in increasing yield and its components, as well as improving fruit quality **[38]**.

The effects of yeast extract may be related to the fact that yeast is a natural component (harmless and non-polluting) that contains a significant number of minerals, especially N, P, and K, proteins, vitamin B, and natural hormones, such as cytokinin and IAA. Axuins, hormones, vitamins, chelating agents, and enzymes generated by yeast were found to have stimulating effects on cell division and enlargement, nutrition absorption, protein synthesis, and improving net photosynthesis **[43, 44]**. These effects cause hormones to improve and carbs to accumulate, resulting in higher sugar and anthocyanin levels in the fruit.

Some researchers found similar results about the influence of yeast spray on grapevine fruiting. They concluded that spraying grapevine with yeast increased berry quality by increasing berry size, TSS%, and anthocyanin content, while lowered overall acidity, causing berry ripening to occur earlier and improving physical and chemical features of the fruit **[45-52]**. This could be owing to the impact of these treatments on the grapevine's nutritional conditions, which has been reflected on fruit output and quality.

5. Conclusions

As for the microbiological experiment, the results of multiple incorporation strategies that used for enrichment of yeast with zinc showed that the first method (growth phase) was adopted in order to reduce the chance of medium contamination. The concentration of zinc in yeast cells was raised as the concentration of zinc sulphate increased in the medium. With 0.4 g/l zinc concentration in the medium, zinc incorporation in yeast cells was 81.25% higher than with 0.0195 g/l zinc concentration in the medium. Zinc sulphate has no passive effect on yeast cell biomass, however the OD of yeast cell biomass was increased due to zinc concentration in the medium.

Regarding the horticultural experiment, it could be deduced that spraying active fresh yeast enriched with Zn at 20 cm^3/l followed by 10 cm^3/l were the most effective treatments for improving shoot length, shoot diameter, number of leaves / shoot, leaf area, leaf chlorophyll content, cluster weight, yield per vine, weight of 100 berries, juice TSS%, leaf mineral content of Fe, Zn, and Mn during the two seasons of the study. Active fresh yeast especially as foliar application decreases the utilization rate of zinc fertilizer to obtain a good production, consequently reduces the pollution resulted from applying the chemical fertilizers.

6. Conflicts of interest

The authors declare that they have no conflict of interest.

7. Formatting of funding sources

National Research Centre, El Buhouth St., Dokki, Cairo, Egypt

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