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Influence of Arbuscular Mycorrhizal Fungi, Seaweed Extract and Nano-Zinc Oxide Particles on Vegetative Growth, Yield and Clusters Quality of 'Early Sweet' Grapevines

Bassam E. A. Belal [1], Mosaad A. El-Kenawy [1], Shimaa M. M. El-Mogy [1], and Asmaa S. M. Omar [2] *

[1]*Viticulture Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.*

[2]*Pomology Department, Faculty of Agriculture, Mansoura University, Egypt.*

THE present investigation has been carried out for two consecutive seasons (2020 and 2021) on 'Early Sweet' grapevines in the experimental farm located at El-Baramon station, Horticulture Research Institute, Mansoura, Dakahlia Governorate, Egypt in order to enhance the vegetative growth traits, nutritional status, yield, and cluster quality attributes by the applications of arbuscular mycorrhizal fungi, seaweed extract, and Nano-Zinc oxide particles individually or combined. The soil was inoculated with arbuscular mycorrhiza fungi at 250 g inoculum/vine once during winter agricultural management and foliar spray with seaweed extract at 1% and Nano-Zinc oxide particles at 50 ppm at three times: after the bud burst stage, at fruit set stage and two weeks after fruit set stage. The results demonstrated that the used applications either individually or combined among them had a significant effect on all conducted traits compared to the control in both seasons. Also, the combined applications were the most effective than the individual applications, especially the triple application (arbuscular mycorrhiza fungi + seaweed extract + Nano-Zinc oxide particles) which significantly improved the vegetative growth aspects, leaf content of mineral elements, the weight of pruning wood, and cane content of total carbohydrates, as well as increasing yield and enhancing the quality attributes of clusters and berries especially the percentage of shot berries and the compactness coefficient of clusters. Therefore, it is recommended to apply this application for 'Early Sweet' grapevines vineyards.

Keywords: Grape, Early Sweet, Arbuscular Mycorrhizal Fungi, Seaweed Extract, Nano-Zinc Oxide.

Introduction

'Early Sweet' is a white seedless table grape cultivar successfully cultivated under Egyptian conditions and is considered one of the earliest grape varieties on the market. The berry has good eating quality, with a slight Muscat flavor, creamy color, and crisp texture that add value to its marketability (Uwakiem, 2015), but there are major problems associated with this cultivar, as poor vegetative growth, shot berries formation and clusters compactness (Or et al., 2020). For the newly requested sustainable global food security, the improvement of early grapes is very important, whether for local or external markets, especially through the use of suitable replacements for inorganic fertilizers to obtain high-quality and healthy products (Cataldo et al., 2020).

Biostimulants (PBs) are products that play a vital role in the sustainable development of horticultural crops, as alternatives to reduce the

Corresponding author: Asmaa S. M. Omar, E-mail: asmaa2007@mans.edu.eg, Tel. +2 01010076311
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use of chemical fertilizers (Rouphael & Colla, 2020), and to stimulate the processes of plant nutrition by improving nutrient use efficiency and facilitating the availability of confined nutrients in the soil or the rhizosphere (Samri et al., 2021). PBs applications in the vineyard are a partial alternative to soil fertilization and have become important for improving grape quality (Petoumenou & Patris, 2021 and Cataldo et al., 2022). Arbuscular Mycorrhizal fungi (AMF) are beneficial soil microorganisms that exist in nature and are considered one of the most important PBs which are widely used (Cataldo et al., 2022), colonizing the roots of approximately 80% of land plants, inclusive vegetables, cereals, and fruit trees. AMF contain wide range of vitamins and micronutrients and play major role in food security (Cataldo et al., 2022). The mutualistic interaction between the plant and the fungal partner is based on biotrophic nutrient exchanges: the host plant provisions the biotrophic partner with carbon (C), while the AMF boosts the plant's ability to obtain nutrients and water from the soil, in particular phosphorus and nitrogen, through its expanded mycelium, thus potentially reducing the need for inorganic fertilizer (Agnolucci et al., 2019). The (AMF, grapevine) symbiosis was pointed out as a key component of the vineyard system, as it enhances, 1- water and nutrient acquisition, 2- growth of the grapevine, and 3- absorption of phosphorus and other relatively immobile micronutrients cations, thus producing plant growth hormones such as gibberellins, cytokinins, and auxins (Zhang et al., 2008).

Marine bioactive substances extracted from seaweeds are natural compounds and are considered one of the main and the broader groups of PBs (Shukla et al., 2019 and Rouphael & Colla 2020), because they promote growth and metabolism, increase antioxidant content, and enhance nutrient availability with a positive impact on plant health, growth and yield (Zhang et al., 2008). Seaweed (*Ascophyllum nodosum* L.) is a known source of plant growth regulators like, auxins, auxin-like compounds, and cytokinins, and is a source for many compounds like, vitamins, amino acids, organic matter, complex polysaccharides, sterols, (Khan et al., 2012a). Therefore, seaweed extract (SWE) plays a key role in the metabolism process, plant productivity, and enhancing plant growth, yield, and fruiting. Recently, it has been one of the important strategies to ensure sustainable agriculture, especially in arid and semi-arid regions where soils are poor in organic nutrients (Cataldo et al., 2022). SWE has been studied to impact the growth, productivity, and fruit quality of different grape varieties, like 'Flame Seedless' (Stino et al., 2017), 'Perlette' (Khan et al., 2012a), and 'Sangiovese' (Salvi et al., 2019). Soil microbial studies have shown that the exemplification of the microbiota existing in the soil and the rhizosphere can be altered by the application of seaweed extracts and consequently improve plant growth, i.e. root and shoot elongation, improve nutrient and water absorbance (Nabti et al., 2016) and these changes concurred with higher plant growth and yield (Arioli et al., 2021 and Hussain et al., 2021).

