

## Strawberry quality affected by the nitrate:ammonium ratio in the nutrient solution

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### Abstract

Strawberry (*Fragaria x ananassa* Duch.) production by using the semi-hydroponic fertigation system, is an increasingly adopted technology. One of the main challenges of this system is the nutritional management of nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ), which can affect fruits development and quality. This work aims to determine the  $\text{NO}_3^-:\text{NH}_4^+$  ratio of the nutrient solution for better quality of strawberry fruits grown in a semi-hydroponic system. ‘San Andreas’ strawberry runners were planted in an organic substrate composed by charred rice husk and pine bark. The  $\text{NO}_3^-:\text{NH}_4^+$  proportions evaluated in fertigation were 100:0; 75:25; 50:50; 25:75 and 0:100. Leaf nitrogen contents and aerial dry mass loss showed a proportional increase concomitantly  $\text{NH}_4^+$  increase on the solution. Leaf area and chlorophyll content increased up to 29%  $\text{NH}_4^+$  in the nutrient solution, while higher concentrations negatively affected these characteristics. Chemical fruits’ features were not affected by the  $\text{NO}_3^-:\text{NH}_4^+$  ratio, but the pulp firmness decreased as the  $\text{NH}_4^+$  increased in the nutrient solution. Results obtained showed the best strawberry fruits development and quality were obtained by applying  $\text{NO}_3^-:\text{NH}_4^+$  in fertigation solution at the ratio of 71:29.

**Keywords:** *Fragaria x ananassa* Duch; ammonium nutrition, ‘San Andreas’, soilless cultivation.

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## Introduction

Strawberry cultivation in the semi-hydroponic system, popularly known as soilless cultivation, is a practice applied in several regions of the planet (Recamales *et al.*, 2007; Neri *et al.*, 2012; Rampazzo, 2016). Instead of soil, it uses a wide range of raw materials to produce the substrates, such as perlite, vermiculite, peat, pine bark, charred rice husk, and coconut fiber (Lieten *et al.*, 2004; Jarosz and Konopinska, 2010; Marques, 2016). The substrates used in semi-hydroponic system usually have good physical and chemical characteristics and have good nutrient bioavailability for plant development (Abad *et al.*, 2005; Ilha, 2013). They also, are free of pest and diseases, provide more rational water and nutrients use, and are more ergonomics for labors (Rampazzo, 2016).

One of the most critical points in strawberry semi-hydroponic system is the nitrogen management by fertigation (Othman *et al.*, 2019). Nitrogen availability affects strawberries productivity and fruits quality (Nestby *et al.*, 2004; Jarosz and Konopinska, 2010). Both nitrogen forms, nitrate and ammonium salts, are absorbed and metabolized by plants (Roosta *et al.*, 2009), and play important metabolic functions, such as growth, development, production and affect fruits appearance, durability and flavor (Tabatabaei *et al.*, 2008; Krüger *et al.*, 2012; Samec *et al.*, 2016).

Despite this, there is little information about nitrate and ammonium concentration in nutrient solutions for growing strawberries. In the existing ones, the variation is quite expressive, between 4 and 12 mmol L<sup>-1</sup> for NO<sub>3</sub><sup>-</sup> and 0.2 and 2.5 mmol L<sup>-1</sup> for NH<sub>4</sub><sup>+</sup> (Hennion and Veschambre, 1997; Paranjpe *et al.*, 2003; Furlani and Fernandez, 2004). This research aimed to determine the best NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup> ratio for strawberry production by using the semi-hydroponic fertigation system.

## Material and methods

This trial was performed in Curitiba, Paraná State, Brazil (25° 24' 38.5" S; 49° 14' 57.5" W). Each 'San Andreas' strawberry runners, a neutral cultivar to the photoperiod, was planted in 8-liter black polypropylene pots on 09/07/18, filled with organic substrate (Table 1) composed by rice husk and pine bark.

