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Effect of Some Biostimulants on the Vegetative Growth, Yield, Fruit Quality Attributes and Nutritional Status of Apple

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Abstract: Although the application of chemical fertilizers to crops promotes plant growth and yield, their continuous use affects soil health and creates environmental pollution. On the other hand, plant biostimulants improve nutrients absorption, plant growth, yield and produce quality and are environment-friendly. Therefore, an experiment was conducted during 2021-22 to evaluate the effect of some biostimulants on the performance of the apple cv. Anna, planted in a sandy loam soil at Marsa Matruh governorate, Egypt. Ninety trees were randomly selected and sprayed with 4 or 6% moringa leaf extract (MLE), 0.3 or 0.4% seaweed extract (SWE), 0.1% or 0.2% Fulvic acid (FA), 4% MLE + 0.3% SWE + 0.1% FA (combination 1), or 6% MLE + 0.4% SWE + 0.2% FA (combination 2) before flowering, during full bloom and one month later and compared with a control (untreated trees). The results demonstrated that spraying MLE, SWE or FA or their combinations positively improved the vegetative growth, fruit set %, fruit yield and fruit physical and chemical characteristics as well as leaf nutritional status. The positive effect of MLE, SWE and FA was increased in parallel to an increase in the used concentration of each one of them. The highest increments in the measured parameters were accompanied by the application of combination 2 over the other treatments.

Keywords: *Malus domestica*; fulvic acid; moringa leaf extract; seaweed extract



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1. Introduction

The apple (*Malus domestica* L.) cv. Anna is widely grown in Egypt. It contains total soluble solids (TSS) \approx 12%, total sugars \approx 9.5%, acidity \approx 0.8%, dietary fibers \approx 2.3 g and protein \approx 0.3 g. In Egypt, the apple is grown on an area of 27,417 hectares, with an annual production of 697,936 tons; the total cultivated area of apples worldwide is 4,622,366 hectares and the worldwide total annual production is 86,442,716 tons [1].

Although the usage of mineral fertilization promotes plant growth and fruiting, it has been documented that the excessive and extensive usage of chemical fertilizers reduces soil quality by reducing its organic matter content and increasing soil pollution [2,3]. Moreover, it also leads to an increase in environmental and underground water pollution, and an increase in greenhouse gases released into the atmosphere, which are not only harmful to humans but also reduce crop yield and food safety [4–7], resulting in soil degradation and compaction, which consequently leads to root growth limitation, poor aeration and drainage [8] and a decrease in the soil's biological activities and nutrient absorption [9].

Plant biostimulants are defined as substances that can be applied to plants to enhance nutrient availability, absorption and efficiency, growth, yield and produce quality charac-

teristics and post-harvest shelf life. These also improve plant immune systems, tolerance to abiotic and biotic stresses [10–17] and the degradation of organic compounds in the soil [18].

Soliman and Shanan [19] documented that MLE is equal to or better in its effectiveness than synthetic plant growth regulators. Further, MLE is easy to prepare cheap and environmentally friendly; therefore, it is a potential crop biostimulant [20]. Spraying MLE on different fruit trees remarkably improved vegetative growth attributes, fruit yield, fruit physical and chemical characteristics and plant mineral status as compared with untreated trees [21–23]. As MLE contains numerous necessary elements, auxins, gibberellins, cytokinins, proteins, sugars, proline and vitamins, it is helpful in improving the nutrition of plants, vegetative growth, photosynthesis process, water use efficiency, root development, mineral content, gas exchange traits, flower formation, yield and fruit quality. Besides, it can improve the resistance of plants to abiotic stresses, salinity, drought, heavy metals, etc., by activating antioxidant enzymes and increasing compatible solutes [24,25].

Seaweed extract (SWE) is a good and cheap source of nutrients, organic matter and plant growth stimulators, so a foliar spray of a seaweed extract is an efficient method to increase vegetative growth, the photosynthetic rate, proline content and total soluble sugars, biotic and abiotic stress tolerance, yield, fruit quality and fruit shelf life in fruit crops [26–33]. As SWE is characterized by a high content of auxins, cytokinins, gibberellins, abscisic acid, vitamins, amino acids, antibiotics, polysaccharides, micro- and macro-nutrients, so it has the ability to improve the growth and yield of crop plants [34–37]. Additionally, SWE can modify gene expression, which is accountable for the internal biosynthesis of growth hormones such as auxins, cytokinins and gibberellins [38].

Fulvic acid (FA) is a humic acid with a high oxygen content and low molecular weight, which helps it to pass easily through living or artificial membranes' micro holes [39]. It has been reported that FA is considered as a plant growth and yield biostimulator because it can enhance the permeability of the cell membrane, the nutrient translocation inside plants, the photosynthetic and respiration rates and lessen the uptake of poisonous elements by plants [40,41]. Fulvic acid is a natural material that results from the decay of plants, animals, the remains of microbes and soil microbes' metabolic activity [42]. It can also improve the growth of plants under drought conditions because it is an anti-transpirant organic acid, nontoxic, cheap and not pollutant to the soil. Moreover, FA molecules are small and can bind nutrients easily. These can penetrate the roots, stems and leaves of plants and carry minerals from plant surfaces to plant tissues [43].

Keeping in view the benefits of these natural substances, this study was conducted to examine the spraying effects of MLE, SWE and FA as ecofriendly alternatives and safe biostimulants on the vegetative growth attributes, yield, fruit quality and nutritional status of Anna apple trees.

