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Impact of soil application with humic acid and foliar spray of milagro bio-stimulant on vegetative growth and mineral nutrient uptake of Nonpareil almond young trees under Nubaria conditions

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Abstract

Background: At present, agricultural production management techniques focus on greater commitment to environmental sustainability. As such, this study was carried out during two successive seasons (2018 and 2019) to investigate the impact of two natural bio-stimulant substances: humic acid (H) and milagro (M) on vegetative growth and nutritional status of Nonpareil almond young trees grown in Experimental Research Station of National Research Centre, Nubaria, El Behera governorate, Egypt. Three-year-old uniform trees were treated at the beginning of growth season by soil application of humic acid (H) and foliar spray of milagro (M) bio-stimulant. There were ten treatments as follows: control (untreated seedlings),10 g humic acid plus 10 ml/l milagro, 20 g humic acid plus 10 ml/l milagro, 30 g humic acid plus 10 ml/l milagro,10 g humic acid plus 20 ml/l milagro, 20 g humic acid plus 20 ml/l milagro, 30 g humic acid plus 20 ml/l milagro, 10 g humic acid plus 30 ml/l milagro, 20 g humic acid plus 30 ml/l milagro, and 30 g humic acid plus 30 ml/l milagro.

Results: The results showed that different treatments improved the vegetative growth of seedlings, stem length, diameter, number of branches and leaves, leaf area, leaf fresh and dry weight, and specific leaf dry weight as well as leaf chlorophylls and minerals content comparing with untreated young trees.

Conclusions: Soil application of 30 g humic acid along with 30 ml/l milagro per young tree as foliar spray was the promising treatment as a new fertilization technique that is non-chemical, low-cost, and environmentally safe for improving growth and nutritional status of Nonpareil almond young trees under Nubaria conditions.

Keywords: Nonpareil almond, Humic acid, Milagro, Bio-stimulants, Growth, Soil application, Foliar spray

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Background

Almond (*Prunus amygdalus* B. cv. Nonpareil) is a small deciduous tree belonging to the subfamily *Prunoideae* of the family *Rosaceae*. Almonds are one of the oldest commercial nut crops of the world; from the Middle and West Asia, it has diffused to other regions and continents which include the Middle East, China, the Mediterranean region, and America (Ladizinsky, 1999). Almond kernels are concentrated sources of energy with a significant share of fat, protein, and fiber. Fats are primarily nonsaturated, mostly oleinic and linoleic fatty acids. Nonsaturated fatty acid is important in maintaining low cholesterol levels in the blood and significant amount of micronutrients (Aslanta et al. 2001). In Egypt, commercial almond production is still limited in spite of suitable environmental conditions for growth and fruiting (Abou Rayya et al. 2009; Kasim et al. 2009).

Humic acid is one of bio-stimulants that are known as the organic substances which promote plant growth and help the trees to withstand harsh environments when applied in small amounts (Chen et al. 1994). It is highly beneficial also for both the trees and the soil, since it maintains proper plant growth as well as it increases nutrient uptake, tolerance to drought and temperature extremes, activity of beneficial soil microorganisms, and availability of soil nutrients particularly in alkaline soils and low organic matter such as newly reclaimed land conditions without excessive use of agricultural chemicals which are considered a menace to the environment (Russo and Berlyn, 1990; Eisa et al., 2016; Abd El-Razek et al. 2018). Uses of humic acid as a soil application improve nutrient availability especially microelements in sandy soils because it promotes nutrient uptake in the form of chelating agent. Moreover, humic substances may increase root growth in a similar manner to auxins (O'Donnell, 1973; Khattab et al. 2012). In addition, the humic acid has many effects as it raises of cation exchange capacity which affects the retention and availability of nutrients, as well as due to a hormonal effect, or a combination of both (Chunhua et al. 1998); as a result, it can be used to solve many problems in soils such as soil nutrient availability and chemical reactions that affect the loss or fixation of almost all nutrients. Generally, there is a growing interest of the use of humic acid and K-humate as a substitute to chemical fertilizers which have potential polluting effects in the environment (Senn and Kingman, 2000).