**Table 1. Characteristics of the organic substrate used for cultivation of 'San Andreas' strawberry runners in a semi-hydroponic system.**

pH CaCl <sub>2</sub>	DD (kg m <sup>-3</sup> )	EC (mS cm <sup>-1</sup> )	C (g dm <sup>-3</sup> )	TP	RAW	BS	AW
				(%)			
5.65	266.78	399	151.3	84.4	7.96	1.59	9.55

DD= dry density; TP= total porosity; RAW= readily available water; BS= buffer solution; AW= available water

The experimental design applied was completely randomized with five repetitions per treatment, and each repetition with three pots. The treatments consisted of proportions of nitrate and ammonium (NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup>) in the nutrient solution of 100:0; 75:25; 50:50; 25:75; and 0:100%, with 150 mg L<sup>-1</sup> nitrogen concentration applied in all pots, based and modified by Furlani and Fernandes Júnior (2004) (Table 2).

**Table 2. Macronutrients and chlorine concentration in the nutritional solution used, according to each nitrate: ammonium proportion.**

NO <sub>3</sub> <sup>-</sup> :NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	SO <sub>4</sub> <sup>2-</sup>	Cl <sup>-</sup>
	(mg L <sup>-1</sup> )			
100-0	150	0	48.9	131
75-25	112.5	37.5	90	249.8
50-50	75	75	112.5	423.1
25-75	37.5	112.5	153.6	544.8
0-100	0	150	194.6	666.1

Other nutrients were applied in the same concentrations in all pots: 170 mg L<sup>-1</sup> of Ca, 200 mg L<sup>-1</sup> of K, 40 mg L<sup>-1</sup> of HPO<sub>4</sub><sup>-</sup>, 40 mg L<sup>-1</sup> of Mg, 32 µmol L<sup>-1</sup> of Fe, 10 µmol L<sup>-1</sup> of Mn, 30 µmol L<sup>-1</sup> of B, 3.1 µmol L<sup>-1</sup> of Zn, 1.3 µmol L<sup>-1</sup> of Cu, and 0.17 µmol L<sup>-1</sup> of Mo. The following salts were used to formulate the nutrient solution: Ca(NO<sub>3</sub>)<sub>2</sub>, KNO<sub>3</sub>, KH<sub>2</sub>PO<sub>4</sub>, MgSO<sub>4</sub>, NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, CaCl<sub>2</sub>, KCl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, H<sub>3</sub>BO<sub>3</sub>, CuSO<sub>4</sub>·5H<sub>2</sub>O, MnSO<sub>4</sub>·H<sub>2</sub>O, ZnSO<sub>4</sub>·7H<sub>2</sub>O, Na<sub>2</sub>MoO<sub>4</sub>·2 H<sub>2</sub>O, Fe-EDDHA (6%).

Fertigation was applied in each pot once a day, to maintain the substrate water retention capacity at 60%, in such way approximately 500 mL were daily applied per pot. Electrical conductivity (EC) on the nutritional solution was maintained between 1 300 and 1 800 mS cm<sup>-1</sup> and the pH of 6 ±0.2.

Leaf evaluations were performed at 60 and 120 days after planting (Dap), corresponding to the vegetative and productive stages, respectively (Nepar, 2019). The leaf area was obtained by measuring the length and width of the leaflets of the newly developed 4th leaf of the plant with a digital caliper and using the equation  $La = (\pi / 4) [(L + W) / 2]^2$  (Pires, 1999). Where La= leaf area, L= length and W= width. From the same leaflets, a tissue sample of approximately 0.17 g was collected to evaluate chlorophyll a, b and total leaves (Porra, 2002).

The material was macerated with 10 ml of 80% acetone and centrifuged at 12 000 rpm for 10 minutes and a spectrophotometer reading at 645 and 663 nm wavelengths was performed. The remainder of the 4th collected leaf was used for nutritional analysis (S, Ca, K, Mg, P, Fe and Zn) by wet digestion with nitric acid (HNO<sub>3</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and reading were done with the inductively coupled plasma optical emission spectroscopy (ICP-OES) (Varian 720-EST<sup>TM</sup>). Total N was analyzed by the Dumas combustion method in Elemental Analyzer (Carmo *et al.*, 2000).

All senescent leaves were collected through the plants' growth and dried at 65 °C until reaching constant mass. At the end of trial, all remained leaves were also dried at 65 °C until reaching constant mass. Dry weigh per plant were obtained by adding the senescence dry weight to the final leaves dry weight.