2. Materials and Methods

2.1. Experimental Trees and Treatments Application

The present study was carried out during 2021–22 on the apple (*Malus domestica* L.) cv. Anna trees were budded on Malling-Merton 106 (MM 106) rootstock, planted at 3 × 4 m in a sandy loam soil under drip irrigation in El Omid, Marsa Matruh governorate, Egypt. Ninety trees, ten years old with uniform size and growth, were selected to perform this study. All experimental trees were treated with same agricultural practices in the orchard during the two study years. The physical and chemical composition of the experimental orchard soil was determined according to [44] and is listed in Table 1. Before flowering, during full bloom and one month later, the selected trees were sprayed with 4 or 6% moringa leaf extract (MLE), 0.3 or 0.4% seaweed extract (SWE) and 0.1% or 0.2% Fulvic acid (FA), 4% MLE + 0.3% SWE + 0.1% FA (combination 1), or 6% MLE + 0.4% SWE + 0.2% FA (combination 2). A control (untreated trees) was also included for comparison.

Table 1. Physicochemical properties of the experiment soil.

Depth	Texture		pH		EC (dS/m)		
0–60 cm	Sandy loam		8.17		2.58		
Soluble anions (%)				Soluble Cations (%)			
CaCO ₃ ²⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ²⁻	Na ⁺	Mg ²⁺	K ⁺	Ca ²⁺
26.7	5.0	14.5	6.0	15.2	4.00	1.6	5.00

The aforementioned treatments were distributed in a randomized complete block design, and each treatment was comprised of ten replicates. The influence of the applied treatments was investigated on the following parameters.

2.2. Vegetative Growth Parameters

At the start of vegetative growth (in the month of February during both years 2021 and 2022, eight shoots from each side of each experimental tree were chosen and labeled. At the end of each growing season, shoot length (cm) and diameter (cm) were measured. Relative chlorophyll content was measured in fresh leaves as SPAD by a chlorophyll meter apparatus (SPAD-502; Minolta, Japan). Leaf area (cm²) was measured during the growing seasons according to Equation (1) [45].

$$LA = -0.5 + \left(0.23 \times \frac{L}{W}\right) + (0.67 \times L \times W) \quad (1)$$

where *LA* is a leaf area (cm²), *L* is leaf length (cm) and *W* is leaf width (cm).

2.3. Fruit Set and Fruit Drop Percentages

Four branches from each side of each experimental tree were selected carefully and labeled in March during 2021 and 2022, and the number of flowers on each branch was counted. The fruit set percentage was calculated according to Equation (2).

$$\text{Fruit set (\%)} = \frac{\text{Number of set fruits}}{\text{Number of opened flowers}} \times 100 \quad (2)$$

Estimation of pre-harvest fruit drop was performed by counting the number of dropping fruits after fruit setting until the harvesting date in June each year. Then, the fruit drop was calculated as a percentage as follows.

$$\text{Fruit drop (\%)} = \frac{\text{Number of dropped fruits}}{\text{Number of set fruits}} \times 100 \quad (3)$$

2.4. Fruit Yield

In June 2021 and 2022, all the fruits from each experimental tree were harvested and yield was estimated by using a digital weighing scale (Yongkang Beichen Weighing Apparatus Co., Ltd., Jinhua, China). The yield in tons per hectare was calculated by multiplying the yield of tree with total number of trees in a hectare.

2.5. Fruit Quality

In June 2021 and 2022 (time of harvesting), twenty fruits from each tree were taken randomly to measure their physical and chemical characteristics.

2.5.1. Fruit Physical Characteristics

Fruit weight (g) was recorded using a digital weighing scale, while fruit length (cm) and diameter (cm) were measured using a vernier caliper and fruit size (cm³) was computed. Fruit firmness (kg/cm²) was estimated using a Magness and Taylor pressure tester with 7/18-inch plunger [46].

2.5.2. Fruit Chemical Characteristics

Total soluble solids (TSS %) in the juice of fruits were measured by using a hand refractometer (ATAGO, Tokyo, Japan). Total acidity (%) was determined according to [47], and then TSS-acid ratio was calculated. By using the method of Nelson arsenate—molybdate colorimetric, the percentages of total and reducing sugars were estimated [48]. Non-reducing sugars were calculated by the difference between total sugars and reducing sugars. Anthocyanin (mg/100 g fresh weight peel) was estimated as described by Nangle et al. [49].

2.6. Leaf Mineral Composition

During June 2021 and 2022, after harvesting the fruits, thirty leaves from the middle part of the labeled shoots as described by [50] were selected from each tree and their content of macro- and micro-nutrients was determined. The leaves were first washed with running tap water, then rinsed with distilled water and dried at 70 °C until they reached a steady weight. The dried leaves were ground well, digested by using H₂SO₄ and H₂O₂ and filtered until the solution became clear. The solution was used for the determination of micro-nutrients such as Fe, Mn, Zn and B content by atomic absorption spectrophotometry. Nitrogen content was measured by the micro-Kjeldahl method [51], phosphorus by the vanadomolybdate method [52] and potassium using a flame photometer (SKZ International Co., Ltd., Jinan, China) [53].

2.7. Statistical Analysis

Results of the experiments were statistically analyzed separately for 2021 and 2022 by using one way ANOVA [54] followed by least significant difference (LSD) at 0.05% to make the comparison among the treatment means.