Zinc (Zn) plays important roles in numerous key plant physiological pathways related to the formation of sugar, and photosynthesis by chlorophyll production, protein, and hormone synthesis and plays a main role in fruit set and retention, and also in fruit quality and yield (Cakmak, 2008). Its important role can be attributed to the fact that it synthesizes the amino acid tryptophan which is a precursor of IAA, a phytohormone that dramatically regulates plant growth (Raliya & Tarafdar, 2013). In recent years, many efforts have been made to replace chemical fertilizers with nano fertilizers that do not harm the environment, since they are more effective and used in less quantity than classic fertilizers (Davarpanah et al., 2016 and Kah et al., 2018). Decreasing particle size increases the specific surface area of a fertilizer, which increase the dissolution rate of fertilizers with low water solubility such as zinc oxide (ZnO) (Mortvedt, 1992), resulting in a better distribution of Zn, and the greater surface area of contact of the Zn fertilizer resulting in a better distribution of Zn, increasing the surface area of contact and increasing the efficiency of its absorption with the speed of absorption and movement within the plant's vascular tissues (Liscano et al., 2000).

Foliar spraying of microelements in nano form is considered to be highly efficient compared to soil application because of the low toxicity caused by its accumulation, averting fixation in the soil, and ease of application. (Abdel Wahab et al., 2019). N-ZnO is used in low concentrations and is very important to plant functions as it improves the growth characteristics of many plants (Abou El-Nasr et al., 2021 and Mekawy, 2021) and modifies the effect of auxin by regulating the synthesis of tryptophan reflected in influencing the quality of the fruit (García-López et al., 2019).

Based on the above facts, the main target of this research was to improve vegetative growth traits and cluster quality attributes of 'Early Sweet' grapevines through the use of AMF, SWE, and N-ZnO particles, individually or combined.

Materials and Methods

The present investigation was carried out during 2020 and 2021 seasons on six-years-old 'Early Sweet' grape cultivar grafted on freedom rootstock in El-Baramon experimental farm located at Horticulture Research Institute, Mansoura, Dakahlia Governorate, Egypt. Vines were grown in clay soil (Table 1) under flood irrigation system and at 2 x 3 meters apart space. During the fourth week of January, the vines were spur-pruned under the quadrilateral cordon system with bud load of 56 buds/vine and trellised using the Spanish baron system. After set of berries, crop load at all treatments was adjusted to 24 clusters/vine in both seasons.

Ninety-six uniform vines were chosen on the basis of their growth by weight of pruning wood and vine trunk diameter as an indirect appreciations of vine vigor. Each treatment was repeated three times (four vines per replicate). The experiments were repeated on the same vines in the two seasons.

Eight treatments were performed as follows:

1. Control (untreated vines)
2. Soil inoculation with arbuscular mycorrhiza fungi (AMF) at 250 g inoculum/vine
3. Foliar spray with seaweed extract (SWE) at 1%
4. Foliar spray with Nano-zinc oxide particles (N-ZnO) at 50 ppm
5. AMF + SWE
6. AMF + N-ZnO
7. SWE + N-ZnO
8. AMF + SWE + N-ZnO

Mixed spores of AMF-mycorrhiza (Central Lab of Organic Agriculture, Agricultural Research Center, Giza, Egypt) genera have been prepared after extraction and mixed with sand as a carrier (40-50 spore/g inoculum), after that added to the soil at once during winter agricultural management at rate 250 g inoculum per vine. Seaweed extract (prepared in Central Lab of Organic Agriculture, Agricultural Research Center, Giza, Egypt) which concentrates on a modified *Ascophllum nodosum*

TABLE 1. Physical and chemical analysis of the vineyard soil

Physical	**Sand (%)**	**26.45**
	Silt (%)	**23.21**
	Clay (%)	**50.34**
	Texture	**Clay**
Chemical	**Organic carbon (%)**	**1.89**
	pH (1:2.5)	**7.81**
	EC (mmhos/cm)	**0.66**
	Ca CO_3 (%)	**1.84**
	N (%)	**0.27**
	P (%)	**0.15**
	K (%)	**0.31**

TABLE 2. Components of seaweed extract

Organic matter (%)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Zn (ppm)	Fe (ppm)	Mn (ppm)
53	1.3	0.04	11	0.07	0.04	0.3	55	125	8

marine plant extraction composed of alginate and mineral elements was used (Table 2).

Nano-zinc oxide particles preparation:

Nano-zinc oxide particles were used at 50 ppm (Nanotechnology Lab, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt).

All seaweed extract and Nano-zinc oxide particles applications were foliar sprayed three times: after the bud burst stage, at the fruit set stage, and two weeks after the fruit set stage. Triton B at 0.1% has been added as a wetting agent to all foliar solutions and spraying was done till runoff.

The following characteristics were determined:

Morphological characteristics of vegetative growth

One month after the fruit set, the following morphological traits were measured on four non-fruiting shoots per vine as follows:

- Average shoot diameter (cm)
- Average shoot length (cm)
- Average number of leaves/ shoot
- Total leaf area/vine (m^2) was determined by multiplying the average number of leaves/ shoot by the average leaf surface area then by the number of shoots per vine according to the method of Montero *et al.* (2000).