Milagro is a natural bio-stimulant that extracted from pollen flowers of cabbage (Grove et al. 1979), which is rapid and complete water solubility in water. It has a broad effect on different crops and its impact depends on the time of trans-action. This product is reflecting the effect of auxins, cytokinins, gibberellins, ethylene, and hydrogen sinamed and humic and contains 20% phosphorus, 10% potassium, and 3% boron. It improves plant growth in all parts as a tonic for physiological processes in particular, enhances photosynthesis, increases the yield by 20–25%, and improves quality characteristics (Ebeed et al. 2008; Ramezani and Shekafandeh, 2009; Omaima et al. 2014).

Therefore, the aim of this work was to study the effects of the soil application with humic acid and the foliar spray of milagro on the vegetative growth and mineral nutrient uptake of Nonpareil almond young trees under Nubaria conditions.

Materials and methods

Plant materials and treatments

The present study was conducted during two successive seasons of 2018 and 2019 at the Experimental Research Station of National Research Centre, Nubaria, El Behera governorate, Egypt, on three-year-old Nonpareil almond young trees (*Prunus amygdalus* B.) budded on bitter almond rootstock and planting space 5 × 5 m and grown in sandy soil under drip irrigation system. The soil physical and chemical properties are shown in Table 1. Trees were arranged in randomized complete block design (RCBD), and the following treatments were done with three replicates for each treatment (1 replicate = 3 trees). The experimental treatments were as follow:

1. Control (untreated young trees)
2. 10 g/young trees humic acid + 10 ml/l milagro©
3. 20 g/young trees humic acid + 10 ml/l milagro©
4. 30 g/young trees humic acid + 10 ml/l milagro©
5. 10 g/young trees humic acid + 20 ml/l milagro©
6. 20 g/young trees humic acid + 20 ml/l milagro©
7. 30 g/young trees humic acid + 20 ml/l milagro©
8. 10 g/young trees humic acid + 30 ml/l milagro©
9. 20 g/young trees humic acid + 30 ml/l milagro©
10. 30 g/young trees humic acid + 30 ml/l milagro©

All treatments were applied at the beginning of growth season. Humic acid was added to the soil, and milagro

Table 1 Physical and chemical properties of the experimental soil

Sand (%)	Silt (%)	Clay (%)	Texture	OM (%)	EC dSm^{1}	pH	HCO^{-3}	CO^{-3}
81.71	9.01	7.89	Sandy	2.84	0.54	7.88	1.7	–
Cl^-	**SO_4^{-2}**	**Ca^+**	**Mg^{+2}**	**Na^+**	**K^+**	**N (%)**	**P (%)**	**K (%)**
3.2	0.41	2.3	2.25	0.3	0.45	0.99	0.42	0.54

was a foliar application with Tween-20 (0.1%) as surfactant and applied directly to trees by a handheld sprayer until it runoff in the early morning. Other horticultural practices were carried out as usual. The following parameters were recorded for both seasons.

Vegetative growth measurements

At mid-August of the two seasons, stem length of young trees (cm) and diameter of young trees (mm), numbers of branches/young trees, numbers of leaves/young trees, leaf fresh weight (g), and leaf dry weight (g) were determined. Leaf area (cm^2) was measured by using the Cl-202 portable laser leaf area meter. Specific leaf dry weight (SLDW) (mg/cm^2) was determined by the following equation (Yehia, 1994):

$$Specific\ leaf\ dry\,weight = \frac{Specific\ leaf\ dryweight(g)}{leaf\ area(cm^2)} \times 1000$$

Leaf chlorophylls content

Leave samples were collected at mid-July of each season from the middle portion of the current season growth to each replicate tree to determine chlorophyll-a, chlorophyll-b, and total chlorophyll spectrophotometrically at 645 nm and 663 nm wavelengths, respectively, using the method described by (Arnon, 1949).