3. Results

3.1. Vegetative Growth Parameters

The foliar application of MLE, SWE and FA and their combinations significantly increased the vegetative growth in terms of shoot length and diameter, leaf area and relative leaf chlorophyll content compared with untreated trees during 2021 and 2022 (Table 2). Moreover, combination 2 and combination 1 gave the highest increments in shoot length, shoot diameter, leaf chlorophyll content and leaf area over the untreated trees. The results also demonstrated that the effects of 6% MLE, 0.4% SWE and 0.2% FA were better than the lower applied concentrations of these biostimulants or control during the years 2021 and 2022.

3.2. Fruit Set, Drop and Yield

The results demonstrated that the spraying of MLE, SWE and FA and their combinations significantly improved the fruit set and fruit yields per tree and per hectare over the untreated trees during both the years, i.e., 2021 and 2022 (Table 3). Besides, the same treatments markedly reduced the fruit drop. The most effective treatment, which achieved the most increments in fruit set and fruit yield, was the application of combination 2, compared to the other applied treatments during both the years. Moreover, combination 1 and 6% MLE, 0.4% SWE and 0.2% FA also had a positive influence in increasing the same measured parameters during 2021 and 2022 with respect to the other applied treatments.

Table 2. The influence of the spraying of MLE, SWE and FA and their combinations on some vegetative growth parameters of apple cv. Anna during 2021 and 2022.

Treatments		Shoot Length (cm)		Shoot Diameter (cm)		Leaf Area (cm ²)		Relative Leaf Chlorophyll Content (SPAD)	
		2021	2022	2021	2022	2021	2022	2021	2022
Control	0	40.15 ^f ±1.50	40.67 ^e ±2.17	0.77 ^d ±0.06	0.8 ^e ±0.04	28.81 ^e ±1.48	30.90 ^d ±2.32	43.01 ^e ±2.19	44.12 ^d ±.69
MLE	4%	41.81 ^{ef} ±1.09	43.20 ^{de} ±2.27	0.78 ^d ±0.02	0.82 ^{de} ±0.02	30.57 ^{cde} ±2.59	32.57 ^{cd} ±2.51	45.69 ^d ±1.36	47.62 ^c ±1.19
	6%	44.98 ^{bcde} ±0.81	46.20 ^{cd} ±1.35	0.86 ^c ±0.02	0.88 ^{bc} ±0.01	32.13 ^{bcde} ±1.45	34.43 ^{bc} ±1.07	50.42 ^{bc} ±1.64	51.85 ^b ±2.40
SWE	0.3%	44.03 ^{cde} ±1.00	45.70 ^{cd} ±0.46	0.84 ^c ±0.03	0.82 ^{de} ±0.02	32.33 ^{bcd} ±1.45	33.43 ^{bcd} ±1.93	48.05 ^c ±1.24	50.40 ^b ±1.48
	0.4%	46.35 ^{bc} ±3.32	48.60 ^{bc} ±2.21	0.89 ^{bc} ±0.02	0.90 ^b ±0.02	33.81 ^{abc} ±1.43	35.03 ^{abc} ±1.50	52.53 ^{ab} ±1.78	55.10 ^a ±2.26
FA	0.1%	42.30 ^{def} ±0.39	43.63 ^{de} ±0.45	0.78 ^d ±0.03	0.85 ^{cd} ±0.03	29.71 ^{de} ±3.10	33.43 ^{bcd} ±1.07	49.15 ^c ±1.07	51.00 ^b ±0.78
	0.2%	45.55 ^{bcd} ±1.01	47.27 ^{bc} ±0.95	0.89 ^{bc} ±0.03	0.91 ^b ±0.02	32.66 ^{bcd} ±1.01	34.30 ^{bc} ±1.15	53.39 ^a ±2.40	54.45 ^a ±2.12
Combination	1	47.96 ^b ±3.09	50.27 ^{ab} ±2.00	0.94 ^{ab} ±0.01	0.95 ^a ±0.01	35.46 ^{ab} ±1.29	36.53 ^{ab} ±0.65	52.98 ^a ±1.69	54.85 ^a ±0.78
	2	51.28 ^a ±2.24	52.63 ^a ±2.61	0.95 ^a ±0.02	0.96 ^a ±0.01	36.27 ^a ±1.71	37.87 ^a ±1.53	53.75 ^a ±1.13	56.57 ^a ±1.05
LSD _{0.05}		3.23	3.06	0.05	0.04	3.15	2.81	2.33	2.23

The treatment means that have the same letter(s) in one column indicate that there were non-significant differences between them. The treatment means are followed by ±SE.

Table 3. The influence of the spraying of MLE, SWE and FA and their combinations on fruit set and drop % and yields per tree and per hectare of apple cv. Anna during 2021 and 2022.

