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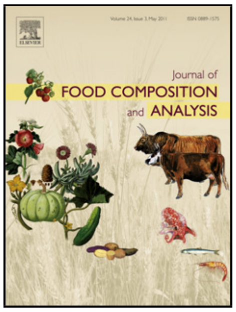

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Original Research Article

Nutritional quality and yield of onion as affected by different application methods and doses of humic substances

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Highlights

- Influence of application method and dose of humic substances was evaluated.
- A field test of onion was assessed.
- Combination of immersion plus foliar pulverization improved bulb yield and quality.
- Increasing nutrient quality of bulbs depended on the dose.

Abstract

Fertilization with humic substances (HS) has been proposed as target tool to improve crop production within a sustainable agriculture framework. The dose and application method are two factors that can influence the effect of HS on nutrient composition and productivity of onion. Therefore, our main objective was to assess the effect of each of the abovementioned factors, separately or interacting, on the quality and productivity of onion bulbs in a field test. The experimental design was completely randomized in a factorial 2 x 3, with two methods of application of HS and three different doses. The combined application method, immersion together with foliar pulverization, showed highest improvement of biomass and nutritional content of bulbs. However, while the intermediate dose of HS exerted greater increases on onion yield, productivity, carbohydrates and proteins levels in bulbs, mineral nutrient accumulation resulted especially when highest doses of HS were added. From a nutritional point of view, higher sweetness (from 113 to 149 mg g^{-1} of soluble sugars in dry matter) and an improved P, K and Mg content of bulbs (4.00, 11.65 and 3.18 g kg^{-1}, respectively) in response to HS addition has been ascribed.

Keywords: *Allium cepa*, bulb yield, carbohydrates, mineral elements, food analysis, food composition, humic substances, proteins, vegetative growth.

1. Introduction

Onion (*Allium cepa* L.) is target vegetable crop worldwide. The harvested area is about 4 million ha with yields greater than 85 million tons and a productivity of 20 thousand kg ha^{-1} in 2013, being China and India the main producing countries (FAOSTAT, 2015). Onion quality is related to the external appearance, bulb size, color, flavor, firmness and chemical composition (Grangeiro et al., 2008). These attributes are defined by factors such as genotype, pre-harvest management, proper harvesting time and post-harvest treatments (Finger & Casali, 2002). Quality parameters as onion bulb pungency level and/or sweetness can be modified due to irrigation strategy (Enciso et al., 2009), postharvest treatment (Nega et al., 2015), K application (Deshpande et al., 2013) or salt stress (Coca et al., 2012). For many years, in order to improve the productivity of crops as onion it was common to increase the planted area and/or use uncontrolled quantities of synthetic fertilizers (Ayala & Rao, 2002). However, over the years, these practices have led to soil depletion, environmental contamination and deforestation, resulting in a large ecological imbalance, affecting the sustainability of the land and food security (Suthar, 2009). Intensive agriculture has been questioned and new strategies have been adopted to improve productivity with a reduction in production costs, increased efficiency of inputs and without compromising environmental sustainability. In this context, the use of humic substances (HS) has been proposed as a viable alternative (Calvo et al., 2014).

Humic substances are composed of humic acids (HA), fulvic acids (FA) and humins, derived from biochemical transformations of compounds of soil organic matter, such as lignin, cellulose, hemicelluloses, sugars and amino acids after microbial decomposition and chemical degradation of dead biota in soils (Schiavon et al., 2010). Humic substances are reported to control nutrient availability and carbon and oxygen exchange between the soil and the atmosphere (Piccolo & Spiteller, 2003). In addition, HS affect plant physiology, promoting plant growth and therefore, considered as plant biostimulants (reviewed by Calvo et al., 2014). Enhanced root growth and nutrient uptake as N, P, Fe and Zn (Baldotto et al., 2009; Chen et al., 2004; Ertani et al., 2011;

Quaggiotti et al., 2004), auxine-like effects (Quaggiotti et al., 2004; Rodda et al., 2006; Zandonadi et al., 2007), increased concentration of chlorophyll (Baldotto et al., 2009; Ertani et al., 2011) and net photosynthesis (Canellas et al., 2002;) are the most commonly reported effects of HS on plants. The use of HS also has effect on the quality of crops, affecting concentrations of solids and soluble sugars (Lima et al., 2011), carbohydrates (Aminifard et al., 2012) and starch (Canellas et al., 2002; Ertani et al., 2011; Nardi et al., 2007).

