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Yield, Leaf Mineral Content, and Quality Properties of Hayward kiwifruit as Influenced by Different Fertilization Methods

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Abstract
Kiwifruit requires a high quantity of nutrients that must be supplied through fertilization to obtain an optimum yield on a sustainable basis. This research was conducted to evaluate different fertilization methods including broadcast application (as control), deep placement of the fertilizer and fertigation, alone or in combination with foliar application and their effects on the yield, leaf mineral contents, and some quality properties of kiwifruit (*A. deliciosa* cv. Hayward). Results showed that fertilization methods had a significant effect on yield and the highest level of yield (76 Kg tree^{-1}) was obtained in the combination of fertigation with foliar spray. In addition, foliar application significantly increased leaf mineral contents of kiwifruit in all treatments. Furthermore, the fertilization method had significant effects on phenol, ascorbic acid contents, and antioxidant capacity of fruits. The highest levels of antioxidant (70.9%), phenol (103.6 mg 100g^{-1}), and ascorbic acid (54.2 mg 100 g^{-1}) in fruits were recorded in 'soil application' method. Total soluble carbohydrate and starch contents of the fruits were reduced following foliar nutrition in all treatments. However, foliar nutrition significantly increased fruit crude protein. In conclusion, based on obtained results, fertilization method directly influenced the yield and leaf mineral status and indirectly influenced the fruit chemical composition of Hayward kiwifruit. In fact, the supplemental foliar application reduced some fruit quality attributes (lower antioxidant activity and carbohydrate content) but enhanced the yield of kiwifruit.

Keywords: Antioxidant capacity, Carbohydrate, Phenol, Starch.

Introduction

The world production of kiwifruit (*Actinidia deliciosa* Planch.) is estimated to be 4.0 million tons in 2017, and the Iran produced 311307 tons of it (FAO, 2017). Among the kiwifruit cultivars, 'Hayward' is the most popular one in the world. Kiwifruit is famous for its taste and aroma, nutritional and medicinal values. Kiwifruit represent a major source of dietary antioxidants for humans. Its antioxidant properties are predominantly associated with phenolic and ascorbate compounds (Hunter et al., 2011).

Kiwifruit has a high nutrient requirement due to its great vegetative growth and high fruit yield (Otero et al., 2007); therefore, it should be grown on the soils, which are well-supplied with the nutrients. This plant absorbs well the nutrients from the soil, and if a shortage of mineral elements occurs, it quickly shows signs of deficiency. Nutrition disorders can lead to a serious reduction in production, and in some cases affect the quality of the fruit during the storage period

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(Smith et al., 1987a). In fact, one of the most important factors affecting the quality and quantity of kiwifruit is fertilization (Peticilaa et al., 2015). It is well known that the fertilizer amount applied by the growers influences the kiwifruit tree growth, yield, fruit quality (Pacheco et al., 2008; Santoni et al., 2013; Santoni et al., 2014) and also leaf mineral composition (Mills et al., 2008; Morton et al., 2013; Santoni et al., 2014). Although the application of excess or unbalanced amount of nutrients will have adverse effects on fruit quality, use of nutrients for fertilization of this plant usually leads to higher yields. As an example of the negative effects of over-fertilization, excess concentration of nitrogen (N) in fruit juice usually promotes flesh softening during storage (Boukouvalas and Chouliaras, 2005). The concentration of mineral elements in fruit trees is influenced by the type (Amodio et al., 2007), amount (Santoni et al., 2014; Ejraei et al., 2018) and application method (Marsh and Stowell, 1993) of fertilizer.

One of the most important factors in the use of fertilizer which needs careful consideration is the method of fertilizer application (Marsh and Stowell, 1993). General practice in kiwifruit orchards in Iran is to apply a single solid application of 200-400 kg ha^{-1} potassium (K) and up to 200 kg ha^{-1} N in the early spring, but there is some debate as to whether other strategies could increase nutrient uptake and make more efficient use of fertilizer (Marsh and Stowell, 1993). By choosing the suitable strategy for fertilizer application, grower can maximize fertilizer uptake efficiency and minimize leaching losses below the root zone. There are four main methods for fertilizer application, including broadcasting, localized placement, fertigation and foliar applications. However, incorrect use of fertilizers can reduce fertilizer use efficiency (Quinones et al., 2003; Dong et al., 2005), which may be followed by adverse effects on the soil productivity and environment.

