Effect of Humic Acid, Nitrogen Concentrations and Application Method on the Morphological, Yield and Biochemical Characteristics of Strawberry ‘Paros’

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ABSTRACT
The current study investigates the effects of humic acid concentration, its application methods as well as nitrogen ratios on the morphological, yield and biochemical characteristics of strawberry cv. Paros, during 2014–2015 in Ardabil Province, Iran. The study has been conducted within the framework of a double split plot randomized complete block design with three replications and density of 10 plants per replications. Different nitrogen levels (50, 100 and 150 kg ha⁻¹) were examined in the main plots while application methods of humic acid (foliar and soil) and its different concentrations (0, 2, 4 and 6 kg ha⁻¹) were considered in the subplots and sub-sub plots, respectively. The results of the analysis of variance (ANOVA) indicated that the interaction effects of different concentrations of nitrogen, humic acid and its application methods are significant on most of the measured variables at 5% probability level. Mean comparisons showed that the highest values for the leaf area, fruit yield, chlorophyll a, carotenoids and titratable acidity are associated with the combined treatments of nitrogen and humic acid with concentrations of 100 kg ha⁻¹ and 4 kg ha⁻¹, respectively. In three-way interactions, the highest shoot and root biomass and total chlorophyll content were obtained from the application of 100 kg ha⁻¹ nitrogen and 4 kg ha⁻¹ foliar humic acid, while the highest nitrogen and protein contents of leaf and root were achieved upon applying 2 kg ha⁻¹ foliar humic acid and 150 kg ha⁻¹ nitrogen. Moreover, the highest amounts of ascorbic acid and total soluble solids were found at a concentration level of 100 kg ha⁻¹ nitrogen along with applying 2 and 6 kg ha⁻¹ foliar and soil humic acids, respectively. Finally, the best results of most of the studied traits were observed at 100 kg ha⁻¹ nitrogen and 4 kg ha⁻¹ foliar humic acid application.

KEYWORDS
Ascorbic acid; chlorophyll; foliar; protein; total soluble solids

Introduction
Strawberry contains a variety of minerals and vitamins, especially vitamin C. ‘Paros’ is one of the most important short–day strawberry cultivars which is suitable for the fresh-fruit market. This cultivar was obtained from Marmolada‘Onebor x Irvine crossing in Metaponto (Matera), Italy, in 1989 (Okie, 2000). The balance of nutrients and organic matter is one of the most important factors affecting the strawberry growth. The presence of arid and semi–arid regions in Iran and soils with poor organic matter contents, makes it impossible for the plant to access nutrients (Bibordi et al., 2000). Therefore, farmers need to apply extra fertilizers, especially nitrogen, in order to increase yield (FAO, 2015).
Nitrogen is the most important nutrient element which plays a vital role in building the protein molecules, enzymes, coenzymes, nucleic acids and cytochromes (Hassegawa et al., 2008). However, the improper use of nitrogen fertilizer not only reduces its efficiency but also causes environmental pollution (Tai-bo et al., 2007). According to FAO (2015), the amount of nitrogen fertilizer consumption has increased from 37.82 million tonnes during 2002–2004 to 87.98 million tonnes within 2011–2013. Organic farming which is known as the use of organic residues and compost to feed the soil, is a method of recycling nitrogen-containing nutrients and consequently reducing the use of nitrogen fertilizer and its entry into the soil (Ho et al., 2008).

The major elements in the humic acid composition are C, H, O, N and S. Regarding the structure, the major functional groups of humic acid include carboxylic, phenolic, carbonyl, hydroxyl, amine, amide and aliphatic moieties (Pena-Mendez et al., 2005). Humic acid is a stable material and its low acidity (pH value ranging from 3.8 to 5.8) leads to the gradual availability and uptake of macro – and micro-elements when used in the alkaline soils. Therefore, it increases the functional groups availability by the plant for a long time, increases the ion capillary capacity (Abbas et al., 2013) and thus improves the quality of products such as watermelon (Salman et al., 2005), grape (Ferrara and Brunetti, 2010) and strawberry (Hosseinifarahi et al., 2013).

The split application of humic acid at three different growth phases of the kinnow mandarin, was observed to stimulate the reproductive vigor and physio-biochemical attributes including the yield indicators, photosynthesis capacity, total chlorophyll content, total soluble solids, ascorbic acid and total sugar, while reducing its acidity (Abbas et al., 2013). Investigation the foliar spray of humic acid on kiwi fruit vines, indicated that the highest yield can be obtained by acid application dose of 0.2% (Mahmoudi et al., 2013). The foliar application of humic acid at a level of 0.05%, was shown to affect the leaf area, chlorophyll a, chlorophyll b, total chlorophyll and sugar contents of the pungent pepper fruit (Denre et al., 2014). In a study conducted by Al-ehvirdizadeh and Nazari Deljou (2014) on the application of different humic acid levels (0, 250, 500, 750 and 1000 mg l⁻¹) for Calendula officinalis, the higher amounts of chlorophyll a, chlorophyll b and total chlorophyll as well as carotenoids were achieved at concentration levels of 500, 250 and 750 mg l⁻¹, compared to the control, respectively. However, a decrease was seen in these contents at the highest concentration level. Eshghi and Garazhian (2015) obtained the highest ascorbic acid and total soluble solids contents of strawberry at 900 and 600 mg l⁻¹ foliar application of humic acid, respectively. Using four doses of urea fertilizer (100, 200, 300 and 400 kg ha⁻¹) for the strawberries, Rodas et al. (2013) obtained the highest amount of total soluble solids (TSS) with the application of 200 and 400 kg ha⁻¹ of nitrogen.

