Application of Glycine, Folic Acid, and Moringa Extract as Bio-stimulants for Enhancing the Production of 'Flame Seedless' Grape Cultivar

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In this study, 130 uniform 'Flame Seedless' grape trees were selected for and subjected to the same cultural practices. The trees were sprayed three times, before flowering, during full bloom, and three weeks later with the following treatments: control (water only), 250, 500, and 750 ppm glycine; 50, 100, and 150 ppm folic acid (FA); 2%, 4%, and 6% leaf moringa aqueous extract (MLAE); and their combinations. High-performance liquid chromatography (HPLC) analysis of moringa leaf aqueous extract (MLAE) showed the presence of the phenolic compounds ellagic acid, vanillic acid, p-hydroxy benzoic acid, catechol, and gallic acid with values of 54.18, 18.79, 14, 12.32, and 12.12 mg/100 g, respectively. The obtained results showed that the foliar spraying of 250, 500, and 750 ppm glycine, 4% and 6% MLAE, and their combinations of glycine 500 ppm + FA 100 ppm + MLAE 4% and glycine 750 ppm + FA 150 ppm + MLAE 6% significantly increased the shoot length, shoot thickness, leaf chlorophyll content, yield, and fruit quality over the control. Glycine at 750 ppm was the best treatment followed by glycine at 500 ppm compared with the other applied treatments and the control in both experimental seasons.

Keywords: Moringa extract; Glycine; Folic acid; Grape; Fruit quality

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INTRODUCTION

Grape (*Vitis vinifera* L.) is a member of the Vitaceae family. In Egypt, the harvested area is 78853 ha, which produces 1759472 tons. It was stated by many authors that berries of grape have been utilized as table fruit, juice, and for the production of wine, and raisins, as well as in the industries of cosmetic from leaf, seed, and skin extracts (Iriti and Faoro 2006; Monagas *et al*. 2006). Moreover, grape skin, pulp, and seeds could be used to produce wine, terpenes, and norisoprenoids, as well as sugars, which could be converted to alcohol (Lund and Bohlmann 2006). It was reported by (Ruberto *et al*. 2007; Hogan *et al*. 2010; Drosou *et al*. 2015) that grapes are characterized by the high content of phenolic compounds, which are a valuable source of natural antioxidants. Grapes are a crucial exporter to potassium and manganese (Torres-Urrutia *et al*. 2011), rich in short-chain carbohydrates (Gibson and Shepherd 2012; Gibson 2017), and vitamin C (Moores 2013; Carr and Maggini 2017), which is important for immunology.

Glycine is an amino acid that has a crucial role in improving total chlorophyll and vegetative growth in grapes, as well as in increasing the availability of Fe, Zn, Mn, and Cu to the plants (Sekhon 2003; Liu *et al.* 2011; Ghasemi *et al.* 2013; Shan *et al.* 2016; Razavi *et al.* 2018). L-glycine works as a signal transduction molecule that contributes to making plant nutrients readily available (Teixeira *et al.* 2017). The plant height, leaf chlorophyll content, and fresh weight of the shoot and root of *Coriandrum sativum* were significantly improved by the application of glycine, especially at higher concentrations (Souri and Hatamian 2019). In addition, the application of glycine increased the total soluble solid and vitamin C contents compared with that in the controls. In comparison with the unfertilized plants, the leaf mineral contents of N, Ca, K, P, Fe, and Zn were significantly increased by the application of glycine (Jiang *et al.* 2020; Lo'ay and Doaa 2020).

Folic acid (FA) has a fundamental role in the metabolism of amino acids and in nucleic acids synthesis (Paucean *et al.* 2018). Moreover, FA was found to increase the productivity of *Pisum sativum* by affecting the leaf content of chlorophyll, as well as the yield, weight, and quality of the seed (Burguieres *et al.* 2007). Foliar spraying of sweet pepper plants with FA increased flowering, yield, and fruit quality (Al-Said and Kamal 2008). Additionally, exogenous FA showed a positive effect on the growth, yield, and quality of soybean and strawberry plants (Mansour 2014; Li *et al.* 2015). Spraying of FA at 0, 10, 20, and 30 mg/L on Luz de otono bean cultivar was investigated by Al-Maliky *et al.* (2019). The obtained results showed that spraying FA raised the height of plant, number of branches, leaf area, and shoot dry weight, as well as length, number and green weight of pods as compared to control. Moreover, 100 seeds weight, the production of green pods, and fresh seeds, the percentage of TSS, and dry matter, as well as protein and vitamin C and the concentration of 30 mg/L was the best concentration. Spraying FA at 150 µM/L under lack of water levels raised significantly plant growth, efficacy of water use, firmness of cell membrane, yield, total soluble solids, and protein content in snap beans (*Phaseolus vulgaris* L.) (Ibrahim *et al*. 2021).