Leaf analysis in combination with soil analysis is the best method for evaluating the absorption of elements. However, plant analysis has distinct advantages over soil analysis as a diagnostic aid for a deep-rooted plant like kiwifruit (Smith et al., 1987b). Leaf tissue testing is a valuable tool to examine the tree nutrient status and can be used to compare fertilizer treatments and to select the best fertilization method. Leaf analysis results are important and support growers to improve the fruit quality. Low leaf mineral content may reduce kiwifruit yield and fruit quality (Coutinho and Veloso, 1997; Parent et al., 2015). Kiwifruit orchards require an optimum nutritional status to maintain high production with acceptable size percentage of fruits. In addition, leaf mineral analysis is a useful tool to diagnose tree deficiencies (Smith et al., 1987a).

There have been few studies about the effect of different strategies of nutrient supply on the growth and production of kiwifruit. Therefore, the aim of this study was to determine whether the fertilization methods including 1) broadcasting; 2) fertigation; 3) deep fertilizer placement; 4) broadcasting plus foliar; 5) fertigation plus foliar, and 6) deep fertilizer placement plus foliar application can influence yield and fruit quality of *A. deliciosa*.

Material and Methods

Site Characteristics and Fertilization Treatments

The study site was located at the Citrus and Subtropical Fruit Research Centre (36°54′11″N 50°39′30″E), Mazandaran province, Iran. Average annual rainfall and annual temperature in this area are1200 mm and 21 °C, respectively.

Kiwifruit vines (*A. deliciosa* cv. Hayward) were planted in 1998 at 5 × 4 m distances. Irrigation was carried out with 90 L hr^{-1} microjet. Tensiometers (in the root zone) were used to schedule irrigations whenever soil tension reached 40 kPa. Soil characteristics of the site are described in Table 1.

Table 1. Soil physicochemical properties of the studied orchard

Depth	pH	EC[a]	CCE[b]	O.C.[c]	Silt	Clay	Total N	P[d]	K[e]
cm	-	dS m^{-1}			%			mg kg^{-1}	
0-30	7.2	0.19	<1	1.9	43	31	0.65	83	116
30-60	7.0	0.20	<1	1.7	43	35	0.16	41	94

[a] EC, electrical conductivity of the 1:5 soil: water extract; [b] CCE, calcium carbonate equivalent; [c] O.C, organic carbon; [d] P_{avail}, Sodium bicarbonate -extractable P; [e] K_{avail}, Ammonium acetate -extractable K.

Experimental Setup

The experiment was arranged in a randomized complete blocks design with six fertilization methods: (1) broadcasting (control); (2) fertigation; (3) deep fertilizer placement; (4) broadcasting plus foliar; (5) fertigation plus foliar, and (6) deep fertilizer placement plus foliar. Six treatments were laid out in four blocks and started in the winter of 2014.

In broadcasting, deep placement and fertigation fertilizer application techniques, total annual nutrient input was 500 g N $tree^{-1}$ yr^{-1}, 100 g P $tree^{-1}$ yr^{-1}, 500 g K $tree^{-1}$ yr^{-1}, 60 g Mn $tree^{-1}$ yr^{-1}, 60 g Zn $tree^{-1}$ yr^{-1} and 10 g B $tree^{-1}$ yr^{-1}, regardless of the method of soil application. Fertilizer materials were derived from urea, mono-ammonium phosphate and phosphoric acid, potassium chloride, manganese sulfate, zinc sulfate and boric acid sources.

In deep fertilizer placement technique, fertilizers were placed in holes prepared at two sides of the trees with 50 cm depth and at the distance of 50 cm from the trunk, in early spring and then refilled by soil. In fertigation method, two vines in each of the blocks received regular inputs of nutrients by fertigation every 2 weeks from April through early December (10 times per year), whereas the others were maintained without fertigation and received the same quantities of water by irrigation. In combined application, the foliar N (0.5% urea) was applied every four weeks (four times per year) following bud burst during April through July. Moreover, aqueous solutions 0.5% of di-ammonium phosphate, and potassium chloride were sprayed on vines (two times per year) in May and July by using hand held sprayer till run off. All the experimental vines were subjected to the same cultural practices such as pollination, training and pruning, irrigation, weeding, insect pest and disease control. In addition, the amounts of fertilizer were close to the values used by farmers.