Physical and chemical fruits features were analyzed on ten fruits harvested per replicate, in three harvest dates (20/12/2017; 20/01/2018; 20/02/2018). Those with at least 3/4 reddish epidermis, minimum weight of 6 g and without injuries, diseases or deformations were chosen. First of all, pulp firmness was determined by using a texture analyzer (Brookfield CT3<sup>TM</sup>), with 2 mm tip, 5

mm penetration and 5 mm s<sup>-1</sup> penetration speed. Afterwards, strawberry juice was extracted, soluble solids content was measured on the by using a refractometer. Juice solution was diluted in water to 10% and titration was performed with 0.1 M sodium hydroxide solution (NaOH) under constant agitation, to reach the pH of 8.2.

The Bartlett test was used to verify the homogeneity of variances ( $p > 0.05$ ) of the data, followed by Anova. In case of significant variation, regression analysis was used to verify the effects of the NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup> ratios. For the statistical analysis, the software Assistat 7.7 (Silva, 2002) was used.

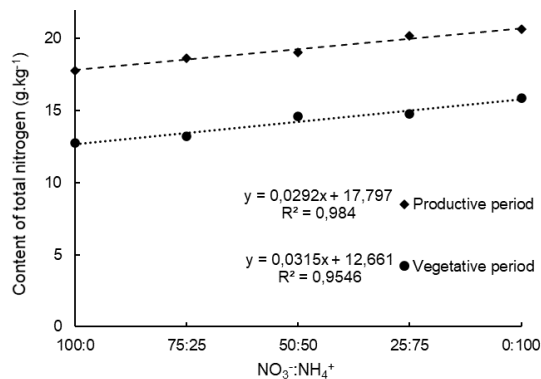
## Results and discussion

The NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup> proportions did not affect the most leaves' nutrient content (S=1.19 g kg<sup>-1</sup>; Ca=10.64 g kg<sup>-1</sup>; K=18.13 g kg<sup>-1</sup>; Mg= 3.75 g kg<sup>-1</sup>; P= 2.89 g kg<sup>-1</sup>; Fe= 69.42 mg kg<sup>-1</sup>; Zn= 23.38 mg kg<sup>-1</sup>). These nutrients levels are the similar foliar level reported in strawberry leaves by other authors (CQFS RS/SC, 2004; Nepar, 2019). It was expected did affect the Ca, K and Mg levels under the highest NH<sub>4</sub><sup>+</sup> level in the nutritive solution, through competition between ions of the same valence, either by a protein channel or for binding to a transport protein in cell membrane (White, 2012), but it not happened in this research.

We also didn't see a change in pH in the rhizosphere due to NH<sub>4</sub><sup>+</sup> absorption (Hawkesford *et al.*, 2012), which has a direct effect on nutrient bioavailability (Waller and Wilson, 1984). The substrate pH changed very few during this trial (5.65 before planting- 5.27 in 0-100% of NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup>), probably due to its organic composition. The substrate composition plays an important role since Choi *et al.* (2011) found a decrease in the substrate pH, from pH 7.0 with the use of nutrient solution without NH<sub>4</sub><sup>+</sup> to pH 5.8 with NH<sub>4</sub><sup>+</sup>, and also a decrease in the levels of K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> in strawberry leaves using a mineral substrate.

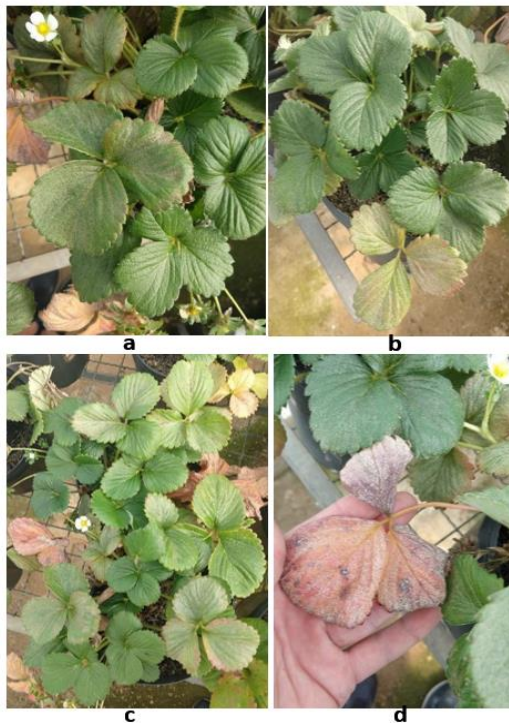
Among all nutrients evaluated in the leaf, only the total nitrogen (N) was affected by the different proportions of NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup> from the nutrient solutions. The content found was 20.64 g kg<sup>-1</sup>, when 100% of the nitrogen applied was in the form of NH<sub>4</sub><sup>+</sup>, representing an increase of approximately 14% of N in relation to the absence of NH<sub>4</sub><sup>+</sup> (Figure 1). A similar result was obtained by Tabatabaei *et al.* (2008), where the application of the maximum dose of 75% of NH<sub>4</sub><sup>+</sup> in the 'Selva' strawberry variety found an increase in the leaf content of N around 16.8%, and by Choi *et al.* (2011) with an increase of 14.2% of N when using 100% NH<sub>4</sub><sup>+</sup> in nutrient solution.