Moringa oleifera contains around forty sex antioxidants such as ascorbate, carotenoids, phenols, and flavonoid (Iqbal and Bhanger 2006). Moringa leaf aqueous extract (MLAE) can be used as a bio-stimulant and contains macro- and micronutrients, amino acids, ascorbic acids, minerals, and growth-enhancing principles such as the hormone of the cytokinin type (Makkar *et al.* 2007). In addition, the growth hormone spray will cause the plants to be firmer and more resistant to pest and diseases. Moreover, *M. oleifera* is one of the best crop treatments with confirmed impacts on growth and yield, and it can be used by farmers as a source for nutrients instead of relying on inorganic fertilizers, which are costly and are associated with both land and soil degradation and environmental pollution (Phiri 2010). Additionally, *M. oleifera* is standardized to contain flavonoid, phenolic, and carotenoid, which can be used as antioxidants, one of them being quercetin (Alhakmani *et al.* 2013; Wang *et al.* 2017). Abbassy *et al.* (2020) reported that *M. oleifera* leaf extract is rich in prime metabolic compounds such as proteins, lipids, carbohydrates, minerals, vitamins, and amino acids. Moringa provides products that improve the growth and yield of various crops (Mohamed *et al.* 2020). Hollywood plum trees sprayed with 6% MLAE had an improved fruit set, fruit yield, fruit weight, firmness, color, total soluble solids, vitamin C, and the content of anthocyanin in the plum cultivar Hollywood, while they had reduced fruit drop compared to the use of 0%, 4%, or 5% MLAE (Shaaban *et al.* 2020).

Therefore, the objective of this study was to investigate the impacts of the foliar application of glycine (amino acid), folic acid, and MLAE as natural biostimulants to vegetative growth, yield, and fruit quality of the grape cv. ‘Flame Seedless’ to minimize the dependency on the chemical fertilization in grape orchards.

EXPERIMENTAL

Preparation of MLAE and HPLC Analysis of Phytochemical

Moringa oleifera Lam. leaves were obtained from Alexandria, Egypt and were shade-dried. The dried leaves were ground to powder using a small laboratory mill. Then, 100 g of the powdered leaves (200 g) were soaked in 2 L of distilled water for 24 h at room temperature (Mohamed *et al.* 2020). The mixture was filtered using Whatman No. 1 filter paper. The dissolved MLAE was concentrated by evaporation, then prepared at the concentrations 2%, 4%, and 6%. An Agilent 1260 Infinity 1260 II LC, System (HPLC Agilent, Santa Clara, CA, USA) was equipped with a Quaternary pump and a Zorbax Eclipse Plus C18 column (100 mm × 4.6 mm i.d.) (Agilent Technologies, Santa Clara, CA, USA) and operated at 30 °C; it was used to identify the phytochemical compounds in the MLAE. Separation conditions and standard phenolic compounds can be found in previously published works (Al-Huqail *et al.* 2019; Behiry *et al.* 2019; Salem *et al.* 2019; Ashmawy *et al.* 2020; Salem *et al.* 2020).

Experimental Location and Treatments

This experiment was carried out during two successive seasons, in 2018 and 2019, on 10-year-old ‘Flame Seedless’ grape trees (*V. vinifera* L.), planted with 3 m between rows and 2 m between trees in the same row, and grown in a calcareous soil under a drip irrigation system in a private orchard located at Nubaria, Beheira Governorate, Egypt. Physiochemical analysis of the experimental soil was performed (Table 1) (Sparks *et al.* 2020).

Table 1. Physical and Chemical Properties of the Experiment Soil

Soil Depth (cm)	Texture	pH	* EC (dS/m)	N (%)	P (%)	K (%)	Fe (mg/L)	Zn (mg/L)	Mn (mg/L)
0 to 60	Sandy loam	8.6	2.22	16.62	23.20	30.25	0.35	0.10	0.08
	$CaCO_3$ (mg/L)		Cations (meq/100 g Soil)				O.M. (%)	Cl^-	HCO_3^-
			Na^+	K^+	Ca_2^+	Mg^{++}			
	28.42		10.18	1.12	6.52	3.63	0.53	12.3	6.2
* Note: EC = electrical conductivity									

A total of 130 uniform trees were selected for this study, and all of them were subjected to the same cultural practices in the two seasons. They were sprayed with the experimental treatments three times in each season: before flowering, during full bloom, and three weeks later (Table 2). These applied treatments were arranged in a randomized complete block design where each treatment was composed of 10 replicates.

Table 2. Treatments Used for Spraying the Trees

Treatment	Concentrations
Control	Water only
Glycine	250, 500, and 750 ppm
Folic acid (FA)	50, 100, and 150 ppm
MLAE	2%, 4%, and 6%
Combination 1	250 ppm glycine+ 50 ppm FA + 2% MLAE
Combination 2	500 ppm glycine + 100 ppm FA + 4% MLAE
Combination 3	750 ppm glycine + 150 ppm FA + 6% MLAE

Measurement of Vegetative Parameters, Leaf Total Chlorophyll, Fruit Yield, Fruit Quality and Leaf Chemical Composition

Shoot length and thickness (cm) were measured at the end of each growing season. Total chlorophyll in the fresh leaves was determined with SPAD units as evaluated by a chlorophyll meter (SPAD-502; Konica Minolta, Osaka, Japan). The number of clusters per each vine and weight of each cluster was measured and recorded. The yield of vine (kg) was determined by the weight of clusters per vine multiplied by the number of clusters per vine. Yield in tons per hectare was estimated by multiplying yield of vine × number of vines per hectare.