Leaf Analysis

Leaf samples (second leaves following the final fruit cluster) were collected for mineral analysis during August, 2015 (Warrington and Weston, 1990) and prepared for nutrient analysis. Leaves oven dried at 65 ˚C for 72 h. Dried leaves were ground and digested by dry ashing method (Kalra, 1998) for P, K and calcium (Ca) analysis. P concentration in plant digests were determined calorimetrically by the ascorbic acid method (Murphy and Riley, 1962). K and Ca in extracts were determined by flame photometry and titration methods, respectively (Kalra, 1998). In addition, nitrogen concentration in oven-dried plants was measured by micro Kjeldahl.

Fruit Analysis

Fruits were harvested and weighted at the stage of physiologic maturity (based on fruit total soluble solids=6.2 Brix) in the second week of November each year. After harvest, 10 fruits per tree were randomly collected and middle part of the fruit tissue was frozen rapidly in liquid nitrogen and stored at -80 ˚C for further analysis. Before the analysis, the fruit tissues were ground to a fine powder in liquid nitrogen. Crude protein, ascorbic acid, phenolics, antioxidant capacity, soluble carbohydrate and starch were analysed in fruit tissue.

The crude protein content in fruits is estimated by multiplying the determined

nitrogen content by a nitrogen-to-protein conversion factor (6.25, Boland 2013). In this method, total nitrogen in fruits was determined by the method of Kjeldahl.

For determination of total soluble carbohydrate content, 0.1 g of fruit powder was extracted using 10 ml of ethanol-distilled water (8:2 v/v), and the supernatants were collected after centrifugation at 5000 g. The residue from ethanol extraction was subsequently used for starch extraction by perchloric acid (52%) (McCready et al., 1950). To follow the standard procedure for carbohydrate determination, 200 µL aliquot of a carbohydrate solution was mixed with 250 µL of 5% aqueous solution of phenol in a test tube. Subsequently, 1 mL of concentrated sulfuric acid was added quickly to the mixture. After allowing the test tubes to stand for 10 min, they were vortexed for 30 s and placed for 20 min in a water bath at room temperature for color development. Then, light absorption at 490 nm is recorded on a spectrophotometer. Soluble carbohydrate concentrations of the samples were calculated using calibration curve that was drawn for glucose standard solutions.

Ascorbic acid was determined according to the method described by Nielsen (2010). Aliquant (1 mL) of kiwifruit juice was mixed with 2.5 ml of 3% metaphosphoric acid-acetic solution. Then each sample was titrated with the indophenol dye solution until a light but distinct rose-pink colour persists for >5 s. Ascorbic acid contents were determined as mg 100 g^{-1} FW.

For determination of total phenol and antioxidant capacity, fruits were homogenized and extracted in 85% methanol for 24 h at 4 ˚C. The filtrates were stored at -20 ˚C until used for analysis of the total phenolics, and antioxidant capacity. The ability to scavenge DPPH free radicals was determined based on the method of Brand-Williams et al. (1995). Total phenolic of the fruits was determined using the Folin–Ciocalteu reagent and gallic acid as the standard as outlined by Ainsworth and Gillespie (2007).

Statistical Analysis

Analysis of variance was performed by ANOVA procedures. Significant differences were calculated according to least significant difference (LSD) tests. Differences at $P \leq 0.05$ were considered statistically significant. The Pearson's correlation was used to evaluate relationship between variables. Statistical analysis was performed with SAS 9.1 (for Windows).

Results

Yield performance

Different fertilization strategies significantly influenced tree yield (Table 2). The highest yield (76 kg $tree^{-1}$) was obtained in kiwifruit vines under fertigation plus foliar treatment, which did not significantly differ from that of vines in control plus foliar (75 kg $tree^{-1}$) and deep placement plus foliar (71 kg $tree^{-1}$) methods. The vines in remaining treatments had lower yields (Table 2). In the present study, no positive responses were recorded for fertigation of kiwifruit vines in comparison with the control and deep placement treatments.

