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(Original Article)

Enhancement of Vegetative and Fruiting Properties of Ruby Seedless Grapevines

Alaa A.B. Masoud*; Rashad A. Ibraim; Ahmed M.M. Abdelghany and Mai E. A. Mansour

Pomology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

*Corresponding author e-mail: alaa.masoud@agr.aun.edu.eg
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Abstract

Experiments were conducted during two consecutive seasons of 2022 and 2023 on Ruby seedless grape cultivar grown in the experimental orchard of Pomology Department, Faculty of Agriculture, Assiut University.

The following applications were carried out on each vine: Nano Zinc oxide at 5 ppm, Nano Zinc oxide at 10 ppm, Methionine at 50 ppm, Methionine at 100 ppm, Fish extract at 0.5 ml/L, and Fish extract at 1.5 ml/L

The study confirmed that foliar spraying with nano-zinc and amino acids gave significant differences compared to the control in shoot length (cm), number of leaves / branch, and leaf area (cm^2) in addition to increasing yield and improving the characteristics of bunches and berries. The study also confirmed improvement of the chemical properties of fruits, such as the ratio of total soluble solids, total acidity, and the ratio of total soluble solids to total acidity. The contents of sugars and anthocyanins also increased compared to the control treatment when foliar spraying with nano-zinc, methionine, and fish oil.

Keywords: *Amino acids, Fish oil, Nano-zinc, Methionine, Ruby seedless.*

Introduction

The world's largest fruit crop, grapevine (*Vitis vinifera* L.), is used to make raisins and wine in addition to fresh consumption. European varieties comprise the majority of grape cultivars cultivated in Egypt, namely table grapes. More grape varieties than any other fruit crop is grown worldwide where grape production makes up nearly half of all fruit produced worldwide. Grapes are the world's most popular fruit crop and Egypt's second most important crop after citrus, with a steady increase in vineyards, particularly on recently reclaimed land. The total area under grape cultivation is 186,404 feddans (1 feddan = 4200m^2), of which 178,485 feddans are under productive vines, with a total production of approximately 1,790,734 tons and average of 10,033 tons per feddan.

The most important application of nanotechnology in agricultural crop production is the field of nano-fertilizers, which can feed plants gradually in a controlled manner, conversely to what occurs in the case of common fertilizers.

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These nano-fertilizers can be more efficient, decreasing soil pollution and other environmental risks that may occur when using chemical fertilizers (Naderi *et al*., 2011). One of the advantages of using nano-fertilizers is that application can be done in smaller amounts than when using other types of fertilizers (Selivanov and Zorin, 2001; Reynolds, 2002; Subramanian *et al*., 2015). The solubility, diffusion, and availability of Zn to plants are affected by the characteristics of nano-particulate Zn-size, specific surface area, and reactivity of nanomaterials, which can be used to design new Zn fertilizers (Subramanian and Sharmila Rahale, 2012; Mosanna and Behrozyar, 2015). Studies have demonstrated the way that amino acids can influence the physiological cycles of the plants straightforwardly or by implication. Amino acids not only help with development, but they also make output quality and quantity better. Amino acids can improve plant cells protection from oxidation and other stresses. They can also assist in the biosynthesis of proteins, growth regulators like IAA, gibberellin and ethylene, and cell division, which improve fruiting in plants. According to Davies, 1982; Yagodin, 1990 and Rai, 2002, fish oil contains two types of omega-3 fatty acids: docosahexaenoic acid (DHA), eosapentaenotic acid (EPA), tocopherols, iron, copper, and vitamin B. It is regarded as a significant antioxidant source. Most recent studies provided strong evidence for the significance of using glutathione and fish oil to protect trees from aging and prevent Reactive Oxygen Species (ROS) induced cell damage. Also, these two treatments have also helped promote organic farming and reduce environmental pollution. Different horticultural crops' yield, fruit quality, and nutritional status were all improved by using fish oils during growth. (Olmo *et al.,* 1981; Osnaya and Schlasser, 1998; Mc-Arthey *et al*., 2006; Yoder *et al*., 2009; Masoud and Abou-Zaid, 2017).