For the leaf chemical composition, samples of 30 leaves were taken from the middle of the shoots and were randomly selected from each replicate after the harvest time in June to determine their content in terms of percentages of nitrogen (N), phosphorus (P), and potassium (K) (Arrobas *et al.* 2018). The leaf samples were washed first with tap water and then with distilled water and dried at 70 °C until a constant weight was obtained. Finally, the dried leaf samples were ground and acid digested using H_2SO_4 and H_2O_2 until the digested solution became clear. The digested solution was used for the determination of nitrogen using the micro-Kjeldahl method (Wang *et al.* 2016), phosphorus by the vanadomolybdate method (Weiwei *et al.* 2017), and potassium using a flame photometer (Banerjee and Prasad 2020).

Measuring of Fruit Quality in terms of Physical, and Chemical Characteristics

At the time of harvesting, 10 clusters from each vine/replicate were chosen randomly to determine their physical and chemical characteristics; cluster weight (g), size (cm^3), length (cm), and width (cm). In addition, 100 berries were selected from all the chosen clusters of each vine to measure their weight (g), size (cm^3), juice (%), berry weight (g), length (mm), and width (mm). Fruit firmness (lb/in^2) using a Magness-Taylor pressure tester (mod. FT 02 (0-2 Lb., Via Reale, 63 - 48011 Alfonsine, Italy) with a 1/16-in plunger. Total soluble solids (TSS) were determined using a hand-held refractometer (ATAGO N-2E Brix 28-62 % made in Japan) and the results were expressed as percentages (%). The percentage of titratable acidity in the fruit juice of 100 berries was determined using the method described by Turner *et al*. (2011). Total sugars were determined calorimetrically using Nelson arsenate-molybdate colorimetric method (Nielsen 2010). Anthocyanin was determined at the stage of coloration (mg/100 g fresh weight peel) (Nangle *et al.* 2015).

Statistical Analysis

The obtained data were subjected to one-way analysis of variance (Ott and Longnecker 2015). A least significant difference at 0.05% was used to compare the means of the treatments and measured with CoHort Software (Pacific Grove, CA, USA).

RESULTS AND DISCUSSION

HPLC Analysis of Polyphenols of MLAE

Figure 1 shows the separation chromatograms of the identified chemical compounds in MLAE. Table 3 shows that ellagic acid (54.18), vanillic acid (18.79), p-hydroxy benzoic acid (14), catechol (12.32), and gallic acid (12.12) were the main identified phenolic compounds.

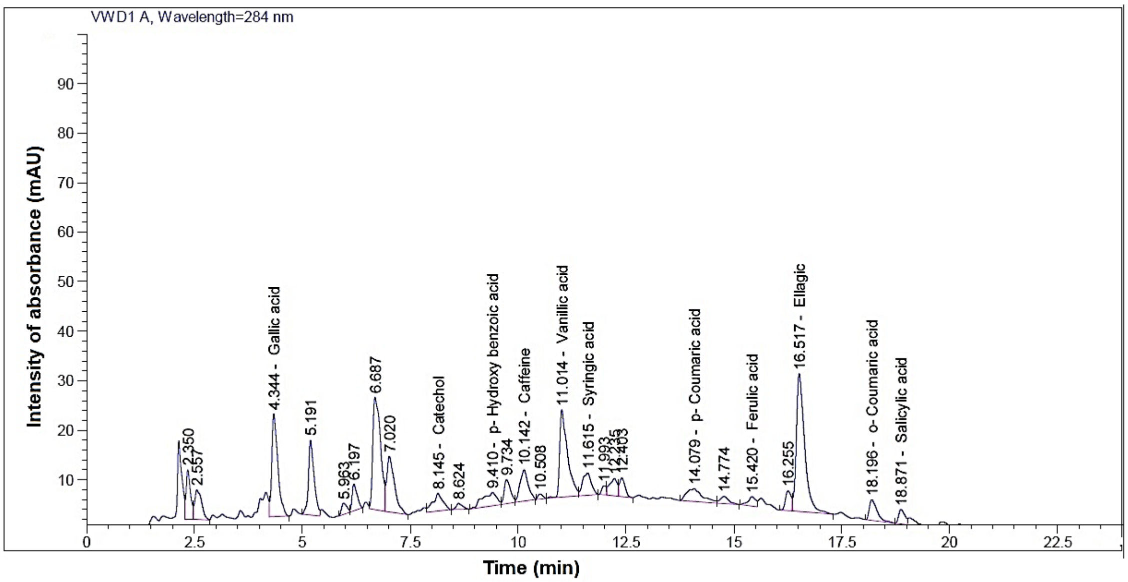

Fig. 1. HPLC chromatogram of phenolic compounds and caffeine identified in MLAE

Table 3. Identified Chemical Composition of Phenolic Compounds and Caffeine of MLAE by HPLC

Compound	Concentration (mg/100 g)
Gallic acid	12.12
Catechol	12.32
p-Hydroxy benzoic acid	14
Caffeine	6.57
Vanillic acid	18.79
Caffeic acid	ND *
Syringic acid	3.69
Vanillin	ND
p-Coumaric acid	1.93
Ferulic acid	1.20
Ellagic acid	54.18
Benzoic acid	ND
o-Coumaric acid	2.37
Salicylic acid	4.70
Cinnamic acid	ND
* ND = not detected	