Chemical characteristics of vegetative growth

Leaf samples were taken one month after fruit set from the sixth and seventh leaves from the tip of the growing shoot, for the determination of leaf content of total chlorophyll and leaves opposite to cluster for the determination of mineral elements.

Total chlorophyll content (mg/g FW) was determined according to the method clarified by Mackinny (1941). Using the modified micro-Kjeldahl method, total nitrogen (%) has been estimated according to Pregl (1945). As reported by Snell and Snell (1967), phosphorus (%) was colorimetrically measured, while potassium (%) was determined according to Jackson (1967) using a Flame photometry instrument. Magnesium (%) was estimated as described by Bowen (1978) and zinc (ppm) was determined according to Brandifeld & Spincer (1965).

Yield and physical properties of clusters

As mentioned by Tourky et al. (1995), representative random samples consisting of six clusters/vines were harvested at maturity when TSS reached approximately 16-17% for the following determination:

- Yield/vine (Kg) which was a calculation by multiplying average cluster weight by the number of clusters/vine
- Average cluster weight (g)
- Average cluster dimensions (cm)
- Coefficient of cluster compactness was determined through dividing the number of berries/cluster by the length of the cluster.
- Shot berries (%) were determined as the percentage by dividing the number of shot berries/cluster by the total number of berries/ cluster

Physical and chemical properties of berries

- Average berry weight (g)
- Average berry size (cm^3)
- Total soluble solids (TSS) were determined as percentage by using a hand refractometer.
- Total acidity as (g tartaric acid/100 ml juice) was determined according to AOAC (2006).
- Total sugars percentage was determined as described by Sadasivam & Manickam (2004).
- Total carotenoids in skin of berries (mg/g FW) have been determined as described by Mackinny (1941).

Dormant season parameters:

During winter, the weight of pruning wood (Kg/vine) was measured and the cane content of total carbohydrates (%) was determined as described by Hodge & Hofreiter (1962).

Experimental design and statistical analysis

The experiment was arranged in a complete randomized block design. According to Snedecor & Cochran (1980), the statistical analysis of the present data has been completed. New LSD values at the 5% level were used, and the averages were compared with these values (Steel & Torrie, 1980). Data were analyzed using The Co-Stat software package, Ver. 6.303 (789 lighthouse Ave PMB 320, Monterey, CA, 93940, USA). Pearson's correlation matrix was employed to analyze the relationships between some analyzed parameters.

Results and Dissuasion

Morphological characteristics of vegetative growth

The response of used applications (AMF, SWE, N-ZnO) either alone or combined, on morphological characteristics of vegetative growth, i.e. shoot diameter, shoot length, number of leaves/shoot, and total leaf area/vine of 'Early Sweet' grapevines in two seasons are presented in Table 3. Initially, the results demonstrate that all used treatments either individually or combined resulted in significant increase of morphological characteristics of vegetative growth compared with the control in 2020 and 2021 seasons. As for individual treatments, it can be noticed that the Nano-zinc oxide particles application used in this study was the most effective than the others in both seasons, and the descending order was as follow: N-ZnO > AMF > SWE. Shifting to the combined treatments, the results showed a significant increase in stimulating morphological vegetative growth traits by the combined treatments compared to the individual treatments. Concerning the dual treatments, it was observed that the dual treatment (AMF + N-ZnO) was the best favorable than the other dual treatments, in both seasons, and the descending order was as follows: AMF + N-ZnO > AMF + SWE > SWE + N-ZnO. Further observing the results showed that the effect of the number of combined treatments is also crucial, where the higher the number of combined treatments, the larger the effect. From this point of view, the highest significant values for these traits were obtained by triple application (AMF + SWE + N-ZnO) compared to all treatments in both seasons.

The positive impact of AMF in improving these parameters may be due to its production of some enzymes that promote root respiration, improve the uptake of elements, and the production of growth-promoting substances such as cytokinins which may be responsible for promoting growth via increased cell division and expansion in plant mycorrhizal association (Edrees, 1982). Moreover, it was recently reported that the inoculation of grapevines with AMF is responsible for the upregulation of nutrient transport genes (Balestrini et al., 2017). Thus, colonization with AMF significantly increased shoot growth (Ozdemir et al., 2010) and improved leaf area (Abdel Latef & Chaoxing, 2011). Also, AMF treatments improved the leaf area of date palms (Anli et al., 2020) and increased the shoot length of treated grape (Arioli et al., 2021) compared to the control. This is may be reflected in improving vegetative growth parameters as shown by our results.

As for SWE, the increment in vegetative parameters might be attributed to SWE content of active organic substances such as cytokinins, IAA, GA3, essential amino acids, macro, and micro nutrients, polysaccharides, alginates, vitamins, enzymes, and 1.3-1.6 D-glucan, oligosaccharides known as laminarin, which perform as elicitors for polyamine (PAs) synthesis, a group of growth regulators which, play a major role in promoting cell division and elongation, thus increasing shoot length and leaf area (Colavita et al., 2011). Our results are in parallel with the findings of Mahmoud et al. (2016) which showed that foliar application of SWE significantly increased the

TABLE 3. Influence of arbuscular mycorrhizal fungi, seaweed extract, and Nano-zinc oxide particles on morphological characteristics of vegetative growth of 'Early Sweet' grapevines during 2020 and 2021 seasons