The aim of this study was to further elucidate the effects of spraying with nano-Zn, amino acids, and fish extract on the vegetative growth, yield, and berry quality of seedless grape variety Ruby.

Materials and Methods

Experiments were conducted during two consecutive seasons of 2022 and 2023 on Ruby seedless grape cultivar grown in the experimental orchard of Pomology Department, Faculty of Agriculture, Assiut University.

Thirty-five standardized grapevines were selected in a completely randomized block design. The seedless variety Ruby was pruned using a traditional head breeding system with 18 fruiting branches, each with three buds, and five replacement branches. This left a total of 64 buds on each vine.

The following applications were carried out on each vine: Nano Zinc oxide at, 5 ppm, Nano Zinc oxide at 10 ppm, Methionine at 50 ppm, Methionine at 100 ppm, Fish extract at 0.5 ml/L and Fish extract at 1.5 ml/L.

The spray solution was added three times: when the shoots were 10 cm long, at full flowering, and 1 month after full flowering. Each treatment included 5 vines (replicates), and horticultural practices such as irrigation, soil management, and fertilization were implemented as recommended.

The following measurements were carried out on the vines under study:

A- Vegetative measurements:

1- Main shoot length of the current season (cm.)

2- Leaf number/main shoot.

3- Leaf height and leaf width (cm.), to determine leaf area (cm^2.) according to the equation published by Ahmed and Morsy (1999).

$$\text{Leaf area} = 0.44\ (\text{L}\times\text{W}) + 18.13$$

B- Yield components:

1-Cluster weight (g).

2-Cluster no. / vine.

3-Yield (kg/vine)

C- Physical properties

1-Berries number / cluster.

2-Berry height and diameter (cm): By a Vernier caliper, to determine berry shape index (H/D).

3-Cluster height, width (cm.): To determine cluster shape index (H/W).

D- Chemical properties

1– Total soluble solids (TSS %): By using a hand refractometer (ATAGO N-IE).

2- Total acidity (T.A) (expressed as % tartaric acid): Was determined by titration of NaOH at 0.1N using phenolphthalene as an indicator. The NaOH was adjusted by using a known volume of oxalic acid 0.1M according to A.O.A.C. (1984).

The total acidity was expressed as tartaric acid according to the following equation:

$$\text{Acidity}(\%) = \frac{\text{NaOH volume used in titration} \times \text{NaOH molarity} \times \text{equivalent weight of tartaric acid}}{1000 \times \text{sample volume}} \times 100$$

Where

Equivalent weight of tartaric acid = 75, NaOH molarity = 0.1M, Sample Vol. = 5 ml.

3- TSS / acid ratio was then calculated.

4- Reducing sugars (%): According to Lane and Eynon procedure outlined in A.O.A.C. (1985).

5- Total Anthocyanin content: Total anthocyanin. The anthocyanin pigment was extracted by ethanolic HCl, a mixture of 95% ethanol and 1.5 N HCl (85:15 v/v).

$$\text{Total Anthocyanin} = \frac{\text{Sample size} \times \text{Spectrophotometer reading}}{\text{Peel weight} \times 98.2} \times 100$$

Analytical statistics

A randomized complete blocked design (RCBD) with five replications for each treatment was used in the experiment. Proc Mixed of SAS package version 9.2 (SAS, 2008) was used to perform the analysis of variance (ANOVA), and the means were compared using the revised L.S.D. test at a probability of 5% (Steel and Torrie, 1980).

Results and Discussion

This study planned to reveal more insight into the impact of splashing Nano zinc, amino acids and fish remove on vegetative development, yield and berry nature of Ruby seedless grape cultivar.

This study reached the following results, which are shown in the following tables and figures:

1-Vegetative and physical components

Table 1 shows the effect of various treatments on shoot length (cm), leaves number/shoot and Leaf area (cm^2) of Ruby seedless grape cultivar during 2022 and 2023 seasons. Fish extract, methionine, and nano-zinc oxide affected shoot length (cm) of the Ruby Seedless grape variety as shown in Table1. The presented data revealed that, during the 1^{st} season of study, Methionine at 50 ppm exhibited the highest shoot length (cm). The recorded shoot length of such treatment was 83.5 cm. The differences between all treatments were not significant compared to the control. During the 2^{nd} season of study, Nano Zinc oxide at 5 ppm represented the highest shoot length (76.9 cm).