Leaf minerals content

Leaf minerals content were determined in leaf samples that were picked in mid-July of each season from the middle portion of the current season growth to each replicate a tree washed and dried at 70 °C till a constant weight for determination of the following nutrient elements: N, P, K, Mg, Zn, Fe, Mn, and Cu (AOAC, 1985).

Statistical analysis

The design of the experiment was completely randomized block design with three replicates each consisted of five young trees. All data were subjected to analysis of variance (ANOVA) as described by (Mstat-C, 1989), and the least significant differences (L.S.D) at 5% were used to compare between treatment means.

Results

Vegetative growth

Results in Table 2 showed that the combination between soil application with humic acid and foliar spray of milagro at the different concentrations significantly increased vegetative growth measurements: stem length, diameter, and number of branches and leaves of Nonpareil almond young trees in both seasons of study than the control. In this respect, the treatment with 30 g humic acid plus 30 ml/l milagro gave the highest values of stem length (110.30 and 112.6 cm), diameter (13.00 and 4.18 mm), number of branches (42.00 and 44.55/young trees), and number of leaves (672.00 and 675.20/young trees) in the first and second season, respectively, while the lowest values of stem length (65.33 and 67.56 cm), diameter (6.50 and 8.09 mm), number of branches (4.66 and 6.58/young trees), and number of leaves (44.00 and 47.75/young trees) were recorded in the control during the both seasons, respectively.

Means within a column followed by different letter(s) are statistically different at 5% level

Concerning the leaf area, the results in Table 3 indicated that humic acid soil application and spraying milagro were not significantly influence on the leaf area in both seasons of the study. The values of different treatments ranged between (2.66 and 3.26 cm^2) in the first season and ranged between (2.09 and 3.53 cm^2) in the second season compare with the control which recorded (3.11 and 3.45 cm^2) in the first and second seasons, respectively.

Regarding to the leaf fresh weight, it was increased significantly by treatments. Humic acid 30 g plus milagro 30 ml/l gave the highest values of leaf fresh weight (5.40

Table 2 Effect of soil application with humic acid and foliar spray of milagro on stem length, diameter, number of branches and leaves/young trees

Treatments	Stem length (cm)		Stem diameter (mm)		No. of branches/young trees		No. of leaves/young trees	
	2018	2019	2018	2019	2018	2019	2018	2019
Control	65.33e	67.56j	6.50f	8.09d	4.66j	6.58i	44.00j	47.75j
10 g H + 10 ml/l M	73.00d	75.56 g	7.00ef	8.56 cd	8.00 h	10.22gh	56.00i	59.14i
20 g H + 10 ml/l M	78.33c	80.56f	8.00de	9.85bcd	7.00i	9.56 h	63.00 h	66.26 h
30 g H + 10 ml/l M	82.00c	82.56e	8.50d	10.06bcd	9.00 g	11.36 g	77.00 g	80.86 g
10 g H + 20 ml/l M	91.00b	89.00d	10.50a	12.12ab	11.00f	13.58f	105.00f	108.30f
20 g H + 20 ml/l M	70.00d	70.56i	9.00 cd	10.48bcd	15.00e	17.45e	117.00e	120.20e
30 g H + 20 ml/l M	72.33d	74.56 h	9.00 cd	10.15bc	22.00d	24.23d	160.00d	163.80d
10 g H + 30 ml/l M	91.00b	93.56c	10.00bc	11.28bc	34.00c	36.27c	443.00c	442.40c
20 g H + 30 ml/l M	107.30a	109.60b	11.00b	12.37ab	40.00b	42.12b	612.00b	615.30b
30 g H + 30 ml/l M	110.30a	112.6a	13.00a	14.18a	42.00a	44.55a	672.00a	675.20a

Table 3 Effect of soil application with humic acid and foliar spray of milagro on leaf area, leaf fresh weight, leaf dry weight, and specific leaf dry weight