Characteristics	Shoot diameter (cm)		Shoot length (cm)		Number of leaves/ Shoot		Total leaf area/ vine (m^2)	
Treatments	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**
Control	0.87	0.92	118.3	119.6	17.4	18.1	8.98	10.06
Arbuscular mycorrhizal fungi (AMF)	1.06	1.09	126.7	128.1	19.5	19.9	11.96	12.26
Seaweed extract (SWE)	1.01	1.05	123.2	126.5	18.3	19.2	10.77	11.85
Nano-zinc oxide (N-ZnO)	1.08	1.10	130.9	129.4	19.7	20.6	12.14	13.03
AMF + SWE	1.18	1.19	136.8	136.7	20.8	21.3	13.78	14.96
AMF + N-ZnO	1.22	1.25	140.3	142.5	21.2	21.7	14.94	15.27
SWE + N-ZnO	1.15	1.17	135.9	134.1	20.3	21.1	13.47	14.71
AMF + SWE + N-ZnO	1.29	1.33	146.1	147.7	21.9	22.8	15.27	16.29
New LSD at 0.05	0.06	0.07	4.3	5.1	0.6	0.9	0.31	0.48

vegetative growth (*i.e*., shoot length, leaf number, and leaf area).

With regards to Zinc, it is helpful for protein synthesis which enhances plant growth, also plays an important role in the assimilation of auxins that increase cell division and elongation by aiding the synthesis of nucleic acids such as tryptophan which is a major component of IAA that necessary for cell division and elongation (Castillo-Gonzalez et al., 2018) resulting in increasing of shoot length and leaf area. Similarly, the results due to the foliar application of N-ZnO demonstrated a significant increase in shoot length (Raliya & Tarafdar, 2013), and leaf area in mango trees (Elsheery et al., 2020).

Chemical characteristics of vegetative growth

As shown in Table 4, it is obvious that leaf content of total chlorophyll and mineral elements including total nitrogen, phosphorus, potassium, magnesium, and zinc were significantly increased with all used applications either alone or combined as compared to control in both seasons. As for individual treatments, it can be noticed that AMF application was the most effective than the others in both seasons, and the descending order was as follow: AMF > N-ZnO > SWE. The dual applications were the best favorable in increasing parameters than the individual applications, especially AMF + SWE application which significantly improved these parameters. Further observing the data, the triple application (AMF + SWE + N-ZnO) used in this study was the best favorable in increasing leaf content of total chlorophyll and mineral elements than the other treatments, where it gives the highest significant values in both seasons.

Increasing leaf content of total chlorophyll could be due to the AMF could increase in the photosynthetic rate as a large proportion of assimilates are translocated toward the roots of plants inoculated with mycorrhizae. This agrees with Bavaresco et al. (2003) who reported that inoculation of grapevines with AMF increased chlorophyll concentrations in the leaves and photosynthesis rate. It was recently reported that the inoculation of grapevines with AMF is responsible for the upregulation of nutrient transport genes (Balestrini et al., 2017). Also, our results are in parallel with the findings of Mahmoud et al. (2016) on apples, Khan et al. (2012a) on Perlette grapevines, and Anli et al. (2020) on date palm, who stated that foliar application of SWE increased significantly chlorophyll content in leaves. The increase in chlorophyll content was due to decreased chlorophyll degradation, which could be due to betaines in the seaweed extract, as reported by Whapham et al. (1993), or by increasing photosynthesis and stomatal conductance in plants treated with SWE, as studied by Salvi et al. (2019). Besides, the application of N-ZnO increased photosynthetic activity which plays an important role in increasing chlorophyll contents in grape leaves (Raliya and Tarafdar, 2013). Similarly, N-ZnO foliar application

TABLE 4. Influence of arbuscular mycorrhizal fungi, seaweed extract, and Nano-zinc oxide particles on leaf total chlorophyll and mineral elements content of 'Early Sweet' grapevines during 2020 and 2021 seasons

Characteristics	Total chlorophyll (mg/g FW)		Total nitrogen (%)		Phosphorus (%)		Potassium (%)		Magnesium (%)		Zinc (ppm)	
Treatments	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**
Control	10.22	11.26	1.53	1.61	0.17	0.21	1.31	1.37	1.09	1.14	29.5	33.2
Arbuscular mycorrhizal fungi (AMF)	12.89	13.19	1.62	1.69	0.26	0.31	1.43	1.48	1.17	1.25	38.1	47.6
Seaweed extract (SWE)	11.95	12.16	1.57	1.64	0.19	0.24	1.35	1.42	1.13	1.17	35.4	42.1
Nano-zinc oxide (N-ZnO)	12.46	12.88	1.59	1.65	0.22	0.28	1.38	1.44	1.16	1.22	37.2	45.3
AMF + SWE	13.91	14.07	1.79	1.86	0.34	0.41	1.56	1.61	1.28	1.39	46.9	57.5
AMF + N-ZnO	13.57	13.85	1.71	1.75	0.31	0.36	1.52	1.57	1.24	1.33	44.1	54.2
SWE + N-ZnO	12.88	13.28	1.66	1.69	0.29	0.33	1.47	1.53	1.21	1.31	41.7	52.9
AMF + SWE + N-ZnO	14.17	14.32	1.87	1.93	0.39	0.47	1.62	1.66	1.31	1.44	52.3	61.4
New LSD at 0.05	0.24	0.21	0.07	0.06	0.04	0.03	0.05	0.04	0.02	0.01	4.9	3.7

increased chlorophyll contents in leaves (Raliya & Tarafdar, 2013 and Pejam et al., 2021).