According to the report of Taibo and Colleagues (2007), the use of urea-humic acid increases the nitrogen uptake of soil by plant roots and significantly increases the protein content as well as production of the ginger rhizome. Applying humic acid (7000 ml ha⁻¹) or NPK (17: 17: 17) (250 kg ha⁻¹) alone on gladiolus leaves, led to the total chlorophyll content reduction, while their simultaneous application was observed to increase this trait (Ahmad et al., 2013). El-Bassioung and colleagues (2014) indicated that the application of humic acid and half of the recommended nitrogen fertilizer dose on the wheat, lead to the significant increase in the amount of total soluble solids and carbohydrates. They revealed that an increment in the total carbohydrates is due to the increasing efficiency of photosynthesis in response to humic acid treatment. As reported by Kazemi (2013), a combination of humic acid (40 ppm) and potassium nitrate (100 mg l⁻¹) is the most effective one in increasing the vegetative and reproductive growth, chlorophyll content, leaf nitrogen and total soluble solids in the pot cucumbers cultivation.

The present study aims to achieve the best treatment combination of nitrogen and humic acid and its method of application in order to improve the qualitative and quantitative properties of the strawberry fruit.
Materials and Methods

Area and Climate Conditions

The present study was conducted at the campus of the Faculty of Agriculture and Natural Resources of Mohaghegh Ardabili University (2014–2015). Ardabil province is placed on a strip stretching from 36° 50’ N, 47° E to 39° 40’ N, 49° E in the northwest of Iran (Naderi et al., 2014). The altitude of this region is more than 1400 m above the sea level (Molaei, 2011) located in one of the cold semi-arid regions. The warmest months of the year are July and August with an average temperature of 26.6°C and the coldest months of the year are January and February with an average temperature of −5.3°C. The average rainfall of this city in the research year is 280 mm with the maximum rainfall in February and November and the minimum rainfall in July (Iran Meteorological Organization, 2016).

Plant Growth Conditions and Treatments

First, the soil and fertilizer analyses were performed to determine the physical and chemical properties (Table 1). The land was prepared in early September and were applied solid base fertilizers containing 50 and 150 kg ha⁻¹ of potassium (Potassium sulfate) and phosphorus (superphosphate), respectively, and 40 t ha⁻¹ of livestock manure (Kashi and Hekmati, 1991). Furrow plots were prepared in the dimensions of 1 × 1.5 m with a distance of 40 cm between the rows. The strawberry plants of Paros cultivar (bare-root chilled transplants) were prepared from reproduction treasury of Marivan Zaribar, transferred to Ardabil and planted in the rows with a distance of 25 cm from each other and density of 10 plants per 1.5 m² in late November. Then black plastic mulch were applied on bed tops.

The experiment was carried out as a split split plot design in a completely randomized block design with both the foliar application and soil treatments of humic acid (Humaster Tob with 85% of Humic and Fulvic acid, Yazd Golsangkavir Company) at four levels (control, 2, 4 and 6 kg ha⁻¹) together with different urea nitrogen fertilizer levels (50, 100 and 150 kg ha⁻¹), each with three replications. Thus, a total number of 72 experimental units have been created. In these experiments, the impact of nitrogen levels were investigated in the main plots while those of the application type and concentration levels of humic acid were illustrated in sub and sub-sub plots, respectively. Half of the nitrogen levels (urea fertilizer) were introduced to the soil at the time of planting, 25% in early May and 25% in early June. In order to apply the foliar and soil treatments, humic acid was used once at the start of the growing season within the intervals of every 10 days (four times totally). In the soil method, humic acid was measured based on the desired treatments and then dissolved with deionized water and added to the irrigation water. However, in the foliar application, after the plot irrigation, the powder was dissolved in 400 ml of deionized water and then sprayed on the leaves of plants in equal amounts with a hand spray. In addition, only irrigation and deionized waters were applied to the control of soil and foliar treatments, respectively.

| Table 1. Results of soil degradation and animal manure test used. |
|-----------------|---|---|---|---|---|---|---|---|---|---|
| characteristics | Ec (M S/cm) | pH | Lime | Organic carbon | Total nitrogen | Sand | Clay | Silt | P | K | Zn | Fe | Cu | Mn |
| animal manure   | 21.30 | 7.85 | 8.24 | 11.15 | 1.10 | 0 | 0 | 0 | 126 | 23775 | 58.70 | 57.06 | 2.80 | 46.06 |
| Soil            | 2.18  | 7.42 | 14.13 | 14.13 | 0.13 | 48 | 14 | 38 | 15.26 | 822 | 8.77 | 1.11 | 1.14 | 7.39 |
Measurements

Leaf Area
To measure the leaf area, two plants were randomly selected from each replicate at the end of the season and then the whole plant leaves were passed through the AM300 leaf area meter device (BioScientific Ltd) to be expressed in cm².