Vegetative Parameters

Table 4 shows that the shoot length significantly increased as a consequence of the foliar spraying with glycine at 750 ppm, which gave the highest increment compared to the control in both seasons. In addition, it was enhanced by the foliar spraying of glycine at 250 and 500 ppm, MLAE at 4 and 6% and with the combinations of glycine 750 ppm + FA 150 ppm + 6% MLAE and glycine 500 ppm + FA 100 ppm + MLAE 4% in the two seasons as compared with the control. Compared with the control in both seasons, the shoot thickness was significantly enhanced by the applications of glycine at 250, 500, and 750 ppm, MLAE at 2%, 4%, and 6%, and FA at 50, 100, and 150 ppm. Additionally, it improved with the combinations of glycine 250 ppm + FA 50 ppm + 2% MLAE, glycine 500 ppm + FA 100 ppm + MLAE 4%, and glycine 750 ppm + FA 150 ppm + MLAE 6%. The total chlorophyll content in the leaves was greatly improved by the spraying with glycine at 250, 500, and 750 ppm, with MLAE at 2, 4, and 6%, and with FA at 100 and 150 ppm. In addition, it was raised with the combinations of glycine 250 ppm + FA 50 ppm + MLAE 2%, glycine 500 ppm + FA 100 ppm + 4% MLAE, and glycine 750 ppm + FA 150 ppm + 6% MLAE to a higher extent than the control in both experimental seasons. The best results were obtained by the foliar spraying of glycine at 750 ppm over the other applied treatments or the control in the two seasons.

Table 4. Influence of the Foliar Application of Glycine, FA, MLAE, and their Combinations on Shoot Length, Thickness, and Total Chlorophyll of Grape cv. 'Flame Seedless' During 2018 and 2019 Seasons

Treatment	Shoot Length (cm)		Shoot Thickness (mm)		Total Chlorophyll SPAD (µ Molm^{-2})	
	2018	2019	2018	2019	2018	2019
Control	96.00b	96.80d	7.82d	8.20h	31.68c	32.69e
Glycine 250 ppm	98.60ab	100.00abc	8.96bc	9.98abc	38.60a	39.44ab
Glycine 500 ppm	98.80ab	101.40ab	9.78a	10.04ab	38.86a	39.66a
Glycine 750 ppm	100.60a	101.80a	10.12a	10.30a	39.72a	40.14a
FA 50 ppm	96.00b	98.40cd	8.52c	8.90g	32.78c	33.70e
FA 100 ppm	97.40ab	98.60bcd	8.56c	8.96fg	34.74b	35.76d
FA 150 ppm	98.20ab	99.20abcd	8.64c	9.34def	35.69b	35.94d
MLAE 2%	97.60ab	100.40abc	8.64c	9.04fg	34.82b	36.19cd
MLAE 4%	98.60ab	100.60abc	8.94bc	9.52de	38.38a	37.88bc
MLAE 6%	98.60ab	100.80abc	9.16b	9.58cde	38.49a	39.89a
Glycine 250 ppm + FA 50 ppm + MLAE 2%	98.20ab	99.60abcd	8.98bc	9.24efg	35.69b	35.86d
Glycine 500 ppm + FA 100 ppm + MLAE 4%	98.60ab	101.00abc	9.14b	9.74bcd	38.91a	38.77ab
Glycine 750 ppm + FA 150 ppm + MLAE 6%	98.80ab	101.20abc	9.80a	10.04ab	39.09a	39.96a
* LSD 0.05	3.47	2.90	0.48	0.40	1.53	1.74

* Note: LSD = least significant difference
The difference between mean values shown on the same column with same letter is not significant according to Duncan's multiple range test at $P < 0.05$.

Fruit Yield

Table 5 demonstrates that the foliar spraying with glycine at 250, 500, and 750 ppm, FA at 50, 100, and 150 ppm, and MLAE at 2, 4, and 6% significantly increased the clusters number, cluster weight, yield per vine (kg) and yield in ton per hectare comparing with the control in the two seasons. Moreover, they also were statistically raised by the foliar application of glycine 250 ppm + FA 50 ppm + MLAE 2%, glycine 500 ppm + FA 100 ppm + MLAE 4%, and glycine 750 ppm + FA 150 ppm + MLAE 6% combinations over the control. The highest increment and the best results were obtained by the foliar spraying of glycine, especially at 750 and 500 ppm, respectively, compared with the other applied treatments and the control in both experimental seasons.

Table 5. Influence of the Foliar Application of Glycine, FA, MLAE, and Their Combinations on the Number and Weight of Clusters, Yield in kg per Vine and Yield in ton per Hectare of Grape cv. 'Flame Seedless' During 2018 and 2019 Seasons