To the interpretation of the results obtained about the increasing mineral elements content in the leaves, it is found that AMF play an important role in breaking down some complex minerals and organic matter in the soil and making them available to the roots and form hyphae enter the root and create vesicles for nutrient storage structures where nutrients are transferred between fungus and plant (Aslanpour et al., 2019), increasing the availability of certain essential plant nutrients such as phosphorus (P), magnesium (Mg) and zinc (Zn). Mycorrhizae were found to play a minor role in nitrogen uptake due to its high emission rate (Aslanpour et al., 2019), while play a major role in increasing phosphorus levels, either in roots or leaves, in all treated vines (Khalil 2013). Production and secretion of enzyme phosphatase by mycorrhizal hyphae converts the insoluble and stabilized phosphate in the soil as a soluble nutrient, which can be absorbed by roots and induction of P transporters in root cortical cells (White & Hammond, 2013). As for K, Abdel Latef & Chaoxingb (2011) on tomato plants found that AMF-inoculated plants had higher concentrations of K in roots and shoots. Also, Giri et al (2003) showed that in the presence of mycorrhiza, there is an increase in Mg uptake. Ozdemir et al. (2010) found that AMF produced remarkable increases in the Zn status of grape leaves. SWE contains plant hormones that could stimulate root growth and enhance nutrient uptake, herewith causing enhanced general plant growth and vigor. Many studies have proved that SWE has characteristic growth-stimulating properties because it may affect the architecture of plant roots and also alter the biochemical, biological, and physical properties of the soil (Taskos et al., 2019). Our results are in agreement with Khan et al. (2012a) and Irani et al. (2021) on grapes and (Anli et al., 2020) on date palm, who reported that treated plants with SWE showed an increase in leaf nutrients contents levels (N, P, K, and Zn). The structure of the small nanoparticles ensures efficient and rapid delivery of zinc, to which the plant quickly responds to it, this is reflected in the promotion of plant growth and development. Many reports provide a clear illustration of the benefits of Zn in enhancing essential nutrients in plants (Khan et al., 2012b). The anatomical study provided supporting evidence on how N-ZnO can improve nutritional status through support in the vascular system, especially the metaxylem tissues (Pejam et al., 2021). This is in coincidence with our data, Elsheery et al (2020) showed that mango leaf NPK contents have predominately increased by spraying N- particles. Also, the N-ZnO foliar application enhanced K and Zn concentration in leaves (Pejam et al., 2021).

Yield and physical properties of clusters

The response of used treatments (AMF, SWE, N-ZnO) either alone or combined on both yield and physical properties of clusters during 2020

TABLE 5. Influence of arbuscular mycorrhizal fungi, seaweed extract, and Nano-zinc oxide particles on yield and physical properties of clusters of 'Early Sweet' grapevines during 2020 and 2021 seasons

Characteristics	Yield/vine (kg)		Cluster weight (g)		Cluster length (cm)		Cluster width (cm)		Coefficient of cluster compactness		Shot berries (%)	
Treatments	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**
Control	12.67	12.82	525.3	534.1	21.4	22.3	12.9	12.7	6.09	5.81	11.43	10.67
Arbuscular mycorrhizal fungi (AMF)	14.25	14.35	594.9	598.7	25.2	25.6	13.8	14.3	5.46	5.24	9.26	8.71
Seaweed extract (SWE)	13.82	14.06	576.2	586.5	24.3	25.2	13.5	14.1	5.69	5.33	10.13	9.94
Nano-zinc oxide (N-ZnO)	14.45	14.62	602.4	609.3	25.8	26.1	14.7	15.9	5.20	5.06	8.86	8.47
AMF + SWE	15.67	15.89	653.7	662.2	27.7	28.5	15.4	16.3	4.89	4.61	7.96	7.41
AMF + N-ZnO	16.32	16.56	681.5	691.9	28.4	29.3	17.1	17.3	4.82	4.55	7.38	7.03
SWE + N-ZnO	15.26	15.63	636.1	649.4	27.3	28.4	15.3	16.1	4.99	4.84	8.13	7.64
AMF + SWE + N-ZnO	17.08	17.27	712.8	721.2	28.9	29.7	17.8	17.9	4.69	4.48	6.71	6.32
New LSD at 0.05	0.43	0.39	18.3	16.9	0.4	0.3	0.6	0.5	0.11	0.09	0.65	0.47

and 2021 seasons are presented in Table 5. In general, all used treatments showed positive significant influence as compared to control in both seasons. More clearly, all treatments either individually or combined gave the greatest significant increase in yield, cluster weight, cluster length, and cluster width as compared to the control. Further observing the results, it can be noticed that the combined treatments used in this study were the most effective than the individual treatments, especially the triple application (AMF + SWE + N-ZnO) which showed the highest significant values. As stated before, the major problems associated with 'Early Sweet' are the phenomenon of clusters compactness and shot berries. It is noticeable that in conjunction with enhancing cluster length and cluster weight, a decrease in coefficient of cluster compactness and percentage of shot berries was noticed, respectively, especially with the triple application (AMF + SWE + N-ZnO) which showed the least significant values.