The data (Table 1) also showed that, during the two seasons of study, Methionine at 50 ppm recorded the highest leaves number/shoot (13.3 and 12.4, respectively). No significant differences were recorded between all treatments. Nano Zinc oxide at 5 ppm registered the highest leaf area (78.318 and 78.97 cm^2) during the two seasons of study, respectively. During the first season of study, Nano Zinc oxide at 5 ppm represented a significant variation compared to the rest of treatments whereas there were no significant differences between most of the treatments during the 2^{nd} season of study.

These results are in agreement with those obtained by (Rasouli Sadaghiani *et al*., 2002), (Serdar *et al*., 2005), (Khayyat *et al*., 2007), (Tariq *et al*., 2007), Obaid and Al-Hadethi (2013), Hamouda *et al.,* (2016), Jumaa and Ali (2016), El-Hak *et al*., (2019), Genaidy *et al*., (2020), Mohamed, (2020), (Taghavi, 2000), they confirmed that foliar spraying with fertilizers, including trace elements such as nano-zinc, gave significant differences compared to the control in shoot length (cm), leaves number/shoot and Leaf area (cm^2), Kassem *et al*., (2011), (El-Khawaga and Mansour, 2014), Belal *et al*., (2016). They found that foliar spraying with amino acids gave significant differences in leaves number/shoot and Leaf area (cm^2) compared to the control.

Table 1. Effect of Nano Zinc oxide, Methionine, and fish extract on shoot length (cm), leaves number/shoot and Leaf area (cm^2) of Ruby seedless grape cultivar during 2022 and 2023 seasons

Treatments	Shoot length (cm)		Leaves number/shoot		Leaf area (cm^2)	
	2022	2023	2022	2023	2022	2023
Control (water only)	62.2 C	57.4 CD	12.3 AB	9.9B	68.002BC	71.228 C
Nano Zinc oxide at 5 ppm	83 A	76.9 A	11.9 AB	11.1 AB	78.318 A	78.97 AB
Nano Zinc oxide at 10 ppm	62.4 C	54.9 D	12.3 AB	9.8 B	71.366 B	73.436 BC
Methionine at 50 ppm	83.5 A	63.2 BC	13.3 A	12.4 A	72.56 B	79.746 A
Methionine at 100 ppm	65.4 C	60.5 BCD	11.6 B	10.5 B	64.608 C	73.036 C
Fish extract at 0.5 ml/L	74.8 B	64.9 B	11.5 B	11.3 AB	69.47 BC	74.048 BC
Fish extract at 1.5 ml/L	76.85 B	73.1 A	12.2 AB	10.7 B	72.102 B	75.828 ABC

Means with the same letters are not significantly different based on LSD of 5%.

Data presented in Table 2 showed the effect of various treatments on clusters number/vine of Ruby Seedless grape cultivar during 2022 and 2023 seasons. The obtained results revealed that Nano Zinc oxide at 5 ppm and fish extract at 1.5 ml/L exhibited the best results (49.8 clusters/ vine for each of them). Other treatments did not significantly differ from the control. During the 2nd season, fish extract at 1.5 ml/L also gave the best results (40.0 clusters/ vine)

The presented data (Table 2) indicated that, during the two seasons, fish extract at 0.5 ml/L represented the highest values of cluster weight (389.08 and 380.79 gm), respectively. During the 1st season of study, there was a clear difference between fish extract at 0.5 ml/L and the remaining treatments. Whereas, during the 2nd season, there were no significant differences between most of the treatments.

Data presented in Table 2 show the effect of nano Zinc oxide, Methionine and fish extract applications on yield of Ruby Seedless grape cultivar. All treatments significantly exceeded the control except for the differences between Methionine at 100 ppm and the control during the 2nd season of study. During the two seasons of study, Fish extract at 0.5 ml/L exceeded the remaining treatments. It recorded (15.26 and 14.44 kg/vine), respectively.