In onion, humic substances can affect both, yield and quality of bulbs. Feibert et al. (2003) reported that soil HS application promoted crop yield and Sajid et al. (2012) observed more productivity and nutrient concentration in onion when HA was added at rates of 2 kg ha^{-1} at sowing. Similarly, foliar application of 18.5% HA increased total and marketable yield of bulbs as well as enhanced average weight of bulbs and its soluble sugars content (Kandil et al., 2013).

Different results have been described related to the influence of the method of HS application tested. Parandian and Samavat (2012) found that the immersion method was more effective than pulverization on nutrient uptake and soluble sugar concentration in *Lilium*. In contrast, Osman et al. (2013) observed positive effect of foliar application of HS in rice. Other authors found that applications of HA as both, foliar or soil treatments, significantly increased yield, total soluble sugars and chlorophyll content in pepper (Karakurt et al., 2009). With reference to HS application rates, Sajid et al. (2012) showed best performance for most of the growth and yield parameters in onion when fertilized with 2 kg ha^{-1} of HA instead of 1 or 3 kg ha^{-1}. According to Kandil et al. (2013), foliar application of 18.5% HA, applied at 60 and 80 days after transplant, increased vegetative growth, bulb yield, quality and chemical composition of onion. Nevertheless, there are results that suggest, at least in experimental conditions, that over-application of HA reduced shoot growth, transpiration and resistance to water stress but not root growth in maize (Asli & Neuman, 2010).

Therefore, the main objective of our study was to assess the effect of each of the abovementioned factors, different application methods and doses of HS, separately or interacting, on yield and nutrient composition of onion bulbs. Special attention was paid to the levels of main carbohydrates (starch and sugars), proteins and proline in bulbs as well as to their mineral analysis.

2. Materials and methods

2.1. Plant material and growth conditions

The experiment was performed from August 2012 to February 2013, in the farming area of organic vegetables of the Canguiri Experimental Station Center, Universidade Federal de Paraná (Brazil), located in the region called First Paranaense Plateau (25°25′ S, 49°08′ W, elevation 930 m). According to the Köppen classification system, the climate is temperate Cfb with marked seasonal variations. Mean field conditions were: 18.5 ^{o}C (maximum of 21.6 ^{o}C in December and minimum of 15.5 ^{o}C in August), a photoperiod of 14 h (maximum of 16 h in December and minimum of 10 h in August). Soil was prepared two weeks before seedlings were transplanted, adding 200 kg ha^{-1} of magnesium thermo phosphate (Yoorin Master 1, with 17% P_2O_5 (Agroganadera Pirapey S.A., Itapúa, Paraguay)) and 8 t ha^{-1} of organic matter (N = 14.4 g kg^{-1}; P = 10.6 g kg^{-1}; K = 11.3 g kg^{-1}; Ca = 31.7 g kg^{-1}; Mg = 6.8 g kg^{-1}; C = 384 g kg^{-1}; pH = 7.1; C/N = 27.6). This fertilization was proposed by Raij et al. (1996). The soil was a Latosol red-yellow alico with clay texture (Embrapa, 2006) and its chemical analysis in the 0-15 cm soil profile resulted in: pH ($CaCl_2$) = 5.9; pH (SMP) = 6.0; Al^{3+} = 0; $H^{+}Al$ = 4.0 C mol_c dm^{-3}; Ca^{2+} = 2.14 g dm^{-3}; Mg^{2+}= 0.55 g dm^{-3}; K^{+} = 0.52 g dm^{-3}; P = 32.6 mg dm^{-3}; C = 23.2 g dm^{-3}; B = 0.98 mg dm^{-3}; V% = 81.0 and CTC= 20.52 C mol_c dm^{-3}.

Allium cepa L. cv. Alpha San Francisco Cycle VIII (Embrapa, Brasília, Brazil) seeds were germinated on August 17th 2012 in polystyrene trays filled with the commercial substrate Plantmax® (Buschle & Lepper S.A., Santa Catarina, Brazil). Trays were kept in a greenhouse with sprinkler irrigation every two hours. When seedlings had 18-20 cm of height (Ferreira & Minami, 2000) were transplanted to field plots (18th October 2012). Four rows of plants per plot were grown, with 30 cm of row spacing and 15 cm of distance between plants in the same row, in plots of 2.16 m^2. A total of 48 seedlings were transplanted per plot and only central plants were collected for growth and chemical analysis.