It has been previously shown that the interaction of plants with AMF can result in an improvement in fruit production in terms of quality and yield, which could be attributed to a higher concentration of phytohormones and photosynthates in plant that is modulated by microorganisms (Bona et al., 2017). It is known that AMF can contribute significantly to the uptake of phosphorous (P) from the soil and this results in increasing in the grape yield and the number of clusters, it also contributes to the stimulation of molecules or enzymes (such as auxins and sugar compounds) responsible for modulating the expansion of fruit cells, which results in large fruits in treated plants (Skinner et al., 1988). The reduction of the cluster compactness coefficient can be attributed to the high efficiency of the applications made in absorbing and transferring water, minerals, and especially nitrogen to the shoots, thus activating more vegetative growth in full bloom, which lead to changing the competitive balance between blossom clusters and vegetative growth in favor of the latter, causing some flowers to fall and thus reduce the coefficient of cluster compactness as well, which also may be due to the increase in the cluster length. Our results are in line with El-Mogy (2017), El-Boray et al. (2018) and Karoglan et al. (2021). In this regard, Samri, et al. (2021) showed that the application of AMF at 250 g inoculum/vine enhanced yield and physical and chemical properties of clusters and berries as compared with non-inoculations with mycorrhiza in different fruit trees. SWE possibly increased the natural peak of polyamine concentration in fruits, improved cell division, and enhanced endogenous levels of growth promoters, macro and micronutrients and carbohydrates, and hormonal substances, especially cytokinins leading to increased size fruit and weight (Khan et al., 2012a). Our results are in line with field trials that demonstrated the positive effects of SWE on yield, fruit quality, and vine vigor in different table grapes cultivars (Khan et al., 2012, Salvi et al. 2019, Arioli et al., 2021 and Irani et al., 2021). The N-ZnO treatment significantly increased crop yield by improving the metabolite translocation (Pejam et al., 2021). Spraying N-Zn before

TABLE 6. Influence of arbuscular mycorrhizal fungi, seaweed extract, and Nano-zinc oxide particles on the berry physical and chemical properties of 'Early Sweet' grapevines during the 2020 and 2021 seasons

Characteristics	Average berry weight (g)		Average berry size (cm^3)		TSS (%)		Total acidity (%)		Total sugars (%)		Total carotenoids (mg/g FW)	
Treatments	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**
Control	4.03	4.12	3.89	3.99	16.3	16.7	0.64	0.60	12.7	13.0	3.32	3.73
Arbuscular mycorrhizal fungi (AMF)	4.32	4.46	4.16	4.31	17.2	17.3	0.56	0.54	13.3	13.5	4.23	4.59
Seaweed extract (SWE)	4.17	4.37	4.04	4.23	16.9	17.2	0.57	0.56	13.2	13.3	3.99	4.56
Nano-zinc oxide (N-ZnO)	4.49	4.61	4.35	4.48	17.3	17.5	0.55	0.49	13.7	13.7	4.41	4.83
AMF + SWE	4.83	5.04	4.70	4.91	17.9	18.3	0.45	0.43	14.7	15.3	5.24	5.71
AMF + N-ZnO	4.98	5.19	4.86	5.06	18.2	18.5	0.43	0.40	15.3	15.5	5.37	5.92
SWE + N-ZnO	4.67	4.72	4.54	4.58	17.7	18.0	0.48	0.44	14.3	14.9	4.95	5.27
AMF + SWE + N-ZnO	5.26	5.42	5.12	5.29	18.6	18.8	0.38	0.35	15.5	15.6	5.89	6.37
New LSD at 0.05	0.21	0.18	0.19	0.17	0.3	0.2	0.05	0.04	0.2	0.1	0.49	0.43

flowering improved the overall fruit yield and pomegranate quality (Davarpanah et al., 2016). Also, spraying mango cv. "Ewais" with N-ZnO improved the overall yield, number, weight, length, and width of fruits (Elsheery et al., 2020). Our data agree with Abou El-Nasr et al. (2021) on the Crimson Seedless grape cultivar and Mekawy (2021) on the Flame Seedless grape cultivar, they revealed that foliar applications of Nano-zinc oxide at 50 ppm improve yield and physical and chemical properties of clusters compared to untreated trees.

Physical and chemical properties of berries

The response of used treatments (AMF, SWE, N-ZnO) either alone or combined on the physical and chemical properties of berries during 2020 and 2021 seasons are presented in Table 6. Data showed that the physical and chemical properties of berries were significantly improved in both seasons by all applications compared to the control.

More obviously, the results demonstrated that all treatments either individually or combined afforded significant increase in physical properties of berries, TSS%, Total sugars%, and total carotenoids of berries compared to the control in both seasons. Also, the combined treatments remarkably enhanced these parameters in both seasons than the individual treatments, and the greatest increase was detected from the berries applied with the triple application (AMF + SWE + N-ZnO). It is also noticed that in conjunction with the increase in TSS%, a decrease in total acidity percentage of berries was noticed in all treatments, especially with the triple application (AMF + SWE + N-ZnO) which showed the least significant values.

The positive impact of AMF can be explained by Karoglan et al. (2021) who found that inoculated grapes tended to have higher soluble solids contents and less titratable acidity concentration. Moreover, AMF caused an increase in the carotenoid content of carrot and tomato fruits (Regvar et al., 2003). In this regard, Samri, et al. (2021) showed that the application of AMF at 250 g inoculum/vine enhanced the physical and chemical properties of berries as compared with non-inoculations with mycorrhiza in different fruit trees.