Shoot and Root Biomass
The dry weights of the shoot and root parts were measured at the end of the season after drying plant biomass in an oven at 80 °C for 72 h and biomass expressed as g plant⁻¹.

Fruit Yield
The fruits were picked when they were completely red. (daily) and the average of fruit total yield was obtained after weighing all harvested ones from each plot, deviding by the number of plants per plot and then expressingin g plant⁻¹.

pH and Total Acidity of Fruit
In the middle of harvesting, 10 fruits were randomly selected from each plot and used in order to measure their qualitative characteristics.

The pH value of the fruit was measured by a potentiometer (Tabatabai, 2013) while total acidity (AT) was calculated by titration containing 0.1 N NaOH solution with pH of 8.2 (Ayala-Zavala et al., 2007).

The total acidity of the fruit is calculated using equation 1 (Tabataba’i, 2013) as

\[
\%\text{Acidity} = \frac{(D \times E \times 0.1\text{N NaOH} \times b)}{a \times c} \times 100
\]  
(eq1)

- a = Fruit weight (5 g)
- b = Initial volume (50 ml)
- c = Sample volume taken for titration (25 ml)
- D = The volume of consumed hydroxide
- E = Equivalent weight of dominant acid (citric acid) = 0.064 g mol⁻¹

Ascorbic Acid of Fruit
Ascorbic acid was measured by titration with color solution of dichlorophenol diphenol (A. O. A. C, 1989). It was calculated using equation 2 and expressed in mg 100 g⁻¹ of fruit.

The amount of ascorbic acid (mg 100 g⁻¹ fresh fruit weight) = \[
\frac{(e \times d \times b)}{c \times a} \times 100
\]  
(eq2)

- a = Sample weight (5 g)
- b = Volume created with metaphosphoric acid (50 ml)
- c = Sample volume of taken for titration (5 ml)
- d = Color Factor (0.054)
- e = Reading number for titration of each sample

Total Soluble Solids of Fruit
The total soluble solids (TSS) were read at room temperature using a french-made manual refractometer model 950–0140 OE – ATC (0 to 30 °Brix) (Ayala-Zavala et al., 2007).

Photosynthetic Pigments
At the end of the growing season, two plants were randomly selected from each plot and average of all leaves and roots used to measure the biochemical characteristics of the leaves and roots.
Chlorophyll a and b and carotenoids pigments were measured by the method of Arnon (1967), and using equation 3 chlorophyll a, b, total and carotenoids were measured in mg g$^{-1}$ of fresh leaf weight.

\[
\text{Chlorophyll a } = [19.3(\text{Absorption at 663 nm}) – 8.6(\text{Absorption at 645 nm})] \times V/(1000 \times W)
\] (eq3)

\[
\text{Chlorophyll b } = [19.3(\text{Absorption at 645 nm}) – 3.6(\text{Absorption at 663 nm})] \times V/(1000 \times W)
\] (eq3)

Total chlorophyll = Chlorophyll a + Chlorophyll b

Carotenoids = [100 (Absorption at 470 nm) – 3.27 (mg chlorophyll a) – 104 (mg chlorophyll b)]/227

In this regard: V: Sample volume of filtrated W: Wet sample weight

**Nitrogen and Protein in Leaves and Roots**

Nitrogen and protein in leaves and roots were measured using the Kjeldahl method which consists of three stages of sample digestion, distillation and titration (Tabataba’i, 2013), using equation 4 we calculated the amounts of total nitrogen and crude protein in %:

\[
\text{T.N } = [(T – B) \times 0.014 \times 100]/S
\] (eq4)

B: Volume of acid used for titration of control (ml)
T: Volume of consumed acid for sample titration
T.N: Total nitrogen (%)
S: The plant sample weight
N: Normality of sulfuric acid (0.05 N)

Crude protein percentage = 6.25 × Total nitrogen

**Statistical Analysis**

The statistical analysis of the data was performed using SAS 9.2 software and comparison of the mean values was carried out by LSD test at a probability level of 5%.