The experimental design was completely randomized in a factorial 2 x 3. Two methods of application of HS were tested. The methods involved foliar pulverization of plants (FP) and immersion of seedlings together with foliar pulverization (IM+FP). Sole treatment of immersion of seedlings was not considered due to the low dose of HS application that it would suppose. The original commercial solution had 10% FA, 90% HS and pH 4.0, originating from leonardite (Nutriplant®, São Paulo, Brazil) with 34.4% C, 3.8% H and 2.3% N. Doses of humic substances in the immersion method were: 0, 10, and 20 mL L^{-1}. For foliar pulverization doses were applied ten times less concentrated (0, 1, and 2 mL L^{-1}) than in immersion. For FP method, plants were first pulverized 60 days after transplanting and afterwards, they were treated every 15 days. For IM+FP method, the immersion of plants was performed at the time of sowing, and repeated 30 and 60 days after sowing, together with the treatment of foliar pulverization. Therefore, six treatments were compared: (1) 0FP; (2) 1FP; (3) 2FP; (4) 0IM+0FP; (5) 10IM+1FP and (6) 20IM+2FP. The dose 0 was equivalent to water application instead of HS.

A final harvest was performed 95 days after transplanting, when about 85% of plants reached the stage called snap, the time that pseudostems becomes of, which is related to the end of the crop cycle.

2.2. Growth parameters and water status

At final harvest, ten plants of each treatment were randomly selected and bulb fresh weight (FW), bulb dry matter (DM) and mean productivity (MP) were determined. Mean productivity was estimated by measuring the fresh weight of ten bulbs and multiplying by 222,222 plants ha^{-1} (planting density). Bulb DM was determined after drying at 80 °C until weight was constant. Water content (WC) of bulbs was calculated: (FW of bulb – DM of bulb)/ DM of bulb, and expressed as g of water g^{-1} DM.

2.3. *Starch, total soluble sugars (TSS), total soluble proteins (TSP) and proline in bulbs*

Starch, total soluble sugars (TSS), total soluble proteins (TSP) and proline were quantified in potassium phosphate buffer (KPB; 50 mM, pH 7.5) extracts of dry bulbs (0.5 g) (n=5 bulbs). These extracts were filtered through four cheesecloth layers and centrifuged at 38720 *g* for 10 min at 4 ^{o}C. The pellet was used for starch determination (Jarvis & Walker, 1993). The supernatant was collected and stored at 4 ^{o}C for TSS, TSP and proline determinations (two replicates per sample). Soluble sugars were analyzed with the anthrone reagent in a Spectronic 2000 (Bausch and Lomb, Rochester, USA) according to Yemm and Willis (1954). Soluble proteins was measured by the protein dye-binding method of Bradford (1976) using bovine serum albumin (BSA) as a standard. The free proline was estimated by spectrophotometric analysis at 515 nm of the ninhydrine reaction (Irigoyen et al., 1992). The results were expressed as mg of starch, TSS, TSP or proline per g of bulb DM. All chemicals and standards were supplied by Panreac Química S.L.U. (Castellar del Vallès, Spain).

2.4. *Mineral analyses*

For phosphorus, potassium, magnesium, calcium, manganese, iron, zinc and copper analyses, samples (0.5 g DM) of three bulbs per treatment were dry-ashed and dissolved in HCl according to Duque (1971). Mineral concentrations were determined using a Perkin Elmer Optima 4300 inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer, Massachusetts, USA) and standards were supplied by Merck KGaA (Darmstadt, Germany). The operating parameters of the ICP-OES were: radio frequency power, 1300 W; nebulizer flow, 0.85 L min^{-1}; nebulizer pressure, 30 psi; auxiliary gas flow, 0.2 L min^{-1}; sample introduction, 1 mL min^{-1} and three replicates per sample.

Carbon and nitrogen content was determined in bulb samples (n=5) previously dried at 60 ^{o}C over 48 h and weighed. One mg aliquots were weighed in small tin capsules and, C and N determinations were carried out with an Elemental Analyser (EA) (CarboErba, Milan, Italy).

2.5. C isotopic composition ($\delta^{13}C$)

The carbono isotope composition was determined in three biological replicates ground to powder, weighed (1.0 mg per sample, n=5 bulbs) and stored in tin capsules. $\delta^{13}C$ of the samples was determined using a Flash 1112 Elemental Analyzer (CarboErba, Milan, Italy) coupled to an IRMS Delta C isotope ratio mass spectrometer through a Conflo III Interface (Thermo-Finnigan, Bremen, Germany). Results of carbon isotope ratio analyses are reported as per mile (‰) on the relative δ-scale, as $\delta^{13}C$ and refer to the V-PDB (Vienna Pee Dee Belemnite) international standard according to the following equation:

$$\delta^{13}\mathrm{C} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}}\right) - 1 \qquad \text{(Eq.1)}$$

Where *R* is the $^{13}C/^{12}C$ ratio.

