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 *Pru-noideae* 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 (Chun-hua 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⁻ | SO₄²⁻ | Ca⁺ | Mg²⁺ | 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 (cm²) was measured by using the CL-202 portable laser leaf area meter. Specific leaf dry weight (SLDW) (mg/cm²) was determined by the following equation (Yehia, 1994):

\[
\text{Specific leaf dry weight} = \frac{\text{Specific leaf dry weight (g)}}{\text{Leaf area (cm}^2\text{)}} \times 1000
\]

**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 cm²) in the first season and ranged between (2.09 and 3.53 cm²) in the second season compare with the control which recorded (3.11 and 3.45 cm²) 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

| Treatments | Stem length (cm) 2018 | Stem length (cm) 2019 | Stem diameter (mm) 2018 | Stem diameter (mm) 2019 | No. of branches/young trees 2018 | No. of branches/young trees 2019 | No. of leaves/young trees 2018 | No. of leaves/young trees 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               | 75.56g               | 7.00ef               | 8.56cd               | 8.00 h               | 10.22gh              | 56.00i               | 59.14h               |
| 20 g H + 10 ml/l M  | 78.33c               | 80.56f               | 8.00de               | 9.85bcd              | 7.00i                | 9.56 h               | 63.00 h              | 66.26h               |
| 30 g H + 10 ml/l M  | 82.00c               | 82.56e               | 8.50d                | 10.06bcd             | 9.00 g               | 11.36 g              | 77.00 g              | 80.86g               |
| 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               | 74.56h               | 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              |
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/cm²) was obtained by humic acid 20 g plus milagro 10 ml/l. In the second season, the highest value of

| Treatments          | Leaf area (cm²) | Leaf fresh weight (g) | Leaf dry weight (g) | Specific leaf dry weight (mg/cm²) |
|---------------------|----------------|-----------------------|---------------------|----------------------------------|
|                     | 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 | 4.10ab | 3.68d | 3.60a | 3.16a | 1158.0a | 934.91c |

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.

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/cm²) was obtained by humic acid 20 g plus milagro 10 ml/l, while the lowest value (544.93 mg/cm²) 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
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 different 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
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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 and 5.

| Treatments | Nitrogen (%) | Phosphorus (%) | Potassium (%) | Magnesium (%) |
|------------|-------------|----------------|---------------|---------------|
|            | 2018        | 2019           | 2018          | 2019          | 2018          | 2019          |
| Control    |             |                |               |               | 2.30 cd       | 1.70b         | 0.28c         | 0.36a         |
| 10 g H + 10 ml/l M | 1.05d       | 1.14d          | 1.47bc        | 1.46b         | 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        | 1.60bc        | 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         |

| 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      | 21.00f       | 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 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 compared 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-Khayaga, 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 bio-stimulant 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. AMS 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. 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.

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**Ethics approval and consent to participate**