As mentioned by Khan et al. (2012) and Petoumenou & Patris (2021), an increase in SSC% and a decrease in TA% in grape juice are associated with specific enzymes in SWE, that promote the synthesis of various phytohormones, proteins, sugars, and amino acids.

In this regard, our results were analogous to those of Davarpanah et al. (2016) and Abul-Nasr et al. (2021) who reported that N-ZnO treatments decreased the concentration of titratable acidity while it enhanced the accumulation of total soluble solids and total sugars in fruit juice. These effects could be due to the role of zinc in the transference and synthesis of carbohydrates and proteins (García-López et al., 2019). The increased sugar content of berries may be due to zinc's role in carbohydrate metabolism by improving photosynthesis and sugar conversion

TABLE 7. Influence of arbuscular mycorrhizal fungi, seaweed extract, and Nano-zinc oxide particles on the weight of pruning wood and cane total carbohydrates content of 'Early Sweet' grapevines during 2020 and 2021 seasons

Characteristics	Weight of pruning wood (kg/vine)		Cane content of total carbohydrates (%)	
Treatments	**2020**	**2021**	**2020**	**2021**
Control	2.14	2.23	21.7	22.5
Arbuscular mycorrhizal fungi (AMF)	2.67	2.58	26.3	27.3
Seaweed extract (SWE)	2.46	2.52	23.5	24.7
Nano-zinc oxide (N-ZnO)	2.51	2.56	25.7	26.3
AMF + SWE	2.72	2.71	29.3	30.7
AMF + N-ZnO	2.81	2.84	30.2	32.3
SWE + N-ZnO	2.64	2.67	27.5	28.7
AMF + SWE + N-ZnO	2.97	3.01	31.3	33.1
New LSD at 0.05	0.13	0.16	0.7	0.4

(Suganya et al., 2020). The concentration of carotenoids in fruits was increased in response to the application of N-ZnO (Pejam et al., 2021).

Dormant season parameters

The data presented in Table 7 showed that all used treatments (AMF, SWE, N-ZnO), either alone or combined, positively influenced the weight of the pruning wood and the total carbohydrate content of the cane compared to the control in both seasons. In descending order, the individual application of AMF, N-ZnO, and SWE significantly improved these parameters. Dual applications were more favorable for improving the weight of pruning wood and cane content of total carbohydrates than using each application alone. The best dual application in this respect was using AMF + N-ZnO followed by, in descending order, AMF + SWE followed by SWE + N-ZnO. As compared to all applications, the triple application (AMF + SWE + N-ZnO) resulted in significantly the highest values of the weight of pruning wood and cane content of total carbohydrates in both seasons.

Our results in this study showed the role of AMF in improving the weight of pruning wood and cane total carbohydrate content of 'Early Sweet' grapevines. Kouadio et al (2017) showed that AMF inoculation increased the content of soluble carbohydrates and starch in palm leaves and roots. This can be explained as the AMF improved the water condition of the plants, which induced an improvement in the photosynthetic performance and promoted the uptake of phosphorus (P), potassium (K), and calcium (Ca) and led to a mobilization of starch reserves in the apex in winter (Karoglan et al., 2021). Also, the use of SWE improves water and nutrient uptake resulting in more activation of carbohydrate metabolism (Nabti et al., 2016). On the other hand, similar results demonstrated the vital role of zinc in carbohydrate metabolism (Garcia-Gomes et al., 2017). Besides, the application of N-ZnO increased photosynthetic activity, which led to increased carbohydrate accumulation in grape canes (Raliya & Tarafdar, 2013). Recently, Elsheery et al (2020) showed that mango treated with nanoparticles, including Zn, had the most pronounced effect in increasing total carbohydrate content in trees.

Pearson's correlation matrix

Data illustrated in Table 8 show that there is a positive correlation between all the studied characteristics, except for coefficient of cluster compactness, shot berries and total acidity.

Conclusion

To conclude, in this study, we have used applications such as arbuscular mycorrhizal fungi, seaweed extract, and Nano-Zinc oxide particles, either individually or combined, to enhance the vegetative growth traits, nutritional status, yield, and cluster quality attributes of 'Early Sweet' grapevines for two consecutive seasons (2020 and 2021). The results demonstrated that the triple application (arbuscular mycorrhiza fungi + seaweed extract + Nano-Zinc oxide particles) significantly improved the vegetative growth aspects, leaf content of mineral elements, the weight of pruning wood, and cane content of total carbohydrates, as well as increasing yield and enhancing the quality attributes of clusters and berries. Therefore, it is recommended to apply the combined application of arbuscular mycorrhiza fungi at 250 g inoculum/vine once during winter agricultural management, and foliar spray with seaweed extract at 1% and Nano-Zinc oxide particles at 50 ppm three times: after the bud burst stage, at fruit set stage and two weeks after fruit set stage on 'Early Sweet' grapevines under Egyptian conditions.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this study.