2.6. Statistical analysis

Data were subjected to a two-factor ANOVA (factorial 2 x 3, Assistant Beta 7.7). The variance was related to the main factors, different application methods of humic substances (IM+FP or just FP) and different doses of humic substances (0, 10 and 20 mL L^{-1} when plants were immersed and 0, 1 and 2 mL L^{-1} for FP) and to the interaction between them (Method × dose). Means ± standard errors (SE) were calculated and, when the F ratio was significant, the Tukey´s test was applied. Tests were considered significant at $p < 0.05$.

3. Results

3.1. Growth parameters and water status

Data shown in Table 1 indicate that HS application increased bulb yield of onion when applied by FP or IM+FP method, being this enhancement mainly due to an improvement in biomass of bulbs (method, $p < 0.01$; dose, $p < 0.01$ and method x dose,

$p < 0.01$ for bulb FW and DM). The highest value of bulb FW and mean productivity of bulbs was achieved by plants grown with 10IM+1FP (with 77.22 g $bulb^{-1}$ and 17.16 t ha^{-1}, respectively). Bulb biomass was specially enhanced with combined application of HS by IM+FP methods. Plants that received an intermediate dose of HS (1FP or 10IM+1FP) showed the greater water content of bulbs.

3.2. Starch, total soluble sugars (TSS), total soluble proteins (TSP) and proline in bulbs

Concentrations of starch in bulbs were always clearly lower than those of TSS (Fig. 1). When HS were applied under IM+FP method, starch levels in bulbs exhibited an additive effect of both factors when compared with their respective controls. Concentrations of TSS were significantly influenced by each factor and the interaction between them (method, $p < 0.01$; dose, $p < 0.01$ and method x dose, $p < 0.01$ for TSS). The highest content of TSS was found in bulbs of plants that received 10IM+1FP (148.92 mg g^{-1} bulb DM). Similarly to findings of TSS, the positive effect of HS in protein levels depended on the application method and dose (method, $p < 0.01$; dose, $p < 0.01$ and method x dose, $p < 0.01$ for TSP). The lowest content of proteins in bulbs corresponded to plants grown without HS application. In contrast, plants that received 10IM+1FP showed the greatest increase due to HS adding.

The significant effect of HS addition and the interaction between the two studied factors was verified for proline (dose, $p < 0.01$ and method x dose, $p < 0.01$ for proline) (Fig. 1). Onions that had grown without HS addition showed the highest proline concentration, independently of the method of application tested.

3.3. Mineral analyses

Data shown in Table 2 indicate the significant effect of the dose of HS added and the interaction with the application method (dose, $p < 0.01$ and method x dose, $p < 0.01$) in P, K, Ca, Mg, Fe, Mn and Na. Nevertheless, the method how HS were applied did not modify concentrations of P, K, Fe, B and Na in bulbs (method, $p > 0.05$). In contrast, the treatment IM+FP improved levels of Ca, Mg, Mn and Zn in bulbs, although it depended on the dose of HS applied (method, $p < 0.01$; dose HS, $p < 0.01$ and method x dose $p < 0.01$). For Cu and Ni no interaction was observed between the two studied factors, but the main effects of application method and doses of HS significantly affected their concentration in bulbs (method, $p < 0.05$ for Cu and Ni; dose, $p < 0.01$ for Cu and doses, $p < 0.05$ for Ni). Boron levels in bulbs did not differ significantly among treatments, with values between 20.13 and 21.89 mg g^{-1} DM.

The lowest values of P, K and Mg were found in control plants grown without HS. Contrariwise, when plants were amended with 20IM+2FP treatment, onion bulbs showed the highest values of these elements (4.00 g kg^{-1} DM of P, 11.65 g kg^{-1} DM of K and 3.18 g kg^{-1} DM of Mg) (Table 2).The treatment 20IM+2FP also induced an increase of Ca and Fe levels (6.13 g kg^{-1} DM of Ca and 4.78 g kg^{-1} DM of Fe).

The intermediate dose of HS, independently of method of application employed (FP or IM+FP), increased the concentrations of Cu and Na when compared to controls. For Ni, only 10IM+1FP treatment affected its concentration in bulbs.

When HS application method was IM+FP, independently of the dose, onion bulbs showed the highest values of Ca, Mn and Zn.