Treatment	Clusters Number		Cluster Weight (g)		Yield (kg)/vine		Yield (ton)/hectare	
	2018	2019	2018	2019	2018	2019	2018	2019
Control	30.80h	32.60i	369.00k	376.60k	11.40j	12.20j	18.20j	19.80j
Glycine 250 ppm	43.80c	45.20c	758.00c	772.00c	33.20c	35.00c	53.00c	55.80c
Glycine 500 ppm	45.40b	47.00b	772.80b	781.60b	35.20b	36.80b	56.20b	59.00b
Glycine 750 ppm	47.60a	50.80a	841.20a	846.00a	40.00a	43.20a	64.00a	68.80a
FA 50 ppm	33.60g	35.00h	456.60j	465.60j	15.40i	16.60i	24.40i	26.20i
FA 100 ppm	34.40fg	36.00h	459.20j	467.20j	15.80i	16.80i	25.40i	26.80i
FA 150 ppm	35.60f	38.20g	511.00i	517.00i	18.40h	19.80h	29.00h	31.40h
MLAE 2%	40.00e	40.60f	576.00h	590.20h	23.00g	23.80g	36.80g	38.40g
MLAE 4%	41.80d	42.40e	654.60g	656.80g	27.20f	28.00ef	43.60f	44.40f
MLAE 6%	43.60c	43.20de	665.20f	666.80f	29.00e	28.80e	46.20e	46.40e
Glycine 250 ppm + FA 50 ppm + MLAE 2%	39.60e	39.00fg	707.00e	705.80e	27.80f	27.40f	44.60f	44.00f
Glycine 500 ppm + FA 100 ppm + MLAE 4%	42.60cd	43.80cde	713.00e	734.00d	30.60d	32.20d	48.60d	51.40d
Glycine 750 ppm + FA 150 ppm + MLAE 6%	43.20cd	44.80cd	723.00d	736.00d	31.20d	33.20d	50.00d	52.80d
LSD 0.05	1.52	1.64	6.57	7.05	1.01	1.22	1.55	1.74
The difference between mean values shown on the same column with same letter is not significant according to Duncan's multiple range test at P < 0.05.								

Fruit Physical and Chemical Characteristics

Table 6 shows that the size, length, and width of the clusters had greatly increased by spraying glycine at 250, 500, and 750 ppm and by the combination of glycine 750 ppm + FA 150 ppm + MLAE 6% compared with the control and the other applied treatments in both seasons. The application of glycine at 750 ppm was superior and gave the best results in the two seasons rather than the other applied treatments.

Table 6. Influence of the Foliar Application of Glycine, FA, MLAE, and Their Combinations on the Size, Length, and Width of Clusters of Grape cv. 'Flame Seedless' During 2018 and 2019 Seasons

Treatment	Cluster Size (cm^3)		Cluster Length (cm)		Cluster Width (cm)	
	2018	2019	2018	2019	2018	2019
Control	348.20j	354.60i	16.90i	16.20i	9.20i	11.16i
Glycine 250 ppm	738.20c	755.60b	23.60b	25.20c	13.48c	14.60c
Glycine 500 ppm	753.20b	761.80b	23.90b	26.60b	14.40ab	15.40b
Glycine 750 ppm	821.60a	824.80a	25.90a	30.80a	14.77a	16.60a
FA 50 ppm	435.00i	448.20h	17.16i	17.40h	10.40h	10.80i
FA 100 ppm	437.80i	448.40h	17.70hi	18.40h	10.40h	12.00h
FA 150 ppm	489.40h	496.60g	18.60gh	19.70g	11.10gh	12.40gh
MLAE 2%	555.20g	570.00f	19.00fg	20.80f	11.50fg	12.58g
MLAE 4%	637.00f	635.80e	19.80ef	23.20e	12.00def	13.50e
MLAE 6%	642.80f	642.60e	21.60d	24.00de	12.40de	14.20cd
Glycine 250 ppm + FA 50 ppm + MLAE 2%	689.60e	690.20d	20.40e	23.60e	11.80efg	13.00f
Glycine 500 ppm + FA 100 ppm + MLAE 4%	695.80e	714.60c	22.00cd	23.20e	12.60d	14.08d
Glycine 750 ppm + FA 150 ppm + MLAE 6%	705.40d	716.00c	23.00bc	24.80cd	13.96bc	15.40b
LSD 0.05	7.01	7.03	1.04	1.02	0.78	0.41

The difference between mean values shown on the same column with same letter is not significant according to Duncan's multiple range test at $P < 0.05$.

Table 7 demonstrates that weight and size of 100 berries and berry length and width were greatly improved by foliar spraying with glycine at 250, 500, and 750 ppm, MLAE at 4 and 6%, the combinations of glycine 500 ppm + FA 100 ppm + MLAE 4% and glycine 750 ppm + FA 150 ppm + MLAE 6% compared with that of the control. The highest obvious effect was obtained by the spraying of glycine at 750 followed by 500 ppm comparing with the other applied treatments, in both experimental seasons.

Table 7. Influence of the Foliar Application of Glycine, FA, MLAE, and Their Combinations on Weight, and Size of 100 Berries, Berry Length, and Width of Grape cv. 'Flame Seedless' During 2018 and 2019 Seasons

Treatment	Weight of 100 Berries (g)		Size of 100 Berries (cm^3)		Berry Length (mm)		Berry Width (mm)	
	2018	2019	2018	2019	2018	2019	2018	2019
Control	196.60i	200.00k	175.60j	178.40j	14.94i	15.17h	14.57j	14.86j
Glycine 250 ppm	265.2bc	273.20c	241.20c	248.00c	17.26c	17.25c	16.27d	17.29c
Glycine 500 ppm	275.00a	287.40a	259.00a	263.40b	17.72b	17.98b	16.57c	17.58b
Glycine 750 ppm	277.00a	288.20a	259.40a	274.60a	18.00a	18.64a	17.14a	18.41a
FA 50 ppm	208.80h	212.00j	188.20i	189.60i	16.17f	16.71e	15.43g	15.69g
FA 100 ppm	215.00g	220.00i	193.60h	197.80h	15.43h	16.05g	14.87i	15.15i
FA 150 ppm	219.00f	226.20h	200.00g	202.40g	15.87g	16.39f	15.21h	15.41h
MLAE 2%	244.40e	247.60g	221.20f	229.00f	15.50h	16.15g	14.97i	15.86g
MLAE 4%	247.20e	259.00e	234.20d	239.60d	16.41e	17.22c	15.71f	16.52e
MLAE 6%	259.40d	268.00d	242.20c	247.80c	16.78d	17.95b	16.20d	16.62e
Glycine 250 ppm + FA 50 ppm + MLAE 2%	244.60e	251.40f	226.20e	233.60e	16.07f	16.93d	15.25h	16.10f
Glycine 500 ppm + FA 100 ppm + MLAE 4%	262.40cd	272.00c	242.00c	251.40c	17.20c	17.77b	16.05e	16.97d
Glycine 750 ppm + FA 150 ppm + MLAE 6%	266.00b	279.00b	251.4b	259.40b	16.71d	18.56a	16.74b	17.49b
LSD 0.05	3.33	3.25	4.81	4.18	0.16	0.22	0.11	0.17