Table 2. Effect of Nano Zinc oxide, Methionine and fish extract on Clusters number/vine, Cluster weight (gm), and Yield (Kg/vine) of Ruby seedless grape cultivar during 2022 and 2023 seasons

Treatments	Clusters number/vine		Cluster weight(gm)		Yield (Kg/vine)	
	2022	2023	2022	2023	2022	2023
Control (water only)	48.4A	31B	255.4C	316.67BC	12.36C	9.66D
Nano Zinc oxide at 5 ppm	49.8A	39.6A	286.14BC	335.47ABC	14.14B	13.04BC
Nano Zinc oxide at 10 ppm	43.6AB	38.8A	324.66B	364.68AB	13.98B	14.04AB
Methionine at 50 ppm	49A	37.4AB	295.34B	336.87ABC	14.36AB	12.38C
Methionine at 100 ppm	43.6AB	33.6AB	311.36B	294.7C	13.48B	9.82D
Fish extract at 0.5 ml/L	39.4B	38.2A	389.08A	380.79A	15.26A	14.44A
Fish extract at 1.5 ml/L	49.8A	40A	285.42BC	321.16BC	14.08B	12.66C

Means with the same letters are not significantly different based on LSD of 5%.

These results are in agreement with those obtained by Mohamed (2020), Kassem *et al*., (2011), Madian and Refaai (2011), Ahmed *et al*., (2011), Marzouk and Kassem (2011), El-Khawaga and Mansour (2014). Abdelaziz *et al*., (2017), and Portu *et al*. (2017). They confirmed that foliar spraying with nano-zinc, amino acids and fish oil led to increased yield, and improved the cluster and berry traits.

Table 3 shows the effect of various treatments on Berry length (L, cm), Berry diameter (D, cm) and berry L/D ratio of Ruby seedless grape cultivar during 2022 and 2023 seasons. The obtained results in Table 3 suggested that during the 1st season the highest berry length was obtained from the vines treated with fish extract at 0.5 ml/L (1.704 cm). However, the differences between the remaining treatments were not significant. However, during the second season of the study, methionine at a concentration of 50 ppm represents the highest berry length (1.72 cm).

The obtained results (Table 3) revealed that during the 1st season of study, Methionine at 50 ppm recorded the highest value for Berry diameter (1.536 cm), While nanozinc oxide at 5 ppm and methionine at 50 ppm produced the highest values (1.538 cm for both treatments) during the second season. All treatments exhibited insignificant differences in Berry diameter during the two seasons of study. During the two seasons of study, the highest berry L/D ratio was obtained from the fruits treated with fish extract at 0.5 ml/L (1.114 and 1.140) respectively. While most of the treatments did not show any significant differences during the two seasons of the study (table 3).

In general, these results are in line with those obtained by Khayyat *et al*., (2007), (Davarpanah *et al*., 2016), Genaidy *et al*., (2020), Shaaban *et al.,* (2024), Madian and Refaai (2011) Kassem *et al*., (2011), Marzouk and Kassem (2011), El-Khawaga and Mansour (2014), Portu *et al*., (2015), Nagy and Pintér (2015), Mostafa *et al*., (2015), Belal *et al*., (2016), Portu *et al*. (2017), Bassiony *et al*., (2018), Mohamed and Qaoud (2019), and El-Sese *et al*., (2020), They reported an increase in the quality of berries and yield components compared to the control treatment when foliar spraying with nano-zinc, methionine, or any amino acid, fish oil, or natural oil applications.

Table 3. Effect of Nano Zinc oxide, Methionine and fish extract on Berry length (cm), Berry diameter (cm) and berry L/D ratio of Ruby seedless grape cultivar during 2022 and 2023 seasons.

Treatments	Berry length(cm)		Berry diameter(cm)		Berry L/D ratio	
	2022	2023	2022	2023	2022	2023
Control (water only)	1.604 C	1.628 BC	1.49 A	1.484 A	1.072 C	1.098CD
Nano Zinc oxide at 5 ppm	1.624 BC	1.712 A	1.51 A	1.538 A	1.076 BC	1.114 BC
Nano Zinc oxide at 10 ppm	1.658 BC	1.596 C	1.51 A	1.484 A	1.1 AB	1.076 D
Methionine at 50 ppm	1.684 A	1.72 A	1.536 A	1.538 A	1.096 ABC	1.12 ABC
Methionine at 100 ppm	1.662 AB	1.644 BC	1.524 A	1.524 A	1.09 ABC	1.08 D
Fish extract at 0.5 ml/L	1.704 A	1.69 AB	1.53 A	1.484 A	1.114 A	1.14 A
Fish extract at 1.5 ml/L	1.656 ABC	1.682 AB	1.522 A	1.498 A	1.088 BC	1.124 AB

Means with the same letters are not significantly different based on LSD of 5%.