3.4. Carbon and nitrogen content and C isotopic composition

Results of C and N concentrations are represented in Table 3. Nitrogen and carbon levels in bulbs were not affected by any of the factors and no significant differences were observed when HS were added, with mean values of 1.96% and 42.24% respectively. The highest value of carbon to nitrogen ratio was found in control plants subjected to IM+FP treatment (26.43), which significantly differed from the treatment 20IM+2FP with the lowest value (19.43).

The data on Table 3 indicate that the two main factors assessed in the study influenced $\delta^{13}C$ in onion bulbs (method, $p < 0.01$ and dose, $p < 0.01$). Obtained data showed that compared with the corresponding FP treatment, plants subjected to immersion (IM+FP) were more depleted in $\delta^{13}C$. In relation to the HS application, regardless of IM, treatments with 1 and 2 FP reduced ^{13}C (Table 3).

4. Discussion

In the present study the application of humic substances promoted growth, productivity and quality of onion. The values of bulb FW and mean productivity of 10IM+1FP plants were greater than the data described by Bettoni et al. (2012) studying the same cultivar (Alfa São Francisco - Cycle VIII). Our results reached 57.25 g bulb^{-1} for bulb FW and 15.46 t ha^{-1} for mean productivity, which are close to worldwide average productivity of 19.31 t ha^{-1} in 2013 (FAOSTAT, 2015). Nevertheless, treatments with highest doses of HS did not exert the most beneficial effect on bulb production of onion plants. Similar results were described by Sajid et al. (2012) in onion plants fertilized with HA. In fact, according to Asli and Neuman (2010), the over-application of HA reduced shoot growth, transpiration and resistance to water stress in maize. In agreement with those findings, the fact that plants fertilized with HS were less depleted in ^{13}C, highlighted that those plants had lower transpiration rates. $\delta^{13}C$ has been frequently described as an integrator of stomatal opening, transpiration and photosynthetic performance of several crops (Araus et al., 2003; Peuke et al., 2006; Yousfi et al., 2010, 2012). In agreement with those studies, the fact that plants fertilized with HS showed fewer reductions in ^{13}C at final harvest reveals that stomatal opening in those plants was lower, with the consequent diminishment in transpiration and photosynthetic rates.

The positive effect of HS on plant growth and productivity is probably related, in part, to their auxin-like activity (Nardi et al., 2002). Auxins activate the H^+-ATPase of the plasma membrane, acidifying the apoplast and activating enzymes that act directly on the cell wall, allowing greater plasticity of this, leading to cell elongation (Aguirre et al., 2009;Quaggiotti et al., 2004; Rodda et al., 2006; Schiavon et al., 2010; Silva et al., 2011a; Zandonadi et al., 2007). Plant growth enhancement may also be due to the presence of polyamines, such as putrescine, spermidine and spermine found in HS (Young & Chen, 1997), that act as growth regulators of plants (Kumar et al., 2007). On the other hand, Dobbss et al. (2007) attributed the growth promotion of organic matter to alkylamides, a new class of compounds with hormonal action, which provide stimulating root growth independently of auxin signal (Ramírez-Chávez et al., 2004).

The combined application method of HS, IM+FP, exhibited a cumulative effect on non-structural sugars (starch and soluble sugars) in bulbs. Increased total soluble sugars content in plants that received HS have been described by other researchers (Ertani et al., 2011; Nardi et al., 2007; Parandian & Samavat, 2012). They attributed such increments to the promotion of photosynthesis with increased chlorophyll content and Rubisco activity (Ertani et al., 2011). Recently, Bettoni et al. (2014) found that HS enhanced chlorophyll concentration in onion plants. However, the increase of chlorophyll alone due to HS addition do not necessarily results in higher yields (Nardi et al., 2002).

From a nutritional point of view, bulbs obtained after applying 10 mL L^{-1} of HS by immersion plus 1 mL L^{-1} of HS by foliar pulverization (10IM+1FP) would be adequate for supplying energy through the diet due to their high concentration of TSS (Abou Azoom et al., 2015). Moreover, as the cv. Alfa San Francisco Cycle VIII is usually consumed in salads, this application method and doses of HS (10IM+1FP) would increase its sweet flavor and presumably its acceptance by the consumers. On the other hand, bulbs from plants that received the highest level of HS (2FP and 20IM+2FP) would be a better food source for diabetic people. According to Boyhan et al. (2001), treatment with humic acids resulted in greater percent of marketable bulbs after controlled atmosphere storage compared to the untreated check, with no influence in yield and soluble solids. In our case, 10IM+1FP treatment enhanced bulb fresh and water content concomitant with increased concentration of soluble sugars. Apart from carbohydrates, bulbs of onions fertilized by immersion (10 mL L^{-1}) and further foliar pulverization (1 mL L^{-1}) with HS (10IM+1FP) accumulated the highest content of soluble proteins, which enhances their nutritional value. Protein concentration in onion bulbs was also higher in 10IM+1FP plants, which indicates that HS influenced N cell metabolism. Some authors have reported that HS improved NO_3^- concentration in plants (Mora et al., 2010).The reduction of the pH on the root surface, thus facilitating H^+/NO_3^- symport uptake (Nardi et al., 2000; Quaggiotti et al., 2004), in addition to increased activity of the enzymes glutamine synthetase (GS) and glutamate synthase