Leaf mineral status

The concentrations of the mineral elements of the kiwifruit leaves in different fertilization methods are reported in Table 2. Values are expressed as the percentage of dry weight.

The fertilization method had significant effects on N concentration in kiwifruit vine leaves. The N concentration differed among fertilization treatments, ranging from 3.7% in control to 4.4% in fertigation plus foliar application. In addition, the fertilization method significantly influenced P concentration of the leaves. The highest amount of P (0.19 %) was found in the leaves under fertigation plus foliar treatment, indicating a significant

difference with control, deep placement, and fertigation treatments, but the difference was not significant compared to other foliar treatments. The leaf K concentration was changed from 2.57% (Control) to a maximum of 4.10% (fertigation plus foliar), that significantly differ with other treatments. Furthermore, results of our study indicated that the control plus foliar treatment produced the highest amount of leaf Ca concentration (3.03%), whereas control generated the lowest Ca concentration (2.57%). Leaf N, P, K, and Ca nutrient concentrations were higher in 'foliar plus soil application' than 'soil application' method (Table 2).

Table 2. Effects of different fertilization strategies on yield and leaf mineral content of Hayward kiwifruit

Fertilization methods[a]	yield	Leaf mineral content			
		nitrogen	phosphorus	potassium	calcium
	Kg tree^{-1}	%			
Control (Broadcasting)	44b	3.77c	0.16cd	2.57d	2.47c
Broadcasting plus foliar	75a	4.17ab	0.18ab	3.74b	3.03a
Fertigation	51b	3.99bc	0.17bc	3.16c	2.69b
Fertigation plus foliar	76a	4.32a	0.19a	4.10a	2.93a
Deep placement plus foliar	48b	3.78c	0.15d	2.74d	2.66bc
Deep placement plus foliar	71a	4.31a	0.18ab	3.45bc	2.72b
Mean	61	4.04	0.17	3.34	2.83
F[b]	5.28*	8.06*	8.93*	24.02*	21.74*
C.V(%)	28	4.1	4.2	6.7	4.5

[a] Different letter in each column indicate significant differences
[b]* indicate significance at $P \leq 0.05$ level.

Fruit chemical composition

The results of carbohydrate analysis are depicted in Figure 1. Various fertilization strategies significantly influenced average fruit total soluble carbohydrate and starch contents. The fruit starch content was varied from 2.21% in deep placement plus foliar to a maximum of 4% in fertigation treatment. Furthermore, the total soluble carbohydrate of the fruits was similar among control, deep placement, and fertigation treatments. However, it sharply decreased when foliar feeding was used; it was reduced from 5.87% to 4.33% for control, 4.85% to 4.27 % for fertigation, and 4.51% to 3.64 % for deep fertilization.

The method of fertilizer application influenced the crude protein contents of kiwifruit (Fig. 1). The amount of crude protein varied from 1.34 g 100 g^{-1} in fertigation treatment to a maximum of 1.57 g 100 g^{-1} found in control plus foliar treatment.

Kiwifruit is regarded as a good source of ascorbic acid and phenol. Ascorbic acid and phenol contents in kiwifruit treated by fertilization methods are reported in Figure 2. The amount of ascorbic acid ranged from 48.7 mg 100 g^{-1} found in fertigation plus foliar to a maximum of 54.2 mg 100 g^{-1} found in the control treatment.

The strategy of fertilization application had significant effects on phenol content of the fruits. Phenol content differed across fertilization treatments, from 84.7 in control plus foliar to 103.6 in fertigation as mg gallic acid 100 g^{-1} fresh weight. In addition, the fertilization strategy significantly affected the antioxidant capacity of the fruits. The antioxidant capacity ranged from 53.2% in the control plus foliar to 70.9% in the fertigation method.