**Results**

**Morphological Characteristics and Yield**

The ANOVA of the morphological characteristics and yield data, indicates that the simple effects of humic acid and nitrogen and also their interactions are significant (P ≤ .05) for the leaf area, the shoot and root biomass and fruit yield.

| Table 2. Mean comparison of interaction effects of humic acid levels and application methods on leaf area and chlorophyll a. |
|---------------------------------------------------------------|
| T. Application methods | T. Humic acid (kg ha$^{-1}$) | Leaf area (cm$^2$) | Chlorophyll a (mg g fw$^{-1}$) |
|-------------------------|-------------------------------|-------------------|-----------------------------|
| Follar                  |                               |                   |                             |
| 0                       | 920.14$^{cd}$                 | 1.89$^{ab}$       |
| 2                       | 1050.26$^{bc}$                | 2.04$^a$          |
| 4                       | 1128.23$^{abc}$               | 1.92$^{b}$        |
| 6                       | 768.75$^d$                    | 1.50$^c$          |
| Soil                    |                               |                   |                             |
| 0                       | 915.16$^{cd}$                 | 1.73$^{b}$        |
| 2                       | 1013.77$^c$                   | 1.88$^{ab}$       |
| 4                       | 1250.43$^{ab}$                | 1.93$^{ab}$       |
| 6                       | 1304.34$^a$                   | 1.68$^{bc}$       |
| CV%                     | 18.70                         | 10.04             |
| LSD 5%                  | 325.02                        | 0.30              |

Different letters indicate significant differences were observed using the LSD test at 5% probability level.

mg g fw$^{-1}$: milligram per gram of fresh leaf biomass
Chemical Composition of Foliar and Soil Humic Acid

Comparison of the mean data indicates that the highest and lowest leaf area are obtained at 6 kg ha$^{-1}$ humic acid through soil and foliar applications, respectively (Table 2). As can be seen, the interaction effect of nitrogen and humic acid resulted in the highest leaf area at the nitrogen and humic acid levels of 100 kg ha$^{-1}$ and 4 kg ha$^{-1}$, respectively. Moreover, the lowest leaf area was achieved at 50 kg ha$^{-1}$ nitrogen level and control of humic acid. For all humic acid levels and 100 kg ha$^{-1}$ nitrogen, the shoot and root biomass were significantly increased (Table 3).

However, the nitrogen level of 150 kg ha$^{-1}$ at all levels of humic acid, except 6 kg ha$^{-1}$ soil application, led to a decrease in these characteristics. The highest shoot and root biomass were observed at 100 kg ha$^{-1}$ nitrogen level and foliar application of 4 kg ha$^{-1}$ humic acid. Also, the lowest shoot and root biomass were achieved at nitrogen levels of 50 kg ha$^{-1}$ and 150 kg ha$^{-1}$ with 6 kg ha$^{-1}$ humic acid foliar application, respectively but not significantly different from the foliar control shoot biomass (see Table 4). Based on the results of mean comparisons, the maximum and minimum fruit yields were recorded through combining the foliar and soil humic acid treatments, respectively. Investigating the interaction effects of nitrogen and humic acid, the highest fruit yield was obtained as 104.43 g plant$^{-1}$ at nitrogen level of 100 kg ha$^{-1}$ and 4 kg ha$^{-1}$ humic acid while its lowest value was estimated as 31.49 g plant$^{-1}$ at 150 kg ha$^{-1}$ nitrogen and 6 kg ha$^{-1}$ humic acid (Table 3).

Biochemical Characteristics

The results of ANOVA of the biochemical characteristics of the leaf and root, illustrates that the interaction effect of nitrogen and humic acid are significant (P ≤ .01) on all studied traits (except for the chlorophyll b content). However, the simple effect of the application method and interaction effects of nitrogen and application method were seen to be significant (P ≤ .01) on the nitrogen and protein characteristics of the leaf and root as well. Moreover, the three-way interaction effect were significant on the total chlorophyll content (P ≤ .05), and nitrogen and protein contents of the root and leaf tissue (P ≤ .01).

The maximum chlorophyll b values were obtained at the nitrogen levels of 100 and 50 kg ha$^{-1}$, respectively (Table 5). According to the mean comparison of data in Tables 6, 4 kg ha$^{-1}$ humic acid treatment was found to yield the highest chlorophyll b content. Furthermore, regarding the interaction effects of humic acid and its application methods, the highest amount of chlorophyll a (2.04 mg g$^{-1}$ fresh leaf weight) was associated with the foliar application of 2 kg ha$^{-1}$ humic

### Table 3. Mean comparison of interaction of nitrogen and humic acid on area, chlorophyll a and carotenoids of leaf, yield and total acidity of fruit.