Treatments		Fruit Set (%)		Fruit Drop (%)		Fruit Yield/Tree (kg)		Fruit Yield/Hectare (tons)	
		2021	2022	2021	2022	2021	2022	2021	2022
Control	0	16.77 ^e ±1.62	17.33 ^e ±1.11	73.38 ^a ±2.45	69.65 ^a ±2.06	50.61 ^e ±2.01	51.90 ^f ±1.54	40.49 ^e ±1.61	41.52 ^f ±1.23
MLE	4%	16.97 ^e ±1.50	18.75 ^{de} ±1.55	66.34 ^b ±0.72	66.01 ^{ab} ±2.25	53.42 ^{de} ±1.73	54.20 ^{ef} ±3.00	42.73 ^{de} ±1.39	43.36 ^{ef} ±2.40
	6%	20.17 ^{cd} ±1.37	22.80 ^{bc} ±1.61	56.64 ^d ±1.69	58.38 ^{cd} ±2.36	59.19 ^{bc} ±1.95	59.5 ^{cd} ±2.64	47.35 ^{bc} ±1.56	47.60 ^{cd} ±2.12
SWE	0.3%	18.83 ^{de} ±1.60	20.83 ^{cd} ±1.62	60.55 ^c ±2.67	62.25 ^{bc} ±3.10	55.48 ^{cd} ±2.33	56.88 ^{de} ±2.07	44.38 ^{cd} ±1.86	45.50 ^{de} ±1.66
	0.4%	24.54 ^b ±1.17	25.29 ^b ±1.10	53.63 ^d ±1.99	55.35 ^{de} ±1.93	60.73 ^b ±2.59	63.61 ^b ±1.46	48.59 ^b ±2.07	50.88 ^b ±1.17
FA	0.1%	18.77 ^{de} ±1.36	20.43 ^{cd} ±1.80	63.75 ^{bc} ±1.91	61.51 ^c ±2.19	55.68 ^{cd} ±2.10	58.03 ^{de} ±2.50	44.55 ^{cd} ±1.68	46.43 ^{de} ±2.00
	0.2%	22.13 ^{bc} ±1.95	23.70 ^b ±1.25	54.18 ^d ±2.87	56.23 ^{de} ±1.57	59.70 ^b ±2.65	63.00 ^{bc} ±1.37	47.76 ^b ±2.12	50.40 ^{bc} ±1.10
Combination	1	24.53 ^b ±1.15	25.33 ^b ±0.96	53.41 ^d ±2.14	53.45 ^e ±2.05	62.42 ^b ±1.64	64.00 ^b ±1.51	49.94 ^b ±1.31	51.20 ^b ±1.21
	2	27.96 ^a ±2.55	30.1 ^a ±2.42	49.07 ^e ±2.36	49.22 ^f ±3.14	66.67 ^a ±1.85	67.97 ^a ±2.60	53.34 ^a ±1.48	54.37 ^a ±2.08
LSD _{0.05}		2.81	2.66	3.73	4.02	3.64	3.71	2.92	2.96

The treatment means that have the same letter(s) in one column indicate that there were non-significant differences between them. The treatment means are followed by ±SE.

3.3. Fruit Quality

3.3.1. Physical Fruit Characteristics

The spraying of 4 or 6% MLE, 0.3 or 0.4% SWE and 0.1% or 0.2% FA had positive influences on increasing the fruit weight, size, length and diameter as well as fruit firmness

(Table 4). The results also indicated that their influences on fruit physical characteristics were much better when their combinations or their higher concentrations were applied during both years, i.e., 2021 and 2022. The most effective treatment, which resulted in the highest values of these parameters, was the application of combination 2 as compared with all the other applied treatments. The fruit weight, size and diameter were also significantly increased by the application of combination 1 compared with the rest of the sprayed treatments and the control during both the years of experimentation.

Table 4. The influence of the spraying of MLE, SWE and FA and their combinations on fruit weight, size, length, diameter and fruit firmness of apple cv. Anna during 2021 and 2022.

Treatments		Fruit Weight (g)		Fruit Size (cm ³)		Fruit Length (cm)		Fruit Diameter (cm)		Fruit Firmness (kg/cm ²)	
		2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
Control	0	175.33 ^e ±5.03	179.33 ^f ±5.03	192.00 ^e ±2.00	193.00 ^f ±4.58	44.67 ^e ±1.56	48.70 ^c ±1.37	44.53 ^f ±3.41	46.43 ^d ±1.10	1.32 ^e ±0.07	1.44 ^c ±0.04
MLE	4%	178.33 ^e ±3.51	184.33 ^{ef} ±3.05	193.33 ^e ±3.05	198.33 ^{ef} ±2.89	49.60 ^d ±2.35	51.23 ^c ±1.21	49.1 ^{de} ±1.68	48.07 ^d ±1.98	1.34 ^{de} ±0.07	1.45 ^c ±0.07
	6%	190.00 ^d ±5.00	197.00 ^d ±3.00	205.00 ^d ±5.00	211.00 ^d ±2.64	53.53 ^c ±1.70	57.20 ^b ±1.2	52.30 ^b ±1.70	54.30 ^b ±2.00	1.55 ^{bc} ±0.03	1.59 ^b ±0.05
SWE	0.3%	182.33 ^e ±2.52	186.00 ^e ±3.60	196.67 ^e ±3.05	199.00 ^e ±2.64	47.93 ^{de} ±2.40	50.33 ^c ±1.90	46.11 ^{ef} ±1.65	51.70 ^{bc} ±1.54	1.45 ^{cd} ±0.08	1.48 ^c ±0.06
	0.4%	200.00 ^c ±5.00	204.00 ^c ±3.60	214.33 ^c ±4.04	217.33 ^c ±4.04	59.20 ^b ±0.87	58.53 ^b ±1.02	51.16 ^{bc} ±1.18	54.13 ^b ±2.50	1.55 ^{bc} ±0.03	1.61 ^b ±0.03
FA	0.1%	178.00 ^e ±2.00	183.00 ^{ef} ±3.60	194.33 ^e ±2.31	197.00 ^{ef} ±3.00	48.53 ^d ±1.78	50.87 ^c ±1.42	47.36 ^{de} ±1.07	49.07 ^{cd} ±2.11	1.42 ^{de} ±0.07	1.46 ^c ±0.10
	0.2%	195.00 ^{cd} ±5.00	201.33 ^{cd} ±4.16	208.67 ^{cd} ±6.03	216.00 ^{cd} ±3.60	54.80 ^c ±1.78	59.17 ^b ±1.64	52.43 ^b ±2.26	54.10 ^b ±1.57	1.57 ^b ±0.06	1.59 ^b ±0.02
Combination	1	210.33 ^b ±2.52	212.67 ^b ±2.52	222.67 ^b ±2.52	227.00 ^b ±2.64	59.37 ^b ±1.62	59.55 ^b ±1.17	56.73 ^a ±1.05	58.07 ^a ±1.70	1.58 ^b ±0.04	1.64 ^b ±0.03
	2	217.67 ^a ±2.52	220.33 ^a ±2.52	231.00 ^a ±3.60	233.67 ^a ±1.53	62.93 ^a ±2.06	63.17 ^a ±2.81	58.21 ^a ±1.93	60.67 ^a ±2.66	1.70 ^a ±0.06	1.76 ^a ±0.03
LSD _{0.05}		6.85	5.63	5.83	5.28	3.29	2.86	2.69	3.24	0.10	0.10