2-Chemical constituents of fruits

Table 4 shows the effect of various treatments on total soluble solids % (TSS), total acidity % (TA), and TSS/TA ratio of Ruby seedless grape cultivar during 2022 and 2023 seasons. Table 4 showed that during the two seasons of study, Methionine at 50 ppm and fish extract at 0.5 ml/L significantly exceeded the control with fish extract at 0.5 ml/L recording the highest value during the 1st season (19.20 and 19.32%), respectively. The remaining treatments did not differ significantly during the second season of the study, except for the treatment fish extract at 0.5 ml/L, (20.44) the difference was significant between it and the rest of the treatments. The data presented in Table 4 indicate that all treatments significantly reduced total acidity compared to the control group. There were no significant differences between these results during the two seasons studied.

During the two seasons of the study, fish extract at 1.5 ml/L had the lowest values (0.310 and 0.306) percent, respectively. The obtained results (Table 4) revealed that; during both seasons of study, fish extract at 1.5 ml/L significantly exceeded the control (61.52 and 65.08), respectively.

Table 4. Effect of Nano Zinc oxide, Methionine and fish extract on total soluble solids %, total acidity % and TSS/TA ratio of Ruby seedless grape cultivar during 2022 and 2023 seasons

Treatments	Total soluble solids %		Total acidity %		TSS/TA ratio	
	2022	2023	2022	2023	2022	2023
Control (water only)	17C	17.76C	0.378A	0.416A	46.08C	42.82D
Nano Zinc oxide at 5 ppm	17.94ABC	19.44AB	0.348AB	0.355BC	52.14BC	55.18BC
Nano Zinc oxide at 10 ppm	17.4C	18.88BC	0.328AB	0.384AB	53.56ABC	49.62C
Methionine at 50 ppm	19.2A	19.08B	0.346AB	0.388AB	55.72AB	49.86C
Methionine at 100 ppm	17.56BC	19.2B	0.33AB	0.324C	53.98ABC	59.56AB
Fish extract at 0.5 ml/L	19.32A	20.44A	0.358AB	0.422A	54.06ABC	48.62CD
Fish extract at 1.5 ml/L	19AB	19.76AB	0.31B	0.306C	61.52A	65.08A

Means with the same letters are not significantly different based on LSD of 5%.

Table 5 shows the effect of various treatments on reducing sugar% and Anthocyanin (mg/g) of Ruby seedless grape cultivar during 2022 and 2023 seasons. The obtained results for the 1st season (Table 5) demonstrated that the best treatment that gave the highest percentage of reducing sugars was Methionine at 50 ppm that is significantly surpassed the check treatments. The percentage of reducing sugars for the abovementioned treatment was 14.17%.

During the second season of the study, spraying with fish extract at a rate of 0.5 ml/L showed the best results. The percentage of reducing sugars for these treatments was 18.45%, while there were no significant differences between the remaining treatments during the two seasons of the study.

The presented data (Table 5) revealed that most treatments, during the first season, significantly enhanced the content of anthocyanin compared to the control. However, during the second season, most treatments did not significantly affect the content of anthocyanin compared to the control.

The best treatment during the 1st season in this respect was Methionine at 50 ppm. Such treatments produced 38.48 mg/g, during the 2nd season of study, fish extract at 1.5 ml/L significantly exceeded all the other treatments and recorded the highest value (29.28 mg/g).

Table 5. Effect of Nano Zinc oxide, Methionine and fish extract on Reducing sugar% and Anthocyanin (mg/g) of Ruby seedless grape cultivar during 2022 and 2023 seasons.