The difference between mean values shown on the same column with same letter is not significant according to Duncan's multiple range test at $P < 0.05$.

Table 8 demonstrates that the foliar spraying with glycine at 250, 500, and 750 ppm and with MLAE at 4 and 6% produced increases in the juice percentage and anthocyanin concentration as compared with the control in both seasons. In addition, these parameters were significantly enhanced by spraying with glycine 500 ppm + FA 100 ppm + MLAE 4% and with glycine 750 ppm + FA 150 ppm + MLAE 6% compared with the other applied treatments and the control in the two experimental seasons. The percentages of TSS and total sugars were remarkably improved by foliar spraying with glycine at 250, 500, and 750 ppm, with MLAE at 2, 4 and 6%, and by the combinations of glycine 500 ppm + FA 100 + MLAE 4% and glycine 750 ppm + FA 150 ppm + MLAE 6%. However, these treatments decreased the percentage of fruit acidity in both seasons compared with the other applied treatments and control.

Table 8. Influence of the Foliar Application of Glycine, FA, MLAE, and Their Combinations on the Percentages of Juice, Anthocyanin, TSS, Total Sugar, and Total Acidity in Fruit of Grape cv. 'Flame Seedless' During 2018 and 2019 Seasons

Treatment	Juice (%)		Anthocyanin (mg/100 g)		TSS (%)		Total Sugar (%)		Total Acidity (%)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Control	138.80i	142.20f	0.28f	0.28g	16.50h	18.00d	13.78g	15.00d	0.73a	0.74a
Glycine 250 ppm	197.00b	210.20b	0.55bc	0.58bc	19.03bcd	19.74abc	16.16bcd	16.09cd	0.45e	0.46e
Glycine 500 ppm	212.40a	218.00a	0.56b	0.59b	19.50abc	20.09ab	16.84bc	16.77bc	0.35fg	0.38f
Glycine 750 ppm	214.80a	224.80a	0.67a	0.69a	19.97ab	20.68a	17.29ab	17.46b	0.32g	0.33g
FA 50 ppm	148.00h	157.00e	0.34f	0.47e	16.90gh	18.10d	14.28fg	15.04d	0.65b	0.63b
FA 100 ppm	148.60h	169.00d	0.44e	0.51de	17.50fgh	18.60cd	14.68efg	15.18d	0.57c	0.58c
FA 150 ppm	160.00.g	169.80cd	0.44e	0.51de	17.53fgh	18.87bcd	14.96efg	15.44d	0.53d	0.56cd
MLAE 2%	164.20fg	175.80cd	0.43e	0.41f	17.90efg	18.90bcd	15.34def	15.56d	0.55cd	0.56cd
MLAE 4%	171.40de	178.20cd	0.48cde	0.54bcd	18.20def	19.47abc	15.73cde	16.82bc	0.54cd	0.53d
MLAE 6%	176.60d	180.20c	0.48cde	0.55bcd	18.83cde	19.68abc	16.26bcd	17.47b	0.46e	0.47e
Glycine 250 ppm + FA 50 ppm + MLAE 2%	167.00ef	170.40cd	0.46de	0.53cde	18.06def	19.10bcd	15.37def	16.07cd	0.54cd	0.55cd
Glycine 500 ppm + FA 100 ppm + MLAE 4%	186.60c	193.60b	0.5bcde	0.55bcd	19.73abc	20.07ab	16.92b	17.83b	0.43e	0.44e
Glycine 750 ppm + FA 150 ppm + MLAE 6%	189.20c	194.60b	0.52bcd	0.55bcd	20.23a	20.62a	18.14a	19.00a	0.36f	0.35fg
LSD 0.05	6.70	10.41	0.08	0.057	1.03	1.28	1.18	1.09	0.03	0.04

The difference between mean values shown on the same column with same letter is not significant according to Duncan's multiple range test at P < 0.05.

Leaf Chemical Composition

Table 9 shows that the leaf composition of N, P, and K, was remarkably increased by spraying glycine at 500 and 750 ppm, MLAE at 4 and 6% comparing with control or the rest treatments in the two seasons. In addition, the highest leaf content from N, P, and K was correlated by spraying the combinations of glycine 500 ppm + FA 100 ppm + MLAE 4% and glycine 750 ppm + FA 150 ppm + MLAE 6%.