| T. Nitrogen (kg ha$^{-1}$) | T. Humic acid (kg ha$^{-1}$) | Leaf area (cm$^2$) | Fruit yield (g m$^{-2}$) | Chlorophyll a (mg g fw$^{-1}$) | Carotenoids (mg g fw$^{-1}$) | Total acidity of fruit (TA) (%) |
|-----------------------------|----------------------------|-------------------|--------------------------|-----------------------------|-----------------------------|-------------------------------|
| 50                          |                            | 0                 | 864.60b                  | 504.48b                     | 0.07                        | 0.19bd                        |
| 2                           |                            | 2                 | 980.33b                  | 426.40b                     | 0.07                        | 0.13bd                        |
| 4                           |                            | 4                 | 1140.75b                 | 623.12bc                    | 0.26bc                      | 1.11ef                        |
| 6                           |                            | 6                 | 1084.16b                 | 264.16e                     | 0.26bc                      | 1.11ef                        |
| 100                         |                            | 0                 | 1005.11b                 | 390.08bc                    | 0.35b                       | 1.45bcd                        |
| 2                           |                            | 2                 | 1075.40b                 | 504.48c                     | 0.36a                       | 1.61a                         |
| 4                           |                            | 4                 | 1491.53a                 | 835.44a                     | 0.37a                       | 1.88b                         |
| 6                           |                            | 6                 | 1115.56b                 | 675.76abc                   | 0.22c                       | 1.06df                        |
| 150                         |                            | 0                 | 883.25b                  | 716.96ab                    | 0.27b                       | 1.00f                         |
| 2                           |                            | 2                 | 1040.36b                 | 753.36ab                    | 0.25bc                      | 1.23def                        |
| 4                           |                            | 4                 | 935.71b                  | 409.12de                    | 0.24bc                      | 1.14ef                        |
| 6                           |                            | 6                 | 909.91b                  | 251.92e                     | 0.15f                       | 1.04g                         |
| CV %                        |                            |                   | 18.70                    | 27.60                       | 10.04                       | 15.70                         | 15.60                         |
| LSD 5%                      |                            |                   | 325.02                   | 188.72                      | 0.30                        | 0.07                          | 0.34                          |

Different letters indicate significant differences were observed using the LSD test at 5% probability level. mg g fw$^{-1}$: milligram per gram of fresh leaf weight.
Table 4. Mean comparison of the three-way interaction between different levels of nitrogen, humic acid and methods of their application on shoot and root biomass, total chlorophyll, nitrogen and protein of leaf and root, total soluble solids and ascorbic acid of fruit.

| T. Nitrogen (kg ha⁻¹) | T. Application methods | T. Humic acid (kg ha⁻¹) | Dry weight (g m⁻²) | Total chlorophyll (mg g fw⁻¹) | Nitrogen (%) | Protein (%) | Total soluble solids (TSS) | Ascorbic acid (mg 100 g fw⁻¹) |
|------------------------|------------------------|-------------------------|------------------|-----------------------------|--------------|-------------|----------------------------|-------------------------------|
|                        |                        |                         | Shoot           | Root                        | Leaf         | Root        | Leaf                      | Root                          |
| 50                     | Folar                  | 0                       | 68.64           | 60.00                      | 2.20         | 0.88        | 5.49                      | 5.96                          | 32.53                       |
|                        |                        | 2                       | 82.16           | 64.64                      | 2.50         | 0.99        | 4.55                      | 4.80                          | 6.73                        |
|                        |                        | 4                       | 152.00          | 112.00                     | 2.59         | 1.11        | 6.22                      | 4.80                          | 6.73                        |
|                        |                        | 6                       | 35.12           | 39.60                      | 2.28         | 1.18        | 6.97                      | 6.55                          | 8.44                        |
|                        | Soil                   | 0                       | 74.64           | 58.64                      | 1.95         | 1.32        | 8.26                      | 4.81                          | 5.93                        |
|                        |                        | 2                       | 123.52          | 83.76                      | 2.52         | 1.44        | 8.99                      | 5.85                          | 7.73                        |
|                        |                        | 4                       | 70.88           | 90.56                      | 2.69         | 1.46        | 9.14                      | 7.08                          | 8.86                        |
|                        | 100                    | Folar                   | 0               | 101.22                      | 62.72        | 1.06        | 6.66                      | 5.99                          | 8.16                        |
|                        |                        | 2                       | 110.92          | 92.08                      | 2.95         | 1.76        | 11.04                     | 6.68                          | 9.56                        |
|                        |                        | 4                       | 168.64          | 130.24                     | 3.02         | 2.37        | 14.80                     | 8.35                          | 10.53                       |
|                        |                        | 6                       | 105.92          | 81.20                      | 2.08         | 1.82        | 13.35                     | 7.45                          | 9.39                        |
|                        | Soil                   | 0                       | 116.32          | 73.20                      | 2.47         | 1.39        | 8.72                      | 7.66                          | 9.68                        |
|                        |                        | 2                       | 117.60          | 94.80                      | 2.68         | 1.34        | 8.37                      | 8.30                          | 9.90                        |
|                        |                        | 4                       | 161.04          | 99.04                      | 2.94         | 1.43        | 8.93                      | 7.86                          | 10.90                       |
|                        |                        | 6                       | 95.68           | 64.24                      | 2.82         | 1.36        | 6.72                      | 6.87                          | 11.57                       |
|                        | 150                    | Folar                   | 0               | 53.92                       | 62.88        | 1.53        | 9.58                      | 9.02                          | 7.28                        |
|                        |                        | 2                       | 97.76           | 68.08                      | 2.83         | 2.54        | 15.89                     | 7.68                          | 9.42                        |
|                        |                        | 4                       | 138.72          | 79.52                      | 2.27         | 2.24        | 14.04                     | 6.76                          | 11.62                       |
|                        |                        | 6                       | 43.52           | 33.92                      | 1.94         | 1.15        | 7.18                      | 5.43                          | 8.50                        |
|                        | Soil                   | 0                       | 64.80           | 38.64                      | 2.38         | 1.62        | 10.16                     | 10.01                         | 7.31                        |
|                        |                        | 2                       | 86.24           | 60.40                      | 2.43         | 1.67        | 10.45                     | 9.62                          | 9.51                        |
|                        |                        | 4                       | 98.73          | 79.92                      | 2.29         | 1.81        | 11.33                     | 8.14                          | 11.07                       |
|                        |                        | 6                       | 161.36          | 105.44                     | 1.93         | 2.18        | 10.41                     | 8.12                          | 32.00                       |
| CV %                   |                        |                         | 14.20           | 13.50                      | 7.50         | 4.40        | 4.50                      | 8.60                          | 7.98                        |
| LSD 5%                 |                        |                         | 23.36           | 16.80                      | 0.36         | 0.12        | 0.74                      | 1.03                          | 1.18                        |