The treatment means that have the same letter(s) in one column indicate that there were non-significant differences between them. The treatment means are followed by ±SE.

3.3.2. Fruit Chemical Characteristics

Concerning the influence of the applied treatments on fruit chemical characteristics (Table 5), the results demonstrated that the fruit juice TSS, TSS-acid ratio and peel anthocyanin content were significantly increased by the application of combination 2, which gave the highest increments in the mean values of these parameters. In contrast, combination 2 registered the lowest value for fruit juice acidity during both the years (2021 and 2022) with respect to the untreated trees. Besides, the application of combination 1, 6% MLE, 0.4% SWE and 0.2% FA also gave good results in increasing the fruit juice TSS, TSS-acid ratio and peel anthocyanin content, but on the other hand these treatments reduced the fruit acidity during both the years. The differences among the influences of combination 1, 6% MLE, 0.4% SWE and 0.2% FA were very slight on the TSS, acidity and anthocyanin content, mostly being statistically non-significant.

The total, reduced and non-reduced sugar contents of the fruits considerably increased as a result of the spraying of MLE, SWE and FA and their combinations with respect to the untreated trees during the two year study (Table 6). The highest percentages of total and reduced sugars were obtained by the application of combination 2 compared with the other treatments during the years 2021 and 2021. The non-reducing sugar content was markedly increased by the spraying of combinations 2 and 1, 0.4% SWE and 0.2% FA as compared to the control (untreated trees) during both the study years. Besides, it was also noticed that the total, reduced and non-reduced sugar contents increased in parallel to the increase in the used doses for each biostimulant, where 6% MLE, 0.4% SWE and 0.2% FA were better than 4% MLE, 0.3% SWE and 0.1% FA, respectively, during both years, i.e., 2021 and 2022.

Table 5. The influence of the spraying of MLE, SWE and FA and their combinations on fruit juice TSS, acidity, TSS-acid ratio and peel anthocyanin content of apple cv. Anna during 2021 and 2022.

Treatments		TSS (%)		Acidity (%)		TSS-Acid (Ratio)		Anthocyanin Content (mg/100 g)	
		2021	2022	2021	2022	2021	2022	2021	2022
Control	0	10.00 ^e ±0.53	10.47 ^d ±0.30	0.84 ^a ±0.04	0.83 ^a ±0.03	11.88 ^e ±1.00	12.68 ^d ±0.71	0.81 ^f ±0.04	0.85 ^f ±0.05
MLE	4%	11.10 ^{cde} ±0.65	10.73 ^{cd} ±0.25	0.76 ^b ±0.03	0.75 ^b ±0.02	14.65 ^d ±1.53	14.31 ^{cd} ±0.14	0.84 ^{ef} ±0.01	0.89 ^{ef} ±0.03
	6%	11.97 ^{bc} ±0.40	11.90 ^{bc} ±0.79	0.67 ^{cd} ±0.02	0.67 ^c ±0.02	17.80 ^c ±1.12	17.81 ^b ±1.81	0.97 ^{bc} ±0.01	0.99 ^{bc} ±0.03
SWE	0.3%	10.77 ^{de} ±0.15	11.00 ^{cd} ±0.40	0.70 ^c ±0.04	0.72 ^b ±0.02	15.34 ^d ±0.89	15.28 ^c ±0.51	0.93 ^{cd} ±0.02	0.97 ^{cd} ±0.01
	0.4%	12.33 ^b ±1.10	12.33 ^b ±0.35	0.66 ^{cd} ±0.01	0.66 ^c ±0.01	18.62 ^{bc} ±2.08	18.78 ^b ±0.54	0.99 ^b ±0.05	1.04 ^b ±0.03
FA	0.1%	10.73 ^{de} ±0.42	10.90 ^{cd} ±0.85	0.76 ^b ±0.04	0.72 ^b ±0.03	14.14 ^d ±0.85	15.25 ^c ±1.66	0.87 ^{de} ±0.02	0.92 ^{de} ±0.02
	0.2%	11.80 ^{bcd} ±1.01	12.16 ^b ±0.68	0.67 ^{cd} ±0.03	0.67 ^c ±0.01	17.59 ^c ±0.83	18.16 ^b ±1.23	0.95 ^{bc} ±0.02	1.03 ^b ±0.03
Combination	1	12.87 ^b ±0.85	12.60 ^b ±0.36	0.64 ^d ±0.01	0.64 ^c ±0.01	20.03 ^{ab} ±1.78	19.69 ^b ±0.56	1.00 ^b ±0.04	1.05 ^b ±0.04
	2	14.00 ^a ±0.6	14.23 ^a ±1.20	0.63 ^d ±0.01	0.63 ^c ±0.02	22.10 ^a ±0.63	22.70 ^a ±1.52	1.08 ^a ±0.02	1.12 ^a ±0.03
LSD _{0.05}		1.06	1.11	0.05	0.04	2.11	1.94	0.05	0.06