TABLE 8. The Pearson's Correlation Coefficient among some parameters of «Early sweet» grape as affected by treatments

	Shoot diameter	Shoot length	Chlorophyll	Cluster weight	Cluster compactness	Shot berries	Berry weight	TSS	Acidity	Total sugar	Carotenoids	Pruning weight	Carbohydrates
Shoot diameter	1.0000**												
Shoot length	0.941**	1.0000**											
Chlorophyll	0.929**	0.881*-	1.0000**										
Cluster weight	0.964**	0.955**	0.891*	1.0000**									
Cluster compactness	-0.874*	-0.848*	-0.879*	-0.802*	1.0000**								
Shot berries	-0.622	-0.586	-0.428	-0.666	0.310	1.0000**							
Berry weight	0.927**	0.915**	0.891*	0.909**	-0.928**	-0.377	1.0000**						
TSS	0.655	0.625	0.464	0.702	-0.358	-0.946**	0.438	1.0000**					
Acidity	-0.618	-0.594	-0.430	-0.662	0.283	0.934**	-0.387	-0.961**	1.0000**				
Total sugar	0.524	0.504	0.293	0.582	-0.208	-0.931**	0.306	0.954**	-0.948**	1.0000**			
Carotenoids	0.519	0.507	0.324	0.573	-0.214	-0.946**	0.285	0.956**	-0.951**	0.934**	1.0000**		
Pruning weight	0.917**	0.938**	0.927**	0.937**	-0.820*	-0.596	0.848*	0.648	-0.593	0.476	0.522	1.0000**	
Carbohydrates	0.938**	0.915**	0.860*	0.966**	-0.771	-0.761	0.848*	0.791	-0.738	0.682	0.676	0.920**	1.0000**

Values express average values of the treatments.
**. Correlation is significant at the 0.01 level.
*. Correlation is significant at the 0.05 level.

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تأثير فطر الميكوريزا ومستخلص الأعشاب البحرية والزنك النانوى على النمو والمحصول وجودة العناقيد لكرمات عنب الإيرلى سويت

بسام السيد عبد المقصود بلال[١] ، مسعد عوض القناوى[١] ، شيماء محفوظ محمد الموجى[١] وأسماء سعيد مصطفى عمر[٢]
١ قسم بحوث العنب - معهد بحوث البساتين - مركز البحوث الزراعية - الجيزة - مصر.
٢ قسم الفاكهة - كلية الزراعة - جامعة المنصورة - مصر.

تم إجراء البحث الحالي فى موسمين متتاليين (٢٠٢٠ و ٢٠٢١) على كرمات عنب الإيرلى سويت المطعوم على أصل الفريدم في مزرعة البرامون التابعة لمحطة بحوث البساتين بالمنصورة- محافظة الدقهلية لتحسين صفات النمو الخضري، الحالة الغذائية والمحصول وجودة العناقيد لكرمات عنب الإيرلى سويت من خلال إضافة فطر الميكوريزا ومستخلص الأعشاب البحرية والزنك فى صورة نانوية. كان عمر الكرمات ست سنوات نامية فى تربة طينية، وتروى بنظام الرى بالغمر منزرعة على مسافة ٢×٣ متر ومرباة تحت نظام التدعيم بالتكاعيب الأسبانية ، كما تم تقليم الكرمات تقليما دابريًا خلال الأسبوع الأخير من شهر يناير مع الحفاظ على حمولة ٥٦ برعم/كرمة.

وقد اشتملت الدراسة على ثمانى معاملات على النحو التالى: التلقيح بفطر الميكوريزا بتركيز ٢٥٠ جم لقاح / كرمة مرة واحدة خلال الخدمة الشتوية والرش الورقي بمستخلص الأعشاب البحرية بتركيز ١ ٪ وأكسيد الزنك النانوي بتركيز ٥٠ جزء في المليون فى ثلاثة مواعيد وهى: عند إكتمال تفتح البراعم، عند مرحلة العقد، وبعد تمام العقد بأسبوعين. أجريت جميع المعاملات إما بصورة فردية أو مشتركة فيما بينهم، بالإضافة إلى معاملة الكنترول (كرمات غير معاملة).

أشارت نتائج الدراسة إلى أن الإضافة بكل من فطر الميكوريزا ومستخلص الأعشاب البحرية وأكسيد الزنك النانوي إما بصورة فردية أو مشتركة فيما بينهم أثرت بشكل إيجابي على جميع الصفات المدروسة مقارنة بالكرمات الغير معاملة في كلا الموسمين. يعتبر الإضافة المشتركة الثلاثية لكل من التلقيح بفطر الميكوريزا بتركيز ٢٥٠ جم لقاح/كرمة والرش الورقي بمستخلص الأعشاب البحرية بتركيز ١ ٪ وأكسيد الزنك النانوي بتركيز ٥٠ جزء في المليون الأكثر فاعلية بين جميع المعاملات في الحصول على أفضل صفات النمو الخضري، وتحسين الحالة الغذائية للكرمات ويشمل محتوى الأوراق من الكلوروفيل الكلى والعناصر المعدنية ومحتوى القصبات من الكربوهيدرات الكلية بالإضافة إلى زيادة المحصول وتقليل معامل تزاحم العنقود ونسبة الحبات الصغيرة وتحسين صفات الجودة للعناقيد والحبات لكرمات عنب الايرلى سويت. وبالتالى يوصى بالتلقيح بفطر الميكوريزا بتركيز ٢٥٠ جم لقاح / كرمة مرة واحدة خلال الخدمة الشتوية والرش الورقي بمستخلص الأعشاب البحرية بتركيز ١ ٪ وأكسيد الزنك النانوي بتركيز ٥٠ جزء في المليون فى ثلاثة مواعيد وهى: عند إكتمال تفتح البراعم، عند مرحلة العقد، وبعد تمام العقد بأسبوعين فى مزارع عنب الإيرلى سويت وذلك لتحسين النمو الخضرى وجودة العناقيد.

الكلمات الدالة: العنب - الايرلى سويت – فطر الميكرويزا – مستخلص الأعشاب البحرية - الزنك النانوى.