Different letters indicate significant differences were observed using the LSD test at 5% probability level.

mg g fw⁻¹: milligram per gram of fresh leaf weight, mg 100 g fw⁻¹: milligram per 100 gram of fresh fruit weight.
acid (Table 2). Given the mean comparison of data (Table 3), the highest chlorophyll a content was obtained as 2.23 mg g⁻¹ fresh leaf weight at nitrogen level of 100 kg ha⁻¹ and 4 kg ha⁻¹ humic acid while the lowest one was achieved as 1.35 mg g⁻¹ leaf fresh weight via applying 150 kg ha⁻¹ nitrogen and 6 kg ha⁻¹ humic acid.

According to the results presented in Table 3, the highest leaf carotenoid content (0.37 mg g⁻¹ leaf fresh weight) was observed at the nitrogen level of 100 kg ha⁻¹ and 4 kg ha⁻¹ humic acid. Besides, the lowest amount of carotenoid pigments was calculated as 0.15 mg g⁻¹ leaf fresh weight at the nitrogen level of 150 kg ha⁻¹ and humic acid one of 6 kg ha⁻¹. According to the mean comparison of data (Table 4), the highest total chlorophyll content was obtained as 3.02 mg g⁻¹ fresh leaf weight at the nitrogen and humic acid concentration levels of 100 kg ha⁻¹ and 4 kg ha⁻¹, respectively. Also, the lowest total chlorophyll content reached 1.93 mg g⁻¹ fresh leaf weight at 150 kg ha⁻¹ nitrogen level and 6 kg ha⁻¹ humic acid soil application.

Comparison of mean values (Table 4) indicates that the highest obtained nitrogen and protein values of the leaves are 2.54 and 15.89%, respectively, corresponding to the treatment with 150 kg ha⁻¹ nitrogen and spraying 2 kg ha⁻¹ humic acid. The lowest amounts of these traits were evaluated as 0.88 and 5.49% at nitrogen level of 50 kg ha⁻¹ without foliar application of humic acid, respectively. Also, the highest amounts of root nitrogen and protein contents were obtained as 1.6 and 10.01% by applying 150 kg of nitrogen per hectare without humic acid soil application. However, the lowest values of these traits were obtained as 0.73 and 4.55% at the nitrogen level of 50 kg ha⁻¹ and without foliar application of humic acid.

**Qualitative Characteristics of Fruit**

According to the results of ANOVA, the fruit quality ((P ≤ .01) is influenced by the humic acid and nitrogen levels. Also, the effects of humic acid application methods on these traits met statistically significant differences except for the acidity. Based on the obtained data, the triple

| Table 5. Mean comparison of different levels of nitrogen on chlorophyll b leaf and fruit acidity. |
|----------------------------------------------------------|
| T. Nitrogen (kg ha⁻¹) | Chlorophyll b (mg g fw⁻¹) | Fruit acidity (pH) |
|------------------------|---------------------------|-------------------|
| 50                     | 0.61ᵇ                    | 4.03ᵃ            |
| 100                    | 0.68ᵃ                    | 3.86ᵇ            |
| 150                    | 0.64ᵇᵃ                   | 3.73ᵇ           |
| CV %                   | 10.20                    | 8.27             |
| LSD 5%                 | 0.05                     | 0.30             |

Different letters indicate significant differences, which were observed using the LSD test at the 5% probability level.