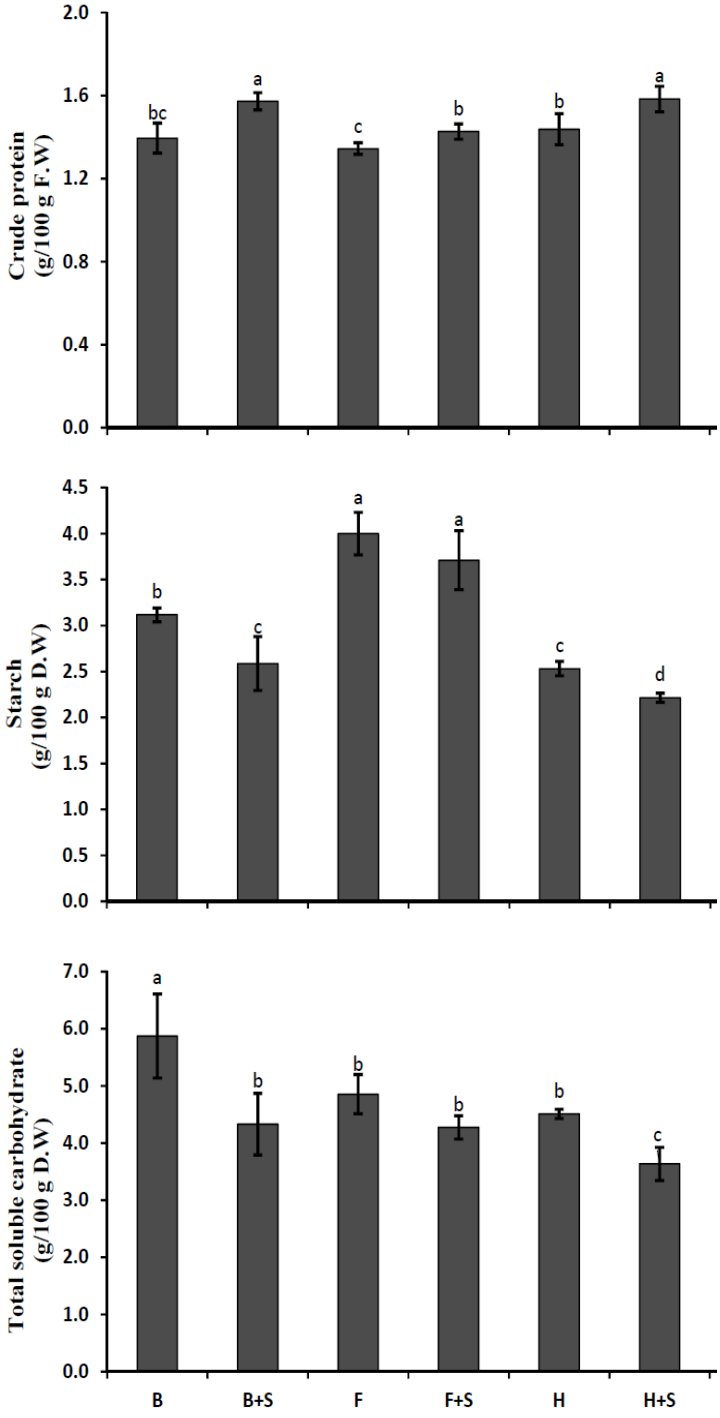

Fig. 1. Effects of different fertilization strategies on total soluble carbohydrate, starch contents and crude protein. ″B″, broadcasting; ″F″, fertigation; ″H″, deep placement; ″B plus S″, broadcasting plus foliar application; ″F plus S″, fertigation plus foliar application; and ″H plus S″, deep placement plus foliar application. Different letters indicate significant differences