Treatments	leaf area (cm^2)		leaf fresh weight (g)		leaf dry weight (g)		Specific leaf dry weight (mg/cm^2)	
	2018	2019	2018	2019	2018	2019	2018	2019
Control	3.11a	3.45a	2.10c	2.68d	1.80 cd	1.88c	578.8i	544.93i
10 g H + 10 ml/l M	2.66a	2.93a	2.10c	2.68d	1.80 cd	1.83c	676.7 h	624.57 h
20 g H + 10 ml/l M	2.82a	2.09a	2.40c	2.98 cd	1.50d	1.33d	531.9j	636.36 g
30 g H + 10 ml/l M	2.93a	3.20a	3.20bc	3.78c	2.30bcd	3.13ab	785.0f	978.13a
10 g H + 20 ml/l M	3.01a	3.28a	3.20bc	3.78c	2.30bcd	3.13ab	764.1 g	954.27b
20 g H + 20 ml/l M	2.57a	2.84a	3.40bc	3.98b	2.51bc	2.33c	972.8c	820.42e
30 g H + 20 ml/l M	2.91a	3.18a	4.00ab	3.58d	2.80ab	2.63b	962.2d	827.04e
10 g H + 30 ml/l M	2.78a	3.05a	4.10ab	3.68d	2.40bcd	2.23c	863.3e	731.15f
20 g H + 30 ml/l M	3.26a	3.53a	5.10a	3.68d	3.20ab	3.03b	981.6b	858.36d
30 g H + 30 ml/l M	3.11a	3.38a	5.40a	4.98a	3.60a	3.16a	1158.0a	934.91c

and 4.98 g) in the first and second season, respectively, while the lowest values (2.10 and 2.68 g) were recorded in the control during the both seasons, respectively (Table 3).

Table 3 clears that leaf dry weight was significantly increased by treatment with humic acid and milagro. The highest significant leaf dry weight (3.60 and 3.16 g) obtained in young trees is treated with humic acid 30 g plus milagro 30 ml/l in both seasons, respectively, while the lowest significant leaf dry weight (1.50 and 1.33 g) obtained in young trees is treated with humic acid 20 g plus milagro 10 ml/l during seasons 2018 and 2019, respectively.

Specific leaf dry weight was significantly affected by soil application with humic acid and foliar spray of milagro (Table 3). In the first season, the highest value of specific leaf dry weight (1158 mg/cm2) was obtained by humic acid 30 g plus milagro 30 ml/l, while the lowest value (531.9 mg/cm^2) was obtained by humic acid 20 g plus milagro 10 ml/l. In the second season, the highest value of