Treatments	Reducing sugar %		Anthocyanin (mg/g)	
	2022	2023	2022	2023
Control (water only)	12.884 BC	15.972 C	28.18 E	26.48 C
Nano Zinc oxide at 5 ppm	13.5384 AB	17.4132 AB	37.64 AB	27.48 B
Nano Zinc oxide at 10 ppm	13.092 BC	16.882 BC	35.22 C	27.6 B
Methionine at 50 ppm	14.1744 A	17.508 AB	38.48 A	27.64 B
Methionine at 100 ppm	12.344 C	17.6556 AB	37.7 AB	28.08 B
Fish extract at 0.5 ml/L	13.3176 ABC	18.4548 A	33.54 D	28.28 B
Fish extract at 1.5 ml/L	13.2832 ABC	17.7498 AB	36.62 BC	29.28 A

Means with the same letters are not significantly different based on LSD of 5%.

These results are in agreement with those obtained by Abdel-Aal *et al*., (2022), Hasani *et al*.,(2012), Hamouda *et al.,* (2016), Jumaa and Ali (2016), Kumar *et al*. (2016), Davarpanah *et al*., (2016), Genaidy *et al*., (2020), Mohamed, (2020), Abou El-Nasr *et al*., (2021), Kassem *et al*., (2011), Marzouk and Kassem (2011), El-Khawaga and Mansour (2014), Portu *et al*., (2015), Nagy and Pintér (2015), Belal *et al*., (2016), Portu *et al*. (2017), Mohamed and Qaoud (2019), and El-Sese *et al*. (2020), They confirmed an increase in the improvement of the chemical characteristics of the fruits, such as total soluble solids %, total acidity % and TSS/TA ratio, sugars ,and anthocyanin content compared to the control treatment when foliar spraying with nano-zinc, methionine, any amino acid, fish oil, or natural oils.

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تحسين الصفات الخضرية والثمرية لكرمات العنب الروبي عديم البذور

علاء عبد الجابر بدوي مسعود، رشاد عبد الوهاب إبراهيم، أحمد محمد محمد عبد الغني، مي ابراهيم عبد الفتاح منصور

قسم الفاكهة، كلية الزراعة، جامعة أسيوط، اسيوط، مصر.

الملخص

أجريت التجارب خلال موسـمين متتاليين 2022 و2023 على صـنف العنب روبى سـيدلس المزروع بمزرعة قسـم الفاكهة بكلية الزراعة جامعة أسـيوط .كان عمر الكروم 16 عامًا في بداية التجربة، وتم زراعتها على مسـافة 2 × 2.5 متر .تم اختيار خمسـة وثلاثين شـجرة عنب موحدة بتصـميم القطاعات العشـوائية الكاملة وتم تقليم الكروم بالطريقة الرأسـية بحيث تحتوي على 18 دابرة ثمرية و3 براعم على كل دابرة و5 دوابر اسـتبدالية. وبذلك يكون مجموع البراعم المتبقية على كل كرمة 64 براعم.

تم تنفيذ التطبيقات التالية على كل كرمة: نانو أكسـيد الزنك عند 5 جزء في المليون، نانو أكسـيد الزنك عند 10 جزء في المليون، ميثيونين عند 50 جزء في المليون، ميثيونين عند 100 جزء في المليون، مستخلص السمك عند 0.5 مل / لتر ومستخلص السمك عند 1.5 مل / لتر.

وأكدت الدراسـة أن الرش الورقي بالزنك النانوي والأحماض الأمينية أعطى فروقاً معنوية مقارنة بالكنترول في طول المجموع الخضـري (سـم)، عدد الأوراق/الفرخ، والمسـاحة الورقية ($سم^2$) .بالإضافة إلى زيادة المحصول وتحسين خصائص العناقيد والثمار .كما أكدت الدراسة زيادة في تحسـن الخواص الكيميائية للحبات مثل نسـبة المواد الصـلبة الذائبة الكلية والحموضـة الكلية ونسبة المواد الصلبة الذائبة إلى الحموضة كما زادت كمية السكريات والأنثوسيانين مقارنة بمعاملة الكنترول عند الرش الورقي بالنانو زنك والميثيونين وزيت السمك.

الكلمات المفتاحية: *الأحماض الأمينية، الميثيونين، روبي سيدلس، زنك، زيت السمك النانو.*