TSS: Total soluble solids. The treatment means that have the same letter(s) in one column indicate that there were non-significant differences between them. The treatment means are followed by ±SE.

Table 6. The influence of the spraying of MLE, SWE and FA and their combinations on fruit total, reduced and non-reduced sugar percentages of apple cv. Anna during 2021 and 2022.

Treatments		Total Sugars (%)		Reducing Sugars (%)		Non-Reducing Sugars (%)	
		2021	2022	2021	2022	2021	2022
Control	0	7.38 ^g ±0.10	7.93 ±0.30	4.57 ^g ±0.08	5.11 ^h ±0.11	2.81 ^{bc} ±0.03	2.82 ^b ±0.42
MLE	4%	7.85 ^f ±0.08	8.08 ^e ±0.12	5.26 ^f ±0.06	5.39 ^g ±0.05	2.59 ^{cd} ±0.06	2.70 ^b ±0.12
	6%	8.59 ^e ±0.21	8.66 ^d ±0.26	5.60 ^e ±0.03	5.79 ^e ±0.14	2.99 ^b ±0.19	2.87 ^b ±0.15
SWE	0.3%	8.07 ^f ±0.19	8.22 ^e ±0.17	5.54 ^e ±0.06	5.68 ^{ef} ±0.06	2.54 ^{cd} ±0.16	2.54 ^b ±0.11
	0.4%	9.96 ^c ±0.20	10.19 ^{bc} ±0.06	6.27 ^c ±0.02	6.34 ^c ±0.01	3.70 ^a ±0.18	3.84 ^a ±0.07
FA	0.1%	7.90 ^f ±0.02	8.02 ^e ±0.08	5.42 ^{ef} ±0.03	5.50 ^{fg} ±0.07	2.48 ^d ±0.02	2.52 ^b ±0.05
	0.2%	9.67 ^d ±0.08	9.94 ^c ±0.06	5.87 ^d ±0.26	6.01 ^d ±0.18	3.80 ^a ±0.18	3.93 ^a ±0.21
Combination	1	10.23 ^b ±0.23	10.37 ^b ±0.04	6.54 ^b ±0.2	6.66 ^b ±0.21	3.69 ^a ±0.21	3.71 ^a ±0.18
	2	10.61 ^a ±0.26	10.67 ^a ±0.19	6.79 ^a ±0.03	6.90 ^a ±0.02	3.82 ^a ±0.23	3.77 ^a ±0.17
LSD _{0.05}		0.26	0.29	0.20	0.20	0.27	0.34

The treatment means that have the same letter(s) in one column indicate that there were non-significant differences between them. The treatment means are followed by ±SE.

3.4. Leaf Mineral Composition

The results presented in Table 7 demonstrate that spraying MLE, SWE and FA markedly improved the leaf N, P, K and Ca contents as compared to the untreated trees during both years (2021 and 2022). The greatest increments in the contents of these nutrients were due to the application of combination 2, which was the best treatment when compared with the other treatments during both years, 2021 and 2022. Further, the treatments of 6% MLE, 0.4% SWE and 0.2% FA and combination 1 also gave a remarkable increase in leaf N, P, K and Ca contents with respect to the untreated trees.

Table 7. The influence of the spraying of MLE, SWE and FA and their combinations on leaf N, P, K and Ca contents of apple cv. Anna during 2021 and 2022.