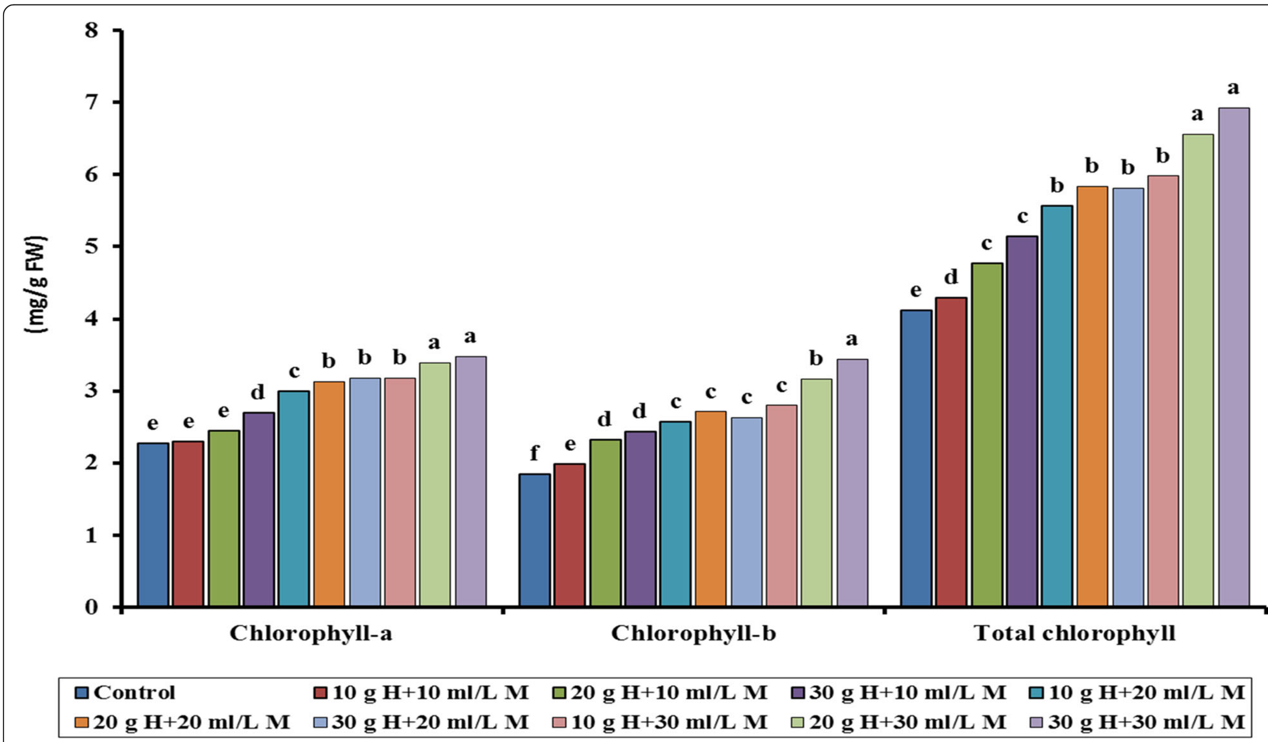

Fig. 1 Effect of soil applications with humic acid and foliar spray of milagro on chlorophyll-a, chlorophyll-b, and total chlorophyll during the first season

specific leaf dry weight (978.13 mg/cm^2) was obtained by humic acid 20 g plus milagro 10 ml/l, while the lowest value (544.93 mg/cm^2) was recorded in the control.

Means within a column followed by different letter(s) are statistically different at 5% level

Leaf chlorophylls content

Figures 1 and 2 present the effect of soil application with humic acid and foliar spray of milagro at the different concentrations on leaf chlorophylls content of Nonpareil almond young trees during the two seasons. All treatments improved leaf content of chlorophyll-a, chlorophyll-b, and total chlorophyll (a + b) compared with control which recorded the lowest values (2.27, 1.85, and 4.12 mg/g FW) in the first season and (2.30, 1.84, and 4.14 mg/g FW) in the second season, respectively.

Results of the 2 years pointed out that there was a clear variation among treatments with the same trend for improving the chlorophyll-a, chlorophyll-b, and total chlorophyll (a + b), where the young trees treated with 30 g humic acid plus 30 ml/l milagro recorded the highest values (3.48, 3.44, and 6.92 mg/g FW) and (3.60, 3.45, and 7.05 mg/g FW) in both seasons, respectively, followed by treatment with humic acid 20 g plus milagro 30 ml/l which gave significant similar results and came in the second arrangement for its impact on improving chlorophyll-a, chlorophyll-b, and total chlorophyll ranged from (3.39, 3.17, and 6.56 mg/g FW) in the 1st season and (3.55, 3.18, and 6.73 mg/g FW) in the 2nd season, respectively.

Leaf minerals content

As for the effect of soil application with humic acid and foliar spray of milagro on some leaf macro element contents, the results in Table 4 presented that the treatments influenced significantly on N, P, K, and Mg leaf contents of Nonpareil almond young trees than the control in both seasons. Regarding to leaf N and K contents, the results showed that the soil applications with humic acid and foliar spray of milagro at the different concentrations gave higher content than the control. The highest values of leaf N and K contents (2.40 and 2.79 and 3.18 and 3.36%) were recorded by 30 g humic acid plus 30 ml/l milagro in 1st and 2nd seasons, respectively, while the lowest value of leaf N content (1.05 and 1.14 and 2.30 and 1.70%) was recorded in the control in 1st and 2nd seasons, respectively.