Most plants absorb the two nitrogen forms which are mediated by specific transport proteins (Roosta *et al.*, 2009). Nitrogen forms can be metabolized in the same root cell where it was absorbed or translocated unchanged to the aerial part of the plant. NH<sub>4</sub><sup>+</sup> is assimilated into root cells and its reduced form is carried to the leaves, while most of NO<sub>3</sub><sup>-</sup> is carried to the leaves and then reduced to be assimilated to amino acids. The energy requirement for NH<sub>4</sub><sup>+</sup> assimilation is lower than for NO<sub>3</sub><sup>-</sup> assimilation, since the first does not need to be reduced for its incorporation into amino acids (Bloom *et al.*, 1992; Hawkesford *et al.*, 2012; Taiz and Zeiger, 2017). Possibly, the NH<sub>4</sub><sup>+</sup> assimilated in the roots and transported in the form of amino acids, ended up accumulating in the leaves and increasing the levels of N in these tissues (Majerowicz *et al.*, 2000).

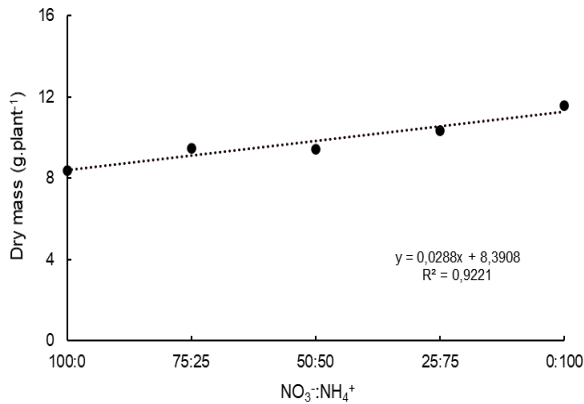


**Figure 1.** Content of total nitrogen in leaves of the ‘San Andreas’ strawberry cultivar according to  $\text{NO}_3^-:\text{NH}_4^+$  proportions in nutritive solution, in vegetative period (60 days after planting) and reproductive period (120 days after planting), in semi-hydroponic cultivation.

High concentrations of  $\text{NH}_4^+$  in the nutrient solution caused necrosis in the leaves (Figure 2), consequently increasing the dry mass of the aerial part (Figure 3), indicating a toxic effect on the plant. Choi *et al.* (2011) also observed that in high concentrations of  $\text{NH}_4^+$  in the nutrient solution, the young strawberry leaves had a matte green color, with withered and curled edges, while the older leaves were dry and tanned. The higher N content in the leaf (Figure 1) favored the production of  $\text{NH}_4^+$  through photorespiration, considered the main metabolic pathway for the production of this compound in plants (Bittsánszky *et al.*, 2015), leading to toxicity.