Table 9. Influence of the Foliar Application of Glycine, FA, MLAE, and Their Combinations on Leaf content from N, P, and K of Grape cv. 'Flame Seedless' during 2018 and 2019 Seasons

Treatment	N%		P%		K%	
	2018	2019	2018	2019	2018	2019
Control	0.22h	0.34def	0.28hi	0.31h	4.14e	3.98i
Glycine 250 ppm	0.35ef	0.38bcde	0.42e	0.44e	5.56c	5.80cd
Glycine 500 ppm	0.43bcd	0.41b	0.49d	0.52d	5.88abc	6.30b
Glycine 750 ppm	0.45bc	0.50a	0.59c	0.62c	5.88abc	6.31b
FA 50 ppm	0.26gh	0.29f	0.22j	0.24i	3.90e	3.84i
FA 100 ppm	0.32fg	0.33ef	0.24ij	0.24i	4.08e	4.42h
FA 150 ppm	0.36ef	0.35cde	0.27hi	0.26i	4.72d	5.04g
MLAE 2%	0.27gh	0.38bcde	0.29h	0.31h	4.86d	5.40ef
MLAE 4%	0.37def	0.39bcd	0.34g	0.37g	5.60bc	5.18fg
MLAE 6%	0.40cde	0.41bc	0.40ef	0.40fg	5.72bc	6.00c
Glycine 250 ppm + FA 50 ppm + MLAE 2%	0.39cde	0.40bc	0.37f	0.42ef	4.92d	5.60de
Glycine 500 ppm + FA 100 ppm + MLAE 4%	0.47ab	0.52a	0.83b	0.88b	5.92ab	6.46b
Glycine 750 ppm + FA 150 ppm + MLAE 6%	0.52a	0.55a	0.91a	0.91a	6.16a	7.24a
LSD 0.05	0.06	0.05	0.03	0.03	0.34	0.26
The difference between mean values shown on the same column with same letter is not significant according to Duncan's multiple range test at $P < 0.05$.						

The data obtained shows that the foliar spraying with glycine at 250, 500, and 750 ppm had an effective role in improving the vegetative growth parameters, yield, and fruit quality characteristics of 'Flame Seedless' grape cultivar. These results were previously explained by Sekhon (2003) and Souri (2016). They reported that glycine amino acid is the smallest amino acid and can produce chelates for different ions of elements and products. The stimulatory effect of glycine amino acid increases under the conditions of stress such as salinity and water stress (Rai 2002; Cerdán *et al.* 2013; Sadak *et al.* 2015; Souri *et al.* 2018). Besides, the application of glycine increased TSS and vitamin C compared with the control (Sekhon 2003; Liu *et al.* 2011; Shan *et al.* 2016; Razavi *et al.* 2018; Mohammadipour and Souri 2019). Moreover, they found that leaf nutrient concentrations of N, Ca, K, P, Mg, Mn, Fe, and Zn, were significantly increased by soil application of glycine compared with the unfertilized control plants. Glycine amino acids can play a crucial role in raising the stability of cell membrane and its protection from peroxidation through the growth of plant under the stresses conditions (Keutgen and Pawelzik 2008; Zobiole *et al*. 2012; Rizwan *et al*. 2017; Teixeira *et al*. 2017). It was mentioned by many authors that the transfer of nutrients in tissues of plants is related greatly to the glycine concentration and status (Marschner 2011; Souri 2016). In addition, glycine has a crucial role in the total chlorophyll, forming vegetative growth, and in increasing the availability of Fe, Zn, Mn, and Cu to plants (Ghasemi *et al.* 2013). The application of nitrogen reduced compounds such glycine amino acid raised the chlorophyll content in the leaves (Fahimi *et al.* 2016; Souri *et al.* 2017). Parameters of coriander (*Coriandrum sativum*) plant growth, plant height, leaf SPAD value, and shoot and root fresh weights were significantly improved by applying glycine, particularly at high concentrations (Souri and Hatamian 2019). Additionally, the same authors reported that, because of the simple process for glycine synthesis, it could be combined with the nutrients producing chelates to increase the absorption of the elements and transfer them into the plants. Spraying glycine amino acids on apple trees (*Malus domestica* L. Borkh) cv. 'Anna' at 25, 50, and 100 ppm improved shoot length, shoot diameter, total chlorophyll, yield, fruit weight, size, length, diameter, TSS, total sugars, anthocyanin, and leaf composition from macro and micronutrients, while it reduced the total acidity and fruit drop percentage. Moreover, they noticed that the effect of 50 and 100 ppm was higher than 25 ppm (Mosa *et al.* 2021).

The results of this study show that the foliar spraying of folic acid (FA) had a good effect on the vegetative growth parameters, yield, and fruit quality of grape cv. 'Flame Seedless', especially when it was applied at 150 ppm. These results were in harmony with the previous findings of Stakhova *et al.* (2000). They stated that foliar spraying of FA enhanced the photosynthetic rate in the leaves, seed weight, and yield of peas (*Pisum sativum* L.). FA at 50 μm raised the yield, leaf mineral content, seed weight, and quality of the seed and leaf chlorophyll rate (Burguieres *et al.* 2007). Moreover, Kim (2007) demonstrated that folate plays an important role in the synthesis and repetition of DNA by arranging the transporting carbon unites, which participates in purines and thymidylate synthesis. In the same trend, Fardet *et al.* (2008) found that FA has a positive influence in increasing the vegetative growth, yield, and fruit quality in many plant species because they can catch the free radicals and active oxygen, which are produced during the processes of photosynthesis and respiration. It was noticed by many authors that folates are an important factor in helping the transferring of carbon as donors and acceptors, which can engage in purines, pyrimidines, and amino acids synthesis (Dhonukshe-Rutten *et al.* 2009; Blancquaert *et al.* 2010). Treating flax plants with FA greatly increases the parameters of growth, photosynthetic rate, number of flowers, maturity index, yield, and quality of fiber and seeds (Emam *et al.* 2011). In *Pisum sativum* L. cv. 'Master-B', Farouk and Abdul Qados (2018) found that spraying FA at 20 mg/L significantly increased the height of plant, weight of shoot and dry shoots, area of plant leaf, pigments of photosynthesis, yield, and fruit quality.