Treatments		N (%)		P (%)		K (%)		Ca (%)	
		2021	2022	2021	2022	2021	2022	2021	2022
Control	0	2.02 ^d	2.04 ^d	0.34 ^d	0.36 ^d	2.05 ^d	2.04 ^f	1.38 ^d	1.40 ^d
		±0.01	±0.04	±0.02	±0.02	±0.01	±0.02	±0.02	±0.03
MLE	4%	2.02 ^d	2.07 ^d	0.36 ^{cd}	0.39 ^{cd}	2.10 ^{cd}	2.06 ^{ef}	1.39 ^d	1.43 ^{cd}
	6%	2.08 ^b	2.15 ^{bc}	0.40 ^{bc}	0.42 ^{bc}	2.16 ^b	2.17 ^d	1.44 ^c	1.48 ^{bc}
SWE	0.3%	2.03 ^{cd}	2.08 ^{cd}	0.37 ^{cd}	0.38 ^{cd}	2.10 ^c	2.10 ^e	1.42 ^{cd}	1.48 ^{bc}
	0.4%	2.07 ^{bc}	2.14 ^{bc}	0.40 ^b	0.44 ^b	2.16 ^b	2.25 ^{bc}	1.50 ^b	1.51 ^b
FA	0.1%	2.04 ^{bcd}	2.09 ^{bcd}	0.35 ^d	0.39 ^{cd}	2.10 ^c	2.09 ^{ef}	1.40 ^d	1.43 ^{cd}
	0.2%	2.07 ^{bc}	2.14 ^{bc}	0.41 ^b	0.43 ^b	2.18 ^b	2.20 ^{cd}	1.45 ^c	1.48 ^{bc}
Combination	1	2.09 ^b	2.15 ^b	0.41 ^b	0.45 ^b	2.18 ^b	2.29 ^b	1.51 ^b	1.51 ^b
	2	2.15 ^a	2.21 ^a	0.45 ^a	0.49 ^a	2.26 ^a	2.38 ^a	1.55 ^a	1.57 ^a
LSD _{0.05}		0.04	0.06	0.03	0.03	0.04	0.05	0.04	0.05

The treatment means that have the same letter(s) in one column indicate that there were non-significant differences between them. The treatment means are followed by ±SE.

Regarding apple leaf micro-nutrient concentrations, it is noticed from the results listed in Table 8 that spraying apple trees with combination 2 markedly raised leaf Fe, Zn, Mn and B concentrations during both years, 2021 and 2022. Besides, the effect of 6% MLE, 0.4% SWE, 0.2% FA and combination 1 on increasing the leaf micro-nutrient concentrations was higher than that of 4% MLE, 0.3% SWE, 0.1% FA and the control, which registered lower values for micro-nutrient concentrations during both study years (2021 and 2022).

Table 8. The influence of the spraying of MLE, SWE and FA and their combinations on leaf Fe, Zn, Mn and B concentrations of apple cv. Anna during 2021 and 2022.

Treatments		Fe (mg L ⁻¹)		Zn (mg L ⁻¹)		Mn (mg L ⁻¹)		B (mg L ⁻¹)	
		2021	2022	2021	2022	2021	2022	2021	2022
Control	0	124.00 ^e	129.00 ^d	29.67 ^f	30.33 ^f	40.33 ^f	40.33 ^e	72.67 ^d	75.33 ^e
		±3.60	±3.60	±1.53	±2.52	2.52	±1.53	±2.52	±2.08
MLE	4%	127.00 ^{de}	132.33 ^{cd}	33.00 ^{ef}	36.33 ^e	43.33 ^{ef}	44.33 ^{de}	76.00 ^{cd}	76.67 ^e
	6%	132.67 ^{bc}	137.67 ^b	38.00 ^{cd}	40.67 ^{cd}	50.67 ^{bc}	51.33 ^{bc}	78.67 ^c	80.67 ^d
LSD _{0.05}		±2.52	±2.52	±2.00	±115	±2.08	±1.53	±4.04	±3.05

Table 8. Cont.

Treatments		Fe (mg L ⁻¹)		Zn (mg L ⁻¹)		Mn (mg L ⁻¹)		B (mg L ⁻¹)	
		2021	2022	2021	2022	2021	2022	2021	2022
SWE	0.3%	130.67 ^{bcd} ±2.30	136.00 ^{bc} ±1.00	35.00 ^{de} ±3.00	39.67 ^d ±2.51	47.33 ^{cd} ±2.52	49.00 ^{bc} ±2.65	77.00 ^{cd} ±3.00	77.67 ^{de} ±2.00
	0.4%	133.67 ^b ±3.51	138.33 ^b ±1.53	41.67 ^b ±1.53	43.00 ^{bc} ±2.00	52.33 ^b ±2.52	52.67 ^b ±2.52	88.00 ^b ±2.00	86.33 ^c ±1.53
FA	0.1%	128.33 ^{cde} ±1.53	135.33 ^{bc} ±2.52	31.67 ^{ef} ±1.53	34.00 ^e ±1.00	46.00 ^{de} ±1.00	48.00 ^{cd} ±2.00	74.67 ^{cd} ±1.53	76.00 ^e ±1.00
	0.2%	133.33 ^{bc} ±3.05	138.33 ^b ±1.53	39.00 ^{bc} ±2.00	43.67 ^{bc} ±1.53	53.00 ^b ±2.00	52.00 ^{bc} ±2.64	85.00 ^b ±2.00	86.00 ^c ±2.00
Combination	1	135.00 ^b ±2.64	139.67 ^b ±2.52	41.67 ^b ±1.53	44.00 ^b ±1.00	52.33 ^b ±2.08	52.67 ^b ±3.05	88.00 ^b ±3.60	90.33 ^b ±3.51
	2	140.00 ^a ±2.00	145.00 ^a ±2.00	45.67 ^a ±2.08	48.00 ^a ±2.00	57.00 ^a ±1.00	58.00 ^a ±2.00	93.33 ^a ±2.89	94.67 ^a ±2.51
LSD _{0.05}		4.65	4.10	3.28	3.05	3.44	4.02	4.78	3.75

The treatment means that have the same letter(s) in one column indicate that there were non-significant differences between them. The treatment means are followed by ±SE.