Concerning leaf P content, the treatment with 30 g humic acid plus 30 ml/l milagro recorded the highest significant value of leaf P content (1.89 and 1.88%) in

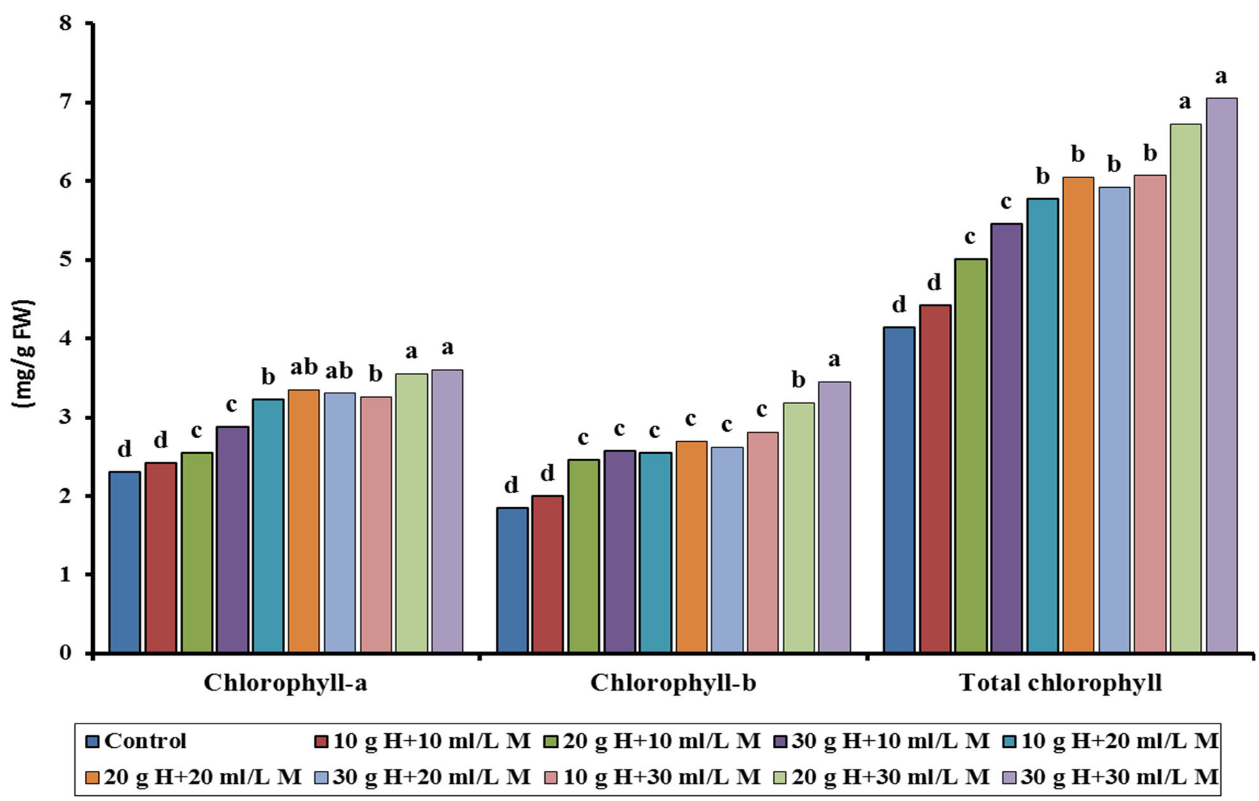

Fig. 2 Effect of soil applications with humic acid and foliar spray of milagro on chlorophyll-a, chlorophyll-b, and total chlorophyll during the second season

Table 4 Effect of soil application with humic acid and foliar spray of milagro on some leaf macro element contents

Treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)		Magnesium (%)	
	2018	2019	2018	2019	2018	2019	2018	2019
Control	1.05d	1.14d	1.47bc	1.46b	2.30 cd	1.70b	0.28c	0.36a
10 g H + 10 ml/l M	1.93ab	2.32ab	1.53bc	1.52ab	2.65abc	2.83ab	0.48a	0.56a
20 g H + 10 ml/l M	1.73bc	2.12abc	1.70ab	1.69ab	2.85de	2.03ab	0.30bc	0.58a
30 g H + 10 ml/l M	1.63bc	2.02abc	1.89a	1.88a	2.73abc	2.91ab	0.46a	0.54a
10 g H + 20 ml/l M	1.10d	1.44 cd	1.60abc	1.56ab	2.44bc	2.61ab	0.42ab	0.50a
20 g H + 20 ml/l M	1.28 cd	1.67bcd	1.60abc	1.59ab	2.53ef	1.71b	0.50a	0.58a
30 g H + 20 ml/l M	1.12d	1.49 cd	1.60abc	1.59ab	2.50f	2.61ab	0.30bc	0.48a
10 g H + 30 ml/l M	1.06d	1.47 cd	1.73ab	1.72ab	2.43bc	2.48ab	0.50a	0.53a
20 g H + 30 ml/l M	1.28 cd	1.67bcd	1.78ab	1.77ab	2.95ab	3.13ab	0.45a	0.53a
30 g H + 30 ml/l M	2.40a	2.79a	1.61abc	1.60ab	3.18a	3.36a	0.52a	0.58a

both seasons, respectively, while the other treatments lacked significance between them in both seasons (Table 4). Meanwhile, the control gave the lowest significant value of leaf P content (1.47 and 1.46%) in both seasons, respectively.

Regarding leaf Mg content, Table 4 explained that it was increased by all treatments than the control which was the lowest content during the two seasons of this study. Also, the analysis of variance at the 5% level showed that the leaf Mg content was slightly higher significant in the first season, while the leaf Mg content was not significantly differ between the different treatments in the second season.

Table 5 presented the effect of soil application with humic acid and foliar spray of milagro on some leaf micro elements of Nonpareil almond young trees during two seasons. In this respect, the treatments influenced significantly on Zn, Fe, Mn, and Cu leaf contents than the control in both seasons. Regarding to the leaf Zn content, the results showed that the treatment with humic acid 20 g plus milagro 20 ml/l gave the highest values (47.67 and 49.21 ppm) in 1st season and 2nd season, respectively.

Concerning to leaf Fe, Mn, and Cu, contents, the treatment with humic acid 30 g plus milagro 30 ml/l gave the highest significant values (129.90 and 131.85 ppm), (80.04 and 81.34 ppm), and (48.38 and 46.84 ppm) in 1st season and 2nd season, respectively.

Meanwhile, the control recorded the lowest significant values of leaf Zn, Fe, Mn, and Cu contents in the both seasons.

Means within a column followed by different letter(s) are statistically different at 5% level

Means within a column followed by different letter(s) are statistically different at 5% level

Discussions

The obtained results cleared that the beneficial effect of combination between soil application with humic acid and foliar spray of milagro at the different concentrations on increasing the uptake of different nutrients and availability of soil nutrients particularly in sandy soil under Nubaria conditions, especially when applied humic acid soil application and spray milagro at high rate 30 g plus 30 ml/l. This explains the improving nutrient status in Tables 4

Table 5 Effect of soil application with humic acid and foliar spray of milagro on some leaf micro element contents

Treatments	Zinc (ppm)		Iron (ppm)		Manganese (ppm)		Copper (ppm)	
	2018	2019	2018	2019	2018	2019	2018	2019
Control	20.00 g	20.00e	100.00f	100.00e	20.00 g	21.00i	20.00f	31.63c
10 g H + 10 ml/l M	28.97e	30.51d	128.08ab	128.86ab	50.46bc	51.76c	35.48de	32.53c
20 g H + 10 ml/l M	20.47 fg	22.01e	105.98e	107.93d	22.62f	23.92f	35.48de	33.20c
30 g H + 10 ml/l M	32.74d	34.28 cd	114.95d	116.90c	43.50 cd	44.80e	45.80ab	35.85bc
10 g H + 20 ml/l M	44.07b	45.61b	120.93c	122.88bc	34.80de	36.10f	40.64bcd	38.69b
20 g H + 20 ml/l M	47.67a	49.21a	123.92bc	125.87ab	59.16b	60.46b	43.22abc	36.43bc
30 g H + 20 ml/l M	36.05c	37.59c	101.00f	101.00e	60.9b	62.20b	38.06cde	30.00d
10 g H + 30 ml/l M	34.16d	35.70 cd	123.92bc	125.87ab	46.98c	48.28d	32.90e	32.40c
20 g H + 30 ml/l M	31.88f	23.42e	114.95d	116.90c	31.32ef	32.62 g	40.64bcd	36.80bc
30 g H + 30 ml/l M	34.10d	36.64 cd	129.90a	131.85a	80.04a	81.34a	48.38a	46.84a