The results show that MLAE plays an important role in improving the vegetative growth, yield, fruit quality, and leaf composition from N, P, and K (%) compared with the control. These results are in parallel with the findings of other studies of (Dillard and German 2000; Siddhuraju and Becker 2003; Aslam *et al.* 2005). They mentioned that moringa leaf extracts have good quantities from β-carotene, protein, vitamin C, Ca, K Mg, K, Mn, P, Zn, Na, Cu, and Fe, as well as natural antioxidants such as ascorbic acid, quercetin, zeatin, kaempferol, β-sitosterol, caffeoylquinic acid, and carotenoids. MLAE increases the content of proline, malondialdehyde, total soluble proteins, and total chlorophyll in spinach (*Spinacia oleracea*) leaves (Aslam *et al.* 2005) and yield, fruit quality, juice percentage, and TSS (%) while it decreases the value of nitrite, including nitrate in the fruit juice of orange trees (*Citrus sinensis*) (El-Enien *et al.* 2015). Additionally, Ndong *et al.* (2007) found that the leaves are a potent source of polyphenols, including quercetin-3-glycoside, rutin, kaempferol, and glycosides. MLAE has increased the growth and yield of a range of plant species such as cereals (Phiri 2010), maize (Basra *et al.* 2011), and wheat (Sarmin 2014). Culver *et al.* (2012) reported that MLAE contains relatively high concentrations of the cytokinin, Zeatin so, tomato leaves' fresh and dry weights were enhanced significantly when MLAE is applied at high rates. Spraying MLAE stimulated the leaf mineral contents from N, P, and K, leaf area, chlorophyll, shoot length and shoot diameter, yield, as well as physical and chemical characteristics of fruits of Le Conte pear (Abd El-Hamied and El-Amary 2015). Furthermore, MLAE significantly enhanced vegetative growth parameters, total leaf chlorophyll content, and the leaf mineral composition of N, P, and K of pomegranate cv. 'Manfalouti' (Kamel 2015) and grape cv. 'Flame Seedless' (*Vitis vinifera* L.) (Bassiony and Ibrahim 2016), as well as fruit quality, color, soluble solids content, vitamin C, anthocyanin content, and antioxidant activity of plum cv. 'Hollywood' (Mahmoud *et al.* 2017). Leone *et al.* (2015) stated that phenolic acids, carotenoids vitamins, polyphenols, flavonoids, isothiocyanates, alkaloids, glucosinolates, tannins, and saponins exist in moringa leaves with high levels. MLAE has been demonstrated to enhance the production of ornamental and medicinal plants as it was used as an agent for biostimulation. Because of the high micro and macronutrient elements of MLAE, it has a simulative effect on plant growth and plays an important role in photosynthesis and carbohydrate synthesis, which are metabolic processes (Sakr *et al.* 2018). This might be due to the significant contribution of nitrogen present in MLAE, which caused cell division, cell enlargement, and the overall plant growth (Kanchani and Harris 2019). Mohamed *et al.* (2020) reported that the application of MLAE on *Origanum majorana* improved its vegetative growth characteristics and the production of oil.

From the above-discussed results, it could be noticed that the foliar spraying of glycine played an essential role in improving attributes of vegetative growth, yield and fruit quality of grape cv. 'Flame Seedless' compared to the control or the rest of the treatments in the two experimental seasons. The positive effect of glycine was increased in parallel by increasing its concentration, where 750 ppm gave better results rather than 250 or 500 ppm during the two seasons of the study.

CONCLUSIONS

1. Shoot length, thickness, and leaf total chlorophyll were significantly increased by foliar spraying with glycine at 500 and 750 ppm and with the combination of glycine 750 ppm + FA 150 ppm + MLAE 6%, which gave the highest increments in the two seasons compared with the control and the other applied treatments.
2. Spraying glycine with 500 and 750 ppm were the best treatments in enhancing the clusters number and weight (kg), thus giving the best yield in kg per vine or in ton per hectare when compared with the control or the other applied treatments.
3. The best results for TSS (%), total sugars, juice (%), and anthocyanin were obtained by the foliar application of glycine at 500 and 750 ppm and by the combination of glycine 750 ppm + FA 150 ppm + MLAE 6% as well as glycine 500 ppm + FA 100 ppm + MLAE 4% when compared with the control and the other applied treatments.
4. The leaf chemical composition of N, P, and K was greatly increased by the foliar spray of glycine 750 ppm + FA 150 ppm + MLAE 6%, which was the superior combination compared with the control and the other applied treatments.
5. In general, the best results were obtained by the foliar spraying of glycine at 750 ppm followed by 500 ppm over all the applied treatments and the control.

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