mg g fw⁻¹: milligram per gram of fresh leaf weight

| Table 6. Mean comparison of different levels of humic acid on chlorophyll b leaf and fruit acidity. |
|----------------------------------------------------------|
| T. Humic acid (kg ha⁻¹) | Chlorophyll b (mg g fw⁻¹) | Fruit acidity (pH) |
|------------------------|---------------------------|-------------------|
| 0                      | 0.58ᵇ                    | 4.03ᵃ            |
| 2                      | 0.68ᵃ                    | 3.86ᵇ            |
| 4                      | 0.70ᵇ                    | 3.62ᵇ            |
| 6                      | 0.62ᵇ                    | 3.98ᵃ            |
| CV %                   | 10.20                    | 8.27             |
| LSD 5%                 | 0.03                     | 0.18             |

Different letters indicate significant differences were observed using the LSD test at 5% probability level.

mg g fw⁻¹: milligram per gram of fresh leaf weight
interaction effects on the ascorbic acid and total soluble solids had statistically significant difference (\( P \leq .01 \)). Also, a significant difference was observed between the bilateral effects of nitrogen and humic acid on the total acidity traits.

The mean comparison of data showed that the highest (4.03) and lowest (3.73) pH values of the fruit are obtained at the nitrogen levels of 50 kg ha\(^{-1}\) and 150 kg ha\(^{-1}\), respectively (Table 5). In the case of humic acid treatment, the highest (4.03) and lowest (3.62) values of the above traits were obtained at the control and 4 kg ha\(^{-1}\) humic acid, respectively (Table 6). Comparison of the means indicates that the total acidity (TA) meets its maximum value (1.88%) at the nitrogen level of 100 kg ha\(^{-1}\) and 4 kg ha\(^{-1}\) humic acid, while its minimum value (1%) is achieved with 150 kg of nitrogen per hectare without the use of humic acid (Table 3).

According to Table 4, the maximum amount of total soluble solids was found to be 11.57 °Brix at nitrogen level of 100 kg ha\(^{-1}\) and 6 kg of humic acid per hectare. However, the minimum value of this trait was obtained as 5.93 °Brix at nitrogen level of 50 kg ha\(^{-1}\) and control of humic acid. Further to these, the highest amount of ascorbic acid was achieved as 64.2 mg 100 g\(^{-1}\) of fruit at nitrogen level of 100 kg ha\(^{-1}\) and the foliar application of 2 kg ha\(^{-1}\) humic acid. Also, the least value of this trait was obtained as 25 mg 100 g\(^{-1}\) of fruit via the nitrogen treatment of 150 kg ha\(^{-1}\) and the foliar application of 6 kg of humic acid per hectare.

**Discussion**

As mentioned earlier, the shoot section, especially leaf area, has increased with the application of foliar humic acid up to 4 kg ha\(^{-1}\) and remarkably decreased at 6 kg ha\(^{-1}\). However, in the case of soil application, the increase was maintained up to 6 kg ha\(^{-1}\) humic acid. This result might be due to the burning of leaves following the high concentration of humic acid in the foliar application. Examining the interaction effects of humic acid and nitrogen, the leaf area in all humic acid treatments with nitrogen level of 100 kg ha\(^{-1}\) was greater than that of 50 kg ha\(^{-1}\) and 150 kg ha\(^{-1}\) concentration levels. Increasing fruit yield up to 100 kg ha\(^{-1}\) nitrogen and 4 kg ha\(^{-1}\) humic acid may depend on the leaf area increment. Similar results were obtained regarding the interaction effects of nitrogen and humic acid on the growth and yield of different plants such as peanut (Moraditouchi, 2012), cucumber (Kazemi, 2013) and tomato (Aman and Rob, 2013), which confirms the present achievements. Investigating the effect of different nitrogen levels (110, 220 and 330 mg l\(^{-1}\)) on the strawberry cultivar Gaviota, Ganjehi and Golchin (2012) indicated that the highest shoot fresh and dry weights, number of fruits and yield are obtained using 110 mg l\(^{-1}\) nitrogen and these traits decrease with the increasing nitrogen levels above this value. It is clear that the foliar application of humic acid increases the permeability of the cells, leading to the rapid entry of minerals into the leafs cells and higher nutrient uptake by the plant (Ehsan et al., 2014) and this improves the vegetative growth of the plants and the increased yield as a consequence (Sarhan et al., 2011).

In the previous studies, it was observed that the treatments of 50 kg ha\(^{-1}\) and 100 kg ha\(^{-1}\) nitrogen levels with 4 kg ha\(^{-1}\) humic acid lead to an increase in the photosynthetic pigments. However, in the treatment with 150 kg ha\(^{-1}\) of nitrogen the increased values of these traits were obtained by applying 2 kg ha\(^{-1}\) humic acid. Allahvirdizadeh and Nazari Deljou (2014) reported that with the application of different humic acid levels on *Calendula officinalis*, the higher contents of chlorophyll a, b, total and carotenoids were observed compared to the control, but these traits decreased at the highest concentration level. The simultaneous use of humic acid and NPK on gladiolus leaves led to an increment in the total chlorophyll content (Ahmad et al., 2013). There are many reports about the positive effects of nitrogen and humic acid and their interactions on the chlorophyll content of various plants, including: Eggplant (Aminifar et al., 2010), Kiinow mandarin (Abbas et al., 2013) and Garlic (Zeinali and Moradi, 2015) whose results are consistent with the findings of this study. The effect of nitrogen fertilizer on the chlorophyll content might be due to the presence of nitrogen in the structure of the chlorophyll molecule (Aminifar et al., 2010). In addition, the increase in the total chlorophyll content of leaf may be due to the accelerated
nitrogen and nitrate absorption, increasing nitrogen metabolism and protein production by humic acid (Haghighi et al., 2012) as well. Ferrara and Brunetti (2010) believe that humic acid either increases the synthesis of chlorophyll or delays the chlorophyll degradation.