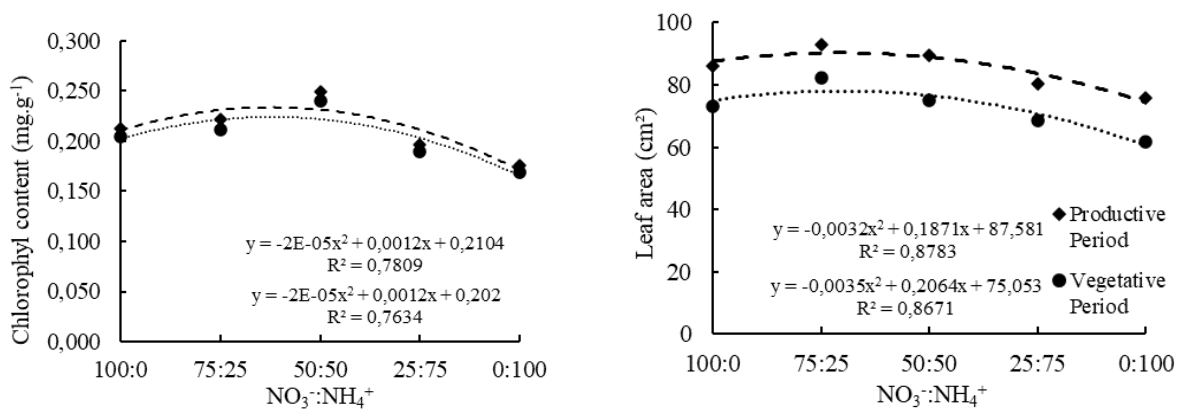
**Figure 2.** Symptoms related to  $\text{NH}_4^+$  toxic effect in strawberry plants under 0:100% treatment of  $\text{NO}_3^-:\text{NH}_4^+$  in nutrient solution, where: a) symptom onset; b) aggravated symptom; c) aerial view of the plant; and d) leaf death.



**Figure 3. Dry mass of ‘San Andreas’ cultivar after the period of 120 days after planting the seedlings, according to NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup> proportions in nutrient solution in semi-hydroponic cultivation.**

Toxicity occurs when the rate of NH<sub>4</sub><sup>+</sup> assimilation into amino acids and amides becomes lower than the rate of absorption. In the leaves, the accumulation of NH<sub>4</sub><sup>+</sup> causes damage to the structures of the chloroplast, lowering the pH of the cells to intolerable levels. This fact dissipates transmembrane proton gradients necessary for electron transport in photosynthesis, which results in a decrease in the photosynthetic rate (Bittsánszky *et al.*, 2015).

Chlorophyll levels varied similarly on 60 DAP and 120 DAP periods, under the proportions of NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup> (Figure 4). The maximum chlorophyll contents were 0.25 mg g<sup>-1</sup>, in the proportions of 36.2% NH<sub>4</sub><sup>+</sup>. Tabatabaei *et al.* (2008) also observed chlorophyll content increase using the proportion of 50% NH<sub>4</sub><sup>+</sup> in nutrient solution. In our study, increasing NH<sub>4</sub><sup>+</sup> concentration above 36% decreased leaf chlorophyll content, probably due the NH<sub>4</sub><sup>+</sup> phytotoxicity discussed earlier. Maximum levels of chlorophyll were obtained with NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> in the fertigation solution, in which the proton generated by NH<sub>4</sub><sup>+</sup> assimilation can be used to reduce NO<sub>3</sub><sup>-</sup>, thus making it easier for plants to regulate intracellular pH when both forms of nitrogen are provided (Hawkesford *et al.*, 2012).



**Figure 4. Chlorophyll content and leaf area on ‘San Andreas’ strawberry leaves according to the proportions of NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup> in nutrient solution in vegetative period (60 days after planting) and reproductive period (120 days after planting) in semi-hydroponic cultivation.**

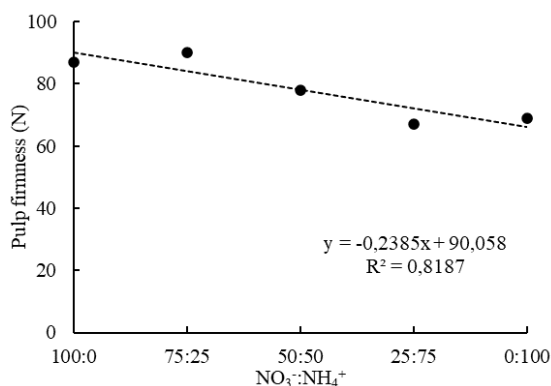


The adequate combination of  $\text{NO}_3^-:\text{NH}_4^+$  has a stimulating effect on plants because reduce the spent of energy in nitrogen uptake process, done by root cells, and in assimilation into amino acids. Therefore, the cell with your metabolism regulated and with greater energy efficiency can assimilate the nitrogen into glutamine and glutamate, as well as incorporate other amino acids that play an important role in protein and molecule formation, such as chlorophyll (Taiz and Zeiger, 2017).