(GOGAT), which act in the availability of NH_4^+, could enhance N organic compounds in plants (Ertani et al., 2011). Moreover, increased nutrient uptake due to HS application has been linked to increased foliar content of some aminoacids (Schiavon et al., 2010).

In contrast, proline levels were greater when plants were not fertilized with HS. This result can be attributed to a metabolic imbalance. In cabbage, similar results were observed, with an accumulation of proline in plants grown under a conventional system in comparison with ones that received humic substances (Vilanova & Da Silva Junior, 2010). Plants under unfavorable growth conditions or metabolic imbalance mobilize carbohydrates for the synthesis of proline (Díaz et al., 2012). In a field study with pistachio subjected to salt stress, humic acids ameliorated negative effects on plant growth related to a reduction in proline accumulation (Moghaddan & Soleimani, 2012).

How humic substances affect plant uptake of ions varies depending on the type and concentration of HS, the pH of the growing medium and plant species (Muscolo et al., 2007; Nardi et al., 2009). In general, the highest dose of HS and the combination of application methods (treatment 20IM+2FP) showed higher levels of mineral nutrients in bulbs such as P, K and Mg. Similarly, Sajid et al. (2012) observed higher nutrient concentrations in onion when HS were applied. Humic substances have the capacity to chelate ions and form complexes (Eyheraguibel et al., 2008), therefore, it is not surprising an increase of plant nutrient uptake. Moreover, according to Canellas and Santos (2005), HS stimulates H^+-ATPase and promotes the acidification of the cell wall, which in turn increases its permeability, thereby allowing the entry of nutrients. On the other hand, as described above, HS have auxin-like effects on plants. In this sense, enhanced root growth and lateral root development are the most commonly reported effects of HS on plant growth (reviewed by Calvo et al., 2014). This fact could also contribute to the increase of nutrient content of onion bulbs.

Eradication of ‘hidden hunger’ (a term used to describe the malnutrition inherent in human diets that are adequate in calories but lack in vitamins and/or mineral nutrients such as Ca, Mg, Fe, Zn, Cu, Se or I), represents a target aspect for food security programs (White & Broadley, 2009). Many people in developed countries (e.g., United Kingdom or USA) do not consume adequate quantities of Cu (Copper Development Association, 2011). Fe deficiency is one of the major public health problems in more than 130 nations, including developed countries, and nearly 50% of the world's population is at risk of inadequate Zn uptake (FAO/WHO, 2001). In this sense, the combination of immersion plus foliar pulverization (IM+FP) at different doses of HS (10+1 and 20+2) appeared as the most adequate method for increasing the levels of several minerals in onion bulbs, including Ca, Mg, Mn and Zn. The application of the highest doses of HS (20IM+2FP) also enhanced the content of Fe in bulbs and plants fertilized by 1FP or 10IM+1FP treatments showed the highest Cu levels.

However, concentrations of N and C in onion bulbs have not been affected due to HS application. Addition of HS can improve the photosynthetic capacity of plants (Calderín et al., 2012; Canellas et al., 2002). According to our results, this fact can be explained by the mobilization of carbon for the synthesis of other compounds such as sugars and starch as explained before. Likewise, HS may increase plant uptake of N (reviewed by Calvo et al., 2014). According to Fatideh and Asil (2012), onion bulb size and weight are increased with intensification of amount of nitrogen fertilizer, while our data showed increased bulb biomass related to greater levels of proteins in plants that received HS. In addition to isotope analyses, the greatest accumulation of ^{13}C was observed when HS were applied by foliar pulverization, which means that bulbs were considered preferred sinks for photoassimilates when compared to other treatments (Silva et al., 2011b).