Not applicable

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**Competing interests**

The authors declare that they have no competing interests.
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References
Abd El-Razek E, Haggag LF, MM, A-M-M, El-Hady Combined ES (2018) Effects of soil applications of humic acid and foliar spray of amino acids on yield and fruit quality of 'Florida Prince' peach trees under calcareous soil conditions. Bioscience Research 15:3270–3282
Abou El Magd MM, Zaki MF, Abo Sedera SA. Bio-fertilization and foliar application of milagro bio-stimulant in relation to growth, head yield and quality as well as mineral K requirements of Chinese. 2018.
Abou Rayya MSM, Kasim NE, Shaheen MA, Yehia TA, Ali EL (2009) Morphological and anatomical evaluation of different budding and grafting methods and times of Neplus ultra almond cultivar. Journal of Applied Sciences Research. 5:253–262
AOAC. Official methods of analysis. Association of Official Agricultural Chemists, 14th ed. Benjamin Farnikl station Washington, Dc, USA. 1985: 490-510
Amon DI (1949) Copper enzyme polyphenoloxidase in isolated chloroplast in Beta vulgaris. Plant Physiology. 24:1–15
Aslanta R, Guleryuz M, Tarun M. Some chemical contents of selected almond (Prunus amygdalus Batsch) types in 11 GREMPA Seminar on Pistachios and Almonds. B. E. Ak, Ed., vol. 56 of Cahiers Options Mediterraneennes. 2001: 347–350.
Chen Y, Avid T (1990) Effect of humic substances on plant growth. In: Humic substances in soil and crop sciences: American Society of Agronomy and Soil Science.161–186
Chen Y, Magen H, Rov J. Humic substances originating from rapidly decomposing organic matter. Proc Int Meet 1994: 427-443 (Chem Abst 121: 229).
Chunhua L, Cooper RJ, Bowman DC (1998) Humic acid application affects photosynthesis, root development and nutrient content of creeping bentgrass. Hort Sci 33:1029–1035
Ebeed S, Mustafa EAM, Saleh MMS (2008) Effect of gibberellic acid and male bud removal on yield and fruit quality of banana plants. Res J Agric Biol Sci. 4:289–292
Eisa RA, Thanaa SM, Nabila EK, Abou Rayya MS (2016) Foliar application of low biuret urea and humic acid influences on the growth and leaf mineral composition of Nonpareil almond young trees s under South Sinai conditions. Journal of Innovations in Pharmaceuticals and Biological Sciences. 3:143–153
Fathy MA, Gabr MA, El Shall SA (2010) Effect of humic acid treatments on 'Caino' apricot growth, yield and fruit quality. New York Sci J. 3:109–115
Fatma KM, Shaaban MWM, Mahmoud TSM (2015) Influence of spraying yeast extract and humic acid on fruit maturity stage and storability of 'Caino' apricot fruits. International Journal of ChemTech Research 8:530-543
Gregor JE, Powell HKU (1998) Protonation reactions of fulvic acids. Journal Soil Science. 39:243–252
Grove MD, Spencer GF (1979) Rohwedder WK. Brassinolide, a plant growth promoting steroid isolated from Brassica napus pollen Nature. 281:216–217
Kasim NE, Abou Rayya MSM, Shaheen MA, Yehia TA, Ali EL (2009) Effect of different collection times and some treatments on rooting and chemical internal constituents of bitter almond hardwood cuttings. Research Journal of Agriculture and Biological Sciences. 5:116–122
Khattab MM, Shaban AE, El-Shrief AH, Mohamed AS (2012) Effect of humic acid and amino acids on pomegranate trees under deficit irrigation. I: Growth, flowering and fruiting. J Hort Sci & Ornam Plants. 4:253–259
Ladzinsky G (1999) On the origin of almond. Genetic Resources and Crop Evolution 46:143–147
Mstat C (1989) Users guide: a micro-computer program for the design, management and analysis of agronomic research experiments. Michigan University, East Lansing, Mc, USA
Mustafa NS, El-Shazly SM (2013) Impact of some biostimulant substances on growth parameters of Washington Navel orange trees. Middle East J of Appl Sci. 3:156–160
O’Donnell RW (1973) The auxin-like effects of humic preparations from Leonardite. Soil Sci 116:106–112
O’Donnell RW, Berlyn GP (1990) The use of organic biostimulants to help low input sustainable agriculture. J Sustainable Agric. 1:9–42
Omaima MH, Malaka A Saleh, Mostafa EAM, El-Shamma MS, Maksoud MA. Improving pollination efficiency, yield and fruit quality of two date palm cultivars using growth activator. International Journal of Agricultural Research. 2014; 9 : 29-37.
Ramezani S, Shekafandeh A (2009) Roles of gibberellic acid and zinc sulphate in increasing size and weight of olive fruit. Afr J Biotechnol. 8:6791–6794
Russo RO, Berlyn GP (1990) The use of organic biostimulants to help low input sustainable agriculture. J Sustainable Agric. 1:9–42
Senn TL, Kingman AR. A review of humus and humic acids. www.australianhumates.com. 2000:1 – 5
Stefano P, Dris R, Rapparini F (2004) Influence of growing conditions and yield and quality of cherry. II. Fruit Journal of Agricultural and Environment. 2:307–309
Tahira A, Ahmed S, Ashraf M, Shadhid MA, Yasin M, Balal RM (2013) Perez MA Abbas. Effect of humic application at different growth stages of Kinnow mandarin (Citrus reticulatiblanco) on the basis of physio-biochemical and reproductive responses. Academy Journal of Biotechnology. 1:14–20
Yehia TA Characterization and prediction of flowering in apples. Ph. D. Thesis, Nottingham University. 1994; UK, 274 p.

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