The ratio 71:29 to  $\text{NO}_3^-:\text{NH}_4^+$  was the one that provided the highest leaf area, both in 60 DAP and 120 DAP, reaching 93  $\text{cm}^2$  (Figure 4). These values approximate those obtained in the studies conducted by Tabatabaei *et al.* (2008) and Choi *et al.* (2011), in which the application of 25% of the nitrogen in the form of  $\text{NH}_4^+$  resulted in larger leaf areas. A greater leaf expansion was possible, since the osmotic and energetic regulation provided by the two forms of N ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) benefited the cell elongation and plant growth (Cao and Li, 2003; Li *et al.*, 2013).

Leaf area results (Figure 4) show the same tendency as chlorophyll contents (Figure 4), since chlorophyll is the basis for the formation of various components of the plant photosynthetic apparatus, providing greater leaf development. However, there was a lower leaf growth on plants supplemented with high  $\text{NH}_4^+$  concentration (75% and 100%), probably due to higher demand of carbohydrates that are channeled for assimilation and detoxification of the large amount of this cation.

The pulp firmness was the only physical-chemical characteristic that guides the quality of the fruit affected by the proportions of  $\text{NO}_3^-:\text{NH}_4^+$  and decreased up to 25% with increasing  $\text{NH}_4^+$  levels in the nutrient solution (Figure 5). Its similar to the results found by Sokri *et al.* (2015) in apples, which the highest concentration of  $\text{NH}_4^+$  tested in the solution decreases the firmness of the pulp.



**Figure 5. Pulp fruit firmness of ‘San Andreas’ strawberry cultivar according to  $\text{NO}_3^-:\text{NH}_4^+$  proportions in nutrient solution in semi-hydroponic cultivation.**

The firmness of the pulp is closely linked to Ca since it is linked to polygalacturonic acids such as pectins. They form the Ca-pectates in the middle lamella, whose function is essential for the formation of cell walls and plant tissues. Pectate degradation is mediated by polygalacturonase, an enzyme strongly inhibited by high Ca concentrations (Wehr *et al.*, 2004). Thus, when Ca deficiency occurs, polygalacturonase activity is increased (Konno *et al.*, 1984). A typical symptom of Ca

deficiency is the disintegration of cell walls and the breakdown of affected tissues, which causes loosening and loss of fruit firmness (Ho and White, 2005). The reduction in pulp firmness may be related to the lower Ca concentration in fruits (Alan, 1989). In this experiment, although leaf concentrations of Ca were not affected by  $\text{NH}_4^+$ , it is likely that Ca concentrations in the fruit may have been influenced.

Fruits had mean values of total soluble solids (TSS) of 8.5, in °Brix, total titratable acidity (TTA) in % of citric acid of 0.97; TSS/TTA (ratio) of 8.9 and pH of 3.5. According to Kader (1999) for the strawberry flavor to be acceptable, the minimum amount of TSS recommended is around 7 °Brix. The maximum values of acidity recommended are between 0.8%, and the SS/TA ratio of at least 8.75. It was expected that SST levels would decrease and TTA levels would increase with higher concentrations of  $\text{NH}_4^+$  in the nutrient solution.

This is probably because, according to Sokri *et al.* (2015) and Zhang *et al.* (2019), the application of  $\text{NH}_4^+$  decreases the influx of K by the plant, due to the antagonism of these cations. Cation concentration is reduced with increasing ammonium concentration, which can lead to an increase in the acidity of the fruits. On the other hand, in soybean seeds and apple fruit (*Malus domestica* Borkh. cv. Fuji) fertilization with K increased TSS (Tu *et al.*, 2017; Zhang *et al.*, 2017). Although leaf tissues show symptoms of  $\text{NH}_4^+$  toxicity, in this trial, leaf K contents did not vary with the increase in  $\text{NH}_4^+$ , maintaining the chemical characteristics of the fruits.

## Conclusions

In this work, the damage caused by the excess of  $\text{NH}_4^+$  in the nutrient solution was mitigated by the use of an organic substrate in strawberry cultivation. Given the above, the exact mechanisms of the toxicity caused by  $\text{NH}_4^+$  are not fully understandable. Future studies are recommended to better elucidate how  $\text{NH}_4^+$  affected the firmness of strawberry pulp. The best  $\text{NO}_3^-:\text{NH}_4^+$  ratio for strawberry development and quality by using the semi-hydroponic fertigation system is 71:29.

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