5. Conclusions

Humic substances fertilization appears as valid horticultural technique for improving productivity and nutritional quality of onion bulbs, although it depended on the dose and method of addition. The immersion of onion plants in a dose of 10 mL L^{-1} associated with 1 mL L^{-1} foliar pulverization results in increases of bulb fresh and dry mass as well as the average water content and productivity. The same treatment also had a positive effect on the chemical composition of onion bulbs, with higher starch, total soluble sugars and proteins. The combined application method together with the highest dose tested (20 mL L^{-1} for IM and 2 mL L^{-1} for FP) was the most effective treatment of HS application for improving main mineral elements in bulbs.

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Figure captions

Fig. 1. Concentrations of starch (mg g^{-1} DM), total soluble sugars (TSS) (mg g^{-1} DM), total soluble proteins (TSP) (mg g^{-1} DM) and proline (mg g^{-1} DM) in onion bulbs after foliar pulverization (FP) or immersion plus foliar pulverization (IM+FP) with different doses of humic substances (HS) (0, 10 and 20 mL L^{-1} HS for IM and 0, 1 and 2 mL L^{-1} HS for FP). Values are means ± SE (n = 5). Within each parameter bars with different letters indicate that values are significantly different at $p < 0.05$. DM = dry matter.

Figures

Figure 1

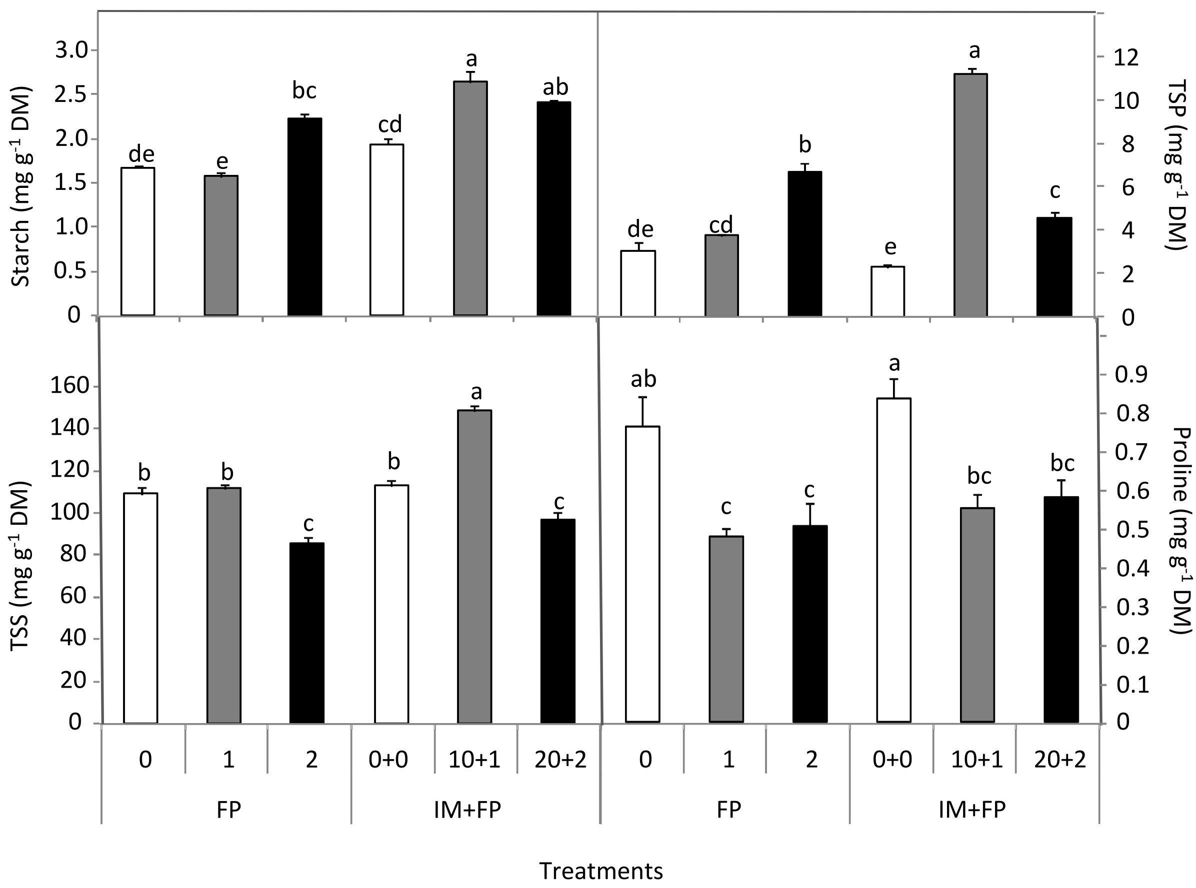

Table 1

Yield parameters and water status in onion bulbs after foliar pulverization (FP) or immersion plus foliar pulverization (IM+FP) with different doses of humic substances (HS) (0, 10 and 20 mL L^{-1} HS for IM and 0, 1 and 2 mL L^{-1} HS for FP).