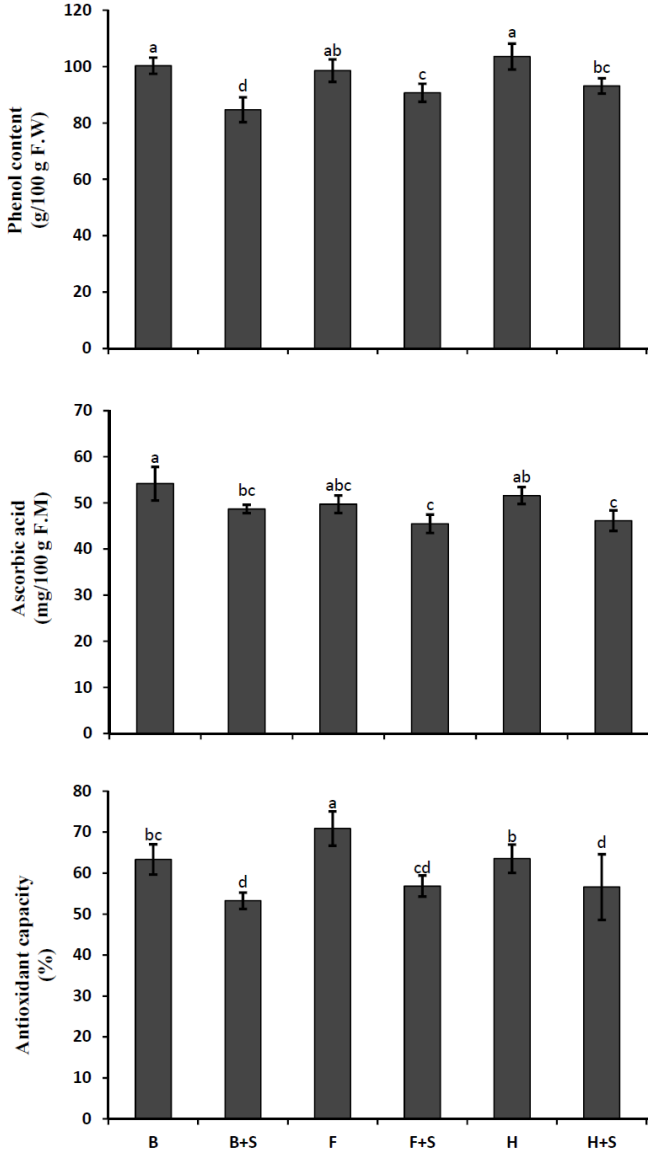

Fig. 2. Effects of different fertilization strategies on ascorbic acid, phenol contents and antioxidant capacity. ″B″, broadcasting; ″F″, fertigation; ″H″, deep placement; ″B plus S″, broadcasting plus foliar application; ″F plus S″, fertigation plus foliar application; and ″H plus S″, deep placement plus foliar application. Different letters indicate significant differences

Table 3. Correlation analysis of phenol and ascorbic acid contents with crude protein, leaf nitrogen (N) and carbon to nitrogen (C/N) ratio of the fruits.

	Crude protein	N-leaf	C/N-Fruit
Phenol	-0.65*	-0.79*	0.62*
Ascorbic acid	-0.47	-0.96*	0.88*

* indicate significance at $P \leq 0.05$ levels.

Discussion

In the present study, no positive responses were recorded for fertigation in kiwifruit vines compared to control and deep placement treatments. Results of the present study are in agreement with the findings of Marsh and Stowell (1993) who also observed that the vines receiving fertigation showed no advantage over those receiving a conventional solid fertilizer

program in terms of yield for kiwifruit. It appears that fertigation may help to overcome soil or nutritional limitations for production or fruit quality. Nevertheless, on soils where fertilizer practices are generally successful, fertigation may be of limited value (Marsh and Stowell, 1993). Results of our study indicated that the supplemental foliar application induced the highest yields per tree in general, whereas the lowest values were found with soil applications (control) without foliar feeding (Table 2). Other studies have also reported beneficial responses to foliar feeding in perennial fruit crops (Amiri et al., 2008; Morton, 2013).

The analysis of nutrient levels in kiwifruit leaves revealed differences among fertilization strategies for supplying nutrients. The concentrations of N, K, P, and Ca that obtained at this study were close to those reported by Velemis et al. (1995), Tarakcioglu et al. (2006), Morton et al. (2013), and Santoni et al. (2014).

Leaf N, P, K, and Ca nutrient concentrations were higher in "foliar plus soil application" than "soil application" (Table 2). As expected, leaf N, P, and K nutrient concentrations indicated a strong increase following the foliar application of these nutrients. Furthermore, supplemental foliar application significantly enhanced leaf Ca concentration. In general, a high rate of N foliar supply induces rapid growth and high vigour in shoots. This, in turn, promotes leaf transpiration due to increased foliage surface area (data not shown), resulting in more xylem Ca being delivered to the leaf (Morandi et al., 2010).