and 5 and leaf chlorophylls content in Figs. 1 and 2 of Nonpareil almond young trees s that reflected on increasing the vegetative growth (Tables 2 and 3).

Increases in vegetative growth can be attributed to the positive effect of humic acid on both plants and soil in increasing microbial activity and enhance soil effectiveness in nutrient uptake as chelating agent and biostimulation of plant growth which improves vegetative characteristics, nutritional status, and leaf pigments. These results are in harmony with those obtained by (Mustafa and El-Shazly, 2013; Fatma et al. 2015; Eisa et al. 2016) who reported that application of humic acid resulted in increment of plant height, lateral shoot number per plant, leaves number per plant, stem diameter, leaf area, dry weight, and total leaf chlorophyll content comparing with the control.

The simulative effect of humic acid on nutrients concentrations might be explained that humic acid enhanced cell permeability, which in turn made more rapid entry of minerals into root cells and so resulted in higher uptake of plant nutrients and promoted the accumulation (N, P, K, and Mg) in leaves than the untreated ones. On the other hand, the least growth in the control was probably due to nutrition status deficiency which could probably reduce number of functional leaves and subsequently the photosynthetic efficiency. This observation is in agreement with the findings of (Stefano et al. 2004) who reported that number of leaves per plant was dependent on fertilizer rate as it increased with increasing fertilizer rate. These results are also in harmony with (Fathy et al. 2010; El- Khawaga, 2011; Tahira et al. 2013; Abd El-Razek et al. 2018). Furthermore, the effects of humic acid on increasing Fe and Zn concentrations in the leaves might be due to their effect on the reduction of Fe^{3+} to Fe^{2+}, making iron chelates that are readily available to the plants and prevent the formation of insoluble complexes of Fe, Zn, and Mn and facilitated their uptake by plants. These results conformed to those obtained by (Gregor and Powerll, 1988; Chen and Aviad, 1990; Eisa et al. 2016).

Also, the increases can be referred in the vegetative growth and nutritional status resulting by foliar application of milagro to its content of many growth regulators. This compound combines the effects of auxins, cytokinins, gibberellic acid, and ethylene; when applied at the growth stage, it combines as the effect of cytokinins (cell division) and gibberellic acid (elongation cell) especially in the meristematic tissues. In addition, milagro provides all the nutrients needed by the plant in the stages of root growth which can give an explanation for improving on growth characteristics and nutritional status of Nonpareil almond young trees. These results are in agreement with those obtained by (Omaima et al. 2014; Abou El Magd et al. 2018).

Conclusion

It can be concluded that the foliar spray of natural biostimulant milagro at 30 ml/l combined with soil application of humic acid at 30 g/young trees as a new fertilization technique is non-chemical for improving growth and nutritional status of Nonpareil almond young trees under Nubaria conditions. Besides, its effect is environmentally safe and low-cost.

Abbreviations

H: Humic acid; M: Milagro; SLDW: Specific leaf dry weight

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Authors' contributions

This work was carried out in collaboration between all authors. NEK designed this study and wrote the protocol. ERA and OAA applied the field works, following up the growth of almond young trees, collected samples, and measured its physical measurements. AMR performed the chemical analysis of the samples and the statistical analysis. TSMM prepared the samples for analysis, managed the analyses of the study and the literature searches, and wrote the first draft of the manuscript. AMS consulted the study design, protocol, and treatments and reviewed the manuscript. All authors read and approved the final version.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are included in this published.

Ethics approval and consent to participate

Not applicable

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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