4. Discussion

The foliar application of MLE, SWE, FA and their combinations positively affected the vegetative growth, fruit set, yield, fruit physical and chemical attributes as well as the leaf mineral status of the apple cv. Anna during a two year study.

The findings of Nagar et al. [55] previously explained the reasons; they documented that moringa leaf extract is characterized by a high content of sugars, vitamins A, B1, B2, B3, C and E, amino acids and phenolic compounds as well as a high content of macro- and micro-nutrients such as calcium, magnesium, sodium, iron, phosphorus and potassium [56]. Besides, MLE also contains cytokinin (zeatin), auxins, gibberellins, ascorbate and other minerals such as S, Zn, Cu, Fe and Se [57–59]; therefore, it has a great impact on the growth and productivity of crops and could be used as an alternative to chemical fertilizers [7]. Anwar et al. [60] reported that MLE application enhanced the photosynthesis process, and improved carbon-nitrogen metabolism. Further, the existence of phytohormones in MLE has an important role in increasing the division of cells, and consequently their elongation, and thus increases plants' growth and yield [61]. Moringa leaf extract helps plants to absorb mineral elements, improve fruit quality and survive under adverse weather conditions [62]. As MLE contains adequate amounts of mineral elements, proteins, vitamins A and C, amino acids, sugars, phenolics, free prolines and plant hormones, it can contribute to increasing the growth and development of many economically important crops, improve fruit post-harvest shelf life and reduce the detrimental effects of environmental stresses [62–65]. Spraying MLE on grape cv. Flame Seedless markedly improved shoot length and thickness, chlorophyll content, fruit yield, cluster weight and number, size, the length and diameter of clusters, juice content, soluble solids, sugars and anthocyanins pigment as well as the leaf mineral content of N, P and K as compared to untreated trees [66].

Seaweed extract is rich in cytokinins; thus, it plays an important role in delaying leaf senescence by minimizing the degeneration of chlorophyll, and it can also regulate the relation between the rates of photosynthesis and respiration in plants [67,68]. As SWE contains high amounts of vitamins, amino acids, antioxidants, PGRs (cytokinins, IAA, IBA and GA₃), macro-nutrients (N, P, K, Mg, Ca and S), as well as micro-nutrients (Cu, Fe, Mn, B, Co, Ni and Mo), it is considered a plant growth stimulant and has a notable role in increasing plant cell division [69–74]. Zhang and Ervin [75] stated that the foliar application of SWE plays a crucial role in enhancing cell membrane permeability and improving plant efficacy in up-taking mineral elements such as nitrogen and phosphorous that are directly linked to chlorophyll formation in plant leaves. The foliar application

of SWE at 0.2 and 0.4% on peach trees resulted in a significantly greater total leaf area and higher chlorophyll content over the control [76]. The foliar spray of SWE was more efficacious in increasing vegetative growth, yield, fruit chemical and physical characteristics and leaf nutritional status than untreated trees in grape cultivars [77–80]. Seaweed extract contains numerous nutrients and has a phytostimulatory influence on improving plant growth, yield and yield components in numerous important crop plants via improving plant survival against abiotic constraints such as drought, salinity and cold [81]. In recent years, it was revealed that SWE contains bio-motivational substances such as carbohydrates, amino acids, phytohormones, osmoprotectants and proteins [12,82]. Moreover, SWE can break seed dormancy, enhance stress tolerance, improve nutrient uptake and increase growth, yield, root system development, flowering [38], fruit quality and taste [31] and consequently the crop productivity. Further, the salicylic acid (SA) content significantly increases in the roots, both under drought and salt stress conditions [83–85].

Fulvic acid can enhance the availability, absorption and translocation of the mineral elements in plants, improve soil properties [86–88], the photosynthesis process and reduce the transpiration rate by regulating stomata opening [89,90], so it can positively influence root development [91] and consequently plant growth and development [92]. Abd El-Hameed et al. [93] stated that FA has an important role in inducing vegetative growth by enhancing the availability of hormones such as IAA, GA₃ and cytokinins and vitamins (such as vitamin B). The influence of FA is similar to the effect of an auxin in plants that aids the plants to uptake potassium and consequently the starch metabolism [94]. Further, it has been noticed that FA has the ability to catch water molecules and makes calcium, magnesium, iron, copper and zinc movement to the plant roots easier and increases plant productivity [95]. Additionally, Wang et al. [87] stated that FA can significantly facilitate nutrient translocation from roots to shoots, particularly iron, zinc and manganese, which are engaged in the photosynthesis process. Fulvic acid helps in transferring the nutrients through the cell membrane into the plant cells, so it is considered ideal for foliar spraying where it is necessary that the nutrients such as Cu, Fe, Mn and Zn should be absorbed well through the plant leaves [96]. In a study, spraying red delicious (*Malus domestica* Borukh.) with FA at 1.5, 2.5 and 3.5% increased fruit yield [97].

5. Conclusions

The obtained results indicate that the foliar application of MLE, SWE and FA individually or in combinations improved the vegetative growth, productivity, fruit quality and leaf mineral composition of apple cv. Anna as compared with untreated trees; therefore, these are ecofriendly potential biostimulants and safe alternatives to chemical fertilizers for apple orchards. Moreover, the best results were obtained when these were applied in combination at 6% MLE + 0.4% SWE + 0.2% FA, indicating that this combination with higher doses was more effective in improving the measured parameters than the other combination with lower doses (4% MLE + 0.3% SWE + 0.1% FA).

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