Based on Figure 1, various fertilization strategies significantly influenced average fruit total soluble carbohydrate and starch contents. In general, the evaluation of starch and total soluble carbohydrate contents in the fruits showed a clear decrease when foliar feeding was applied in control and deep placement treatments. By increasing N application, we increase vegetative growth. In kiwifruit, the vegetative sink is stronger than the fruit (Greer et al., 2003). This may suggest a greater competition for available carbohydrates by vegetative plant portions compared with fruit in 'Hayward' kiwifruit under N foliar nutrition. In agreement with the result of current study, Mills et al. (2009) found that in 'Hort16A' kiwifruit vines supplied with a high level of N (145 kg N ha^{-1} $year^{-1}$) fruit sugar content was reduced. In addition, Aminifard and Bayat. (2016) showed that application of high amounts of vermicompost (especially with 15 t ha^{-1}) causes a decrease in carbohydrate content of bell pepper.

Moreover, the results illustrated that more crude protein was accumulated as a result of 'foliar plus soil application' than the other fertilization methods. Therefore, in response to foliar urea application, part of the carbon from the total non-structural carbohydrate is incorporated into proteins, leading to a decrease in the amount of carbon stored in the total non-structural carbohydrate and an increase in the carbon stored in proteins. The significant reduction in total non-structural carbohydrate in response to foliar urea application clearly showed that the assimilation of inorganic N utilizes carbohydrate for carbon skeleton and energy supply (Faust, 1989; Taylor et al., 1975). These results are in lines with previously published data on grapevine (Xia and Cheng, 2004) and apple (Cheng et al., 2004). The protein content of kiwifruit ranged between 0.49% and 1.13% according to the reports by Richardson et al. (2018), and Fourie and Hansmann (1992).

Kiwifruit is regarded as a good source of ascorbic acid and phenol (Fig. 2). The ascorbic acid content in fruits that obtained at this study was similar to those reported by Lintas et al. (1991) and Selman (1983) (from 37 to 200 mg 100 g^{-1} of FW).

The fertilization method had significant effects on phenol content in the fruits. In general, the highest levels of antioxidant, phenol, and ascorbic acid in fruits were recorded in the 'soil application' treatments

compared with the 'soil plus foliar application' treatments (Fig. 2). Based on the results, kiwifruit was abundant in phenolics and displayed significant antioxidant capacity. The correlation analysis was employed to explore the relationships of antioxidant capacity with phenol and ascorbic acid. The results of the correlations of antioxidant capacity with total phenol and ascorbic acid content were 0.44 ($P \leq 0.05$) and 0.22 ($P > 0.05$), respectively. Therefore, total phenol is a major contributor to the total antioxidant capacity in kiwifruit. There is a particular interest in understanding the potential effects of fertilizers on phenolic production in plants.

The previously published data implied that the decrease in the production of phenolic compounds under foliar feeding may be due to the tendency of phenylalanine for protein synthesis compared to the production of secondary metabolites (Ibrahim and Jaafar, 2011). Winger et al. (2006) indicated that increase in the carbon-nitrogen (C/N) availability ratio in plants is an indication of increase in the synthesis of plant secondary metabolites, especially phenolics and flavonoids. In the present study, this statement was confirmed when the C/N ratio of fruits displayed a significant positive relationship with total phenol ($r=0.62$; $P \leq 0.05$). Based on Table 3, it is evident that fruit protein content has a significant negative relationship with the phenolic content ($r=-0.65$; $P \leq 0.05$). Furthermore, the leaf N had a negative relationship with total phenol. These results indicate that, when the plants are under a limitation of N, the production of plant secondary metabolites may be upregulated. Therefore, in the present study, it has been assumed that relative differences among fertilization methods in supplying nutrients may lead to various C/N ratios and, consequently, differences in the production of secondary metabolites (such as phenolics) in the fruit of kiwifruit vines.

In conclusion, based on the obtained results, fertilization method directly influenced the yield and leaf mineral status and indirectly influenced the fruit chemical composition of Hayward kiwifruit. In fact, the supplemental foliar nutrition decreased fruit quality (lower antioxidant activity and carbohydrate content) but enhanced the yield of kiwifruit vines. Since all kiwifruit orchards in the North of Iran are equipped with micro jet irrigation system, fertigation and foliar application not only can save time and money but also cause an increase in yield and improve some quality attributes of fruits.

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