The nitrogen and protein contents of the leaf and root increase with the increasing nitrogen levels for most humic acid levels and application methods, except for the nitrogen level of 150 kg ha\(^{-1}\) with 4 kg ha\(^{-1}\) and 6 kg ha\(^{-1}\) humic acid foliar application, where a varying trend is observable. The positive and significant effects of nitrogen and humic acid and their interactions on the amount of nitrogen and protein in the leaves and roots of various products such as: Strawberry (Ameri and Tehranifar, 2012), Ginger (Tai-bo et al., 2007), Melon (Ferrante et al., 2008) and Cucumber (El-Nemr et al., 2012; Kazemi, 2013), have been reported which are consistent with the results of this study. Tai-bo et al. (2007) reported that humic acid significantly increases the protein content and protein production of ginger rhizome. Also, the use of humic acid-urea increases the absorption efficiency of soil nitrogen and activity of nitrate reductase as well. Chen and Aviad (1990) argued that the stimulating effects of humic acid are related to the increased absorption of macro-elements. Adani et al. (1998) found that humic acid increases the nitrogen uptake. Pinton et al. (1999) suggested that the humic substances play a role in modulating the absorption of nitrate through interaction with the plasma membrane H\(^+\)-ATPase.

The total fruit acidity was increased with an increment in the nitrogen level up to 100 kg ha\(^{-1}\). However, it decreased at the nitrogen level of 150 kg ha\(^{-1}\) at all humic acid concentration levels. As a result, the highest titratable acidity was observed at nitrogen level of 100 kg ha\(^{-1}\) and 4 kg ha\(^{-1}\) humic acid. The amounts of fruit’s total soluble solids and ascorbic acid were increased up to the nitrogen level of 100 kg ha\(^{-1}\) at all humic acid levels and application methods, except for 6 kg ha\(^{-1}\) humic acid foliar application in which these traits linearly decreased at all nitrogen levels. However, the values of these traits showed a reduction for nitrogen level of 150 kg ha\(^{-1}\) and all humic acid levels and application methods. These increments in the total soluble solids and ascorbic acid contents may be due to the activity of enzymes in response to the application of nitrogen and humic acid. The reduction in the amounts of total soluble solids and ascorbic acid of fruit in the treatment of 150 kg ha\(^{-1}\) nitrogen, may be due to the decreasing activity of the enzymes and molecules necessary for the synthesis of these quality attributes. Many studies have indicated that high doses of nitrogen have a negative effect on the product quality, taste and acidity of the fruits (Kruesekopf et al., 2002). The positive effects of humic acid on the quality of various products such as Kinnon mandarin (Abbas et al., 2013), Sweet pepper (Aminifar et al., 2013), Tomato (Kamari, 2014), Strawberry (Eshghi and Garazhian, 2015; Hosseini Farahi et al., 2013) and Grape (Ferrara and Brunetti, 2010), indicated acceptable agreement with the results of this research. Nardi et al. (2002) expressed that the increase in the quantitative and qualitative properties of fruits are the result of increased respiration, photosynthesis and total protein content of the plants due to the application of humic and fulvic acids. Soil application of humic acid stimulates the photosynthetic pigments and therefore helps improve the plant photosynthesis and growth (Abbas et al., 2013). Also, more assimilations are produced with the increasing photosynthesis, which leads to an increment in the quality of products (Abdel-Mawgoud et al., 2007).

### Conclusion

According to the results, the best treatment combination for the leaf area, shoot and root biomass, fruit yield, chlorophyll a, total chlorophyll, carotenoids and titratable acidity was obtained from treatment of 100 kg ha\(^{-1}\) nitrogen and 4 kg ha\(^{-1}\) humic acid. The highest nitrogen and protein contents of leaf and root were observed at nitrogen level of 150 kg ha\(^{-1}\) and 2 kg ha\(^{-1}\) humic acid foliar application, respectively. Also, it has been observed that the best combinations for the ascorbic acid and total soluble solids are 2 kg ha\(^{-1}\) foliar and 6 kg ha\(^{-1}\) soil applications of humic acid with nitrogen level of
100 kg ha⁻¹, respectively. Therefore, an appropriate treatment combination to improve the quantity and quality of the strawberry fruit is the application of 100 kg ha⁻¹ nitrogen and 4 kg ha⁻¹ foliar humic acid application.

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