Treatment Method	Dose	**Bulb FW** (g)	**Bulb DM** (g)	**Bulb WC** (g H_2O g^{-1} DM)	**MP** (t ha^{-1})
FP	**0**	34.6 ± 0.37 e	3.22 ± 0.02 d	9.73 ± 0.07 c	7.68 ± 0.08 e
	1	56.6 ± 1.48 b	4.03 ± 0.06 b	13.1 ± 0.39 ab	12.6 ± 0.33 b
	2	42.0 ± 1.76 d	3.18 ± 0.05 d	12.2 ± 0.40 b	9.33 ± 0.39 d
IM+FP	**0+0**	33.9 ± 0.19 e	3.58 ± 0.01 c	8.49 ±0.02 c	7.54 ± 0.04 e
	10+1	77.2 ± 1.67 a	5.26 ± 0.05 a	13.7 ± 0.39 a	17.2 ± 0.37 a
	20+2	49.3 ± 1.03 c	5.10 ± 0.03 a	8.68 ± 0.23 c	11.0 ± 0.23 c
Method		**	**	**	**
Dose		**	**	**	**
Method x Dose		**	**	**	**

Values are means ± SE (n= 10). Within each parameter data followed by the same letter indicate that values are similar ($p < 0.05$). ANOVA: ns = not significant; *, ** and *** = significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. FW = fresh weight; DM = dry matter; WC = water content; MP = Mean productivity.

Table 2

Concentrations of mineral nutrients in onion bulbs after foliar pulverization (FP) or immersion plus foliar pulverization (IM+FP) with different doses of humic substances (HS) (0, 10 and 20 mL L^{-1} HS for IM and 0, 1 and 2 mL L^{-1} HS for FP).

Treatment Method	Dose	**P** (g kg^{-1} DM)	**K** (g kg^{-1} DM)	**Ca** (g kg^{-1} DM)	**Mg** (g kg^{-1} DM)	**Fe** (g kg^{-1} DM)	**Cu** (mg g^{-1} DM)	**Mn** (mg g^{-1} DM)	**Zn** (mg g^{-1} DM)	**B** (mg g^{-1} DM)	**Ni** (mg g^{-1} DM)	**Na** (mg g^{-1} DM)
FP	**0**	3.24 ± 0.018 c	10.3 ± 0.020 c	5.10 ± 0.013 cd	1.82 ± 0.048 e	3.93 ± 0.052 c	16.2 ± 0.11 b	25.8 ± 0.29 b	37.8 ± 0.08 b	20.1 ± 0.35 a	7.83 ± 0.19 b	354 ± 9.75 c
	1	3.78 ± 0.028 b	10.8 ± 0.023 bc	5.33 ± 0.025 c	2.84 ± 0.014 bc	4.36 ± 0.047 b	17.9 ± 0.16 a	24.3 ± 0.26 bc	39.0 ± 0.42 b	21.9 ± 0.85 a	8.46 ± 0.27 ab	406 ± 7.90 a
	2	3.85 ± 0.008 ab	11.2 ± 0.245 ab	4.88 ± 0.088 d	2.71 ± 0.034 c	4.46 ± 0.038 b	15.4 ± 0.20 b	21.0 ± 1.16 c	39.0 ± 0.48 b	21.6 ± 0.71 a	7.83 ± 0.37 b	386 ± 5.84 abc
IM+FP	**0+0**	2.97 ± 0.045 d	9.66 ± 0.070 d	5.21 ± 0.037 c	2.00 ± 0.036 d	3.83 ± 0.112 c	16.3 ± 0.26 b	26.6 ± 0.75 b	40.0 ± 0.83 b	20.4 ± 0.35 a	8.50 ± 0.38 ab	397 ± 9.36 ab
	10+1	3.84 ± 0.063 ab	11.2 ± 0.056 ab	5.84 ± 0.093 b	2.89 ± 0.040 b	4.05 ± 0.053 c	18.6 ± 0.40 a	38.3 ± 1.14 a	43.9 ± 0.63 a	20.2 ± 0.42 a	9.41 ± 0.28 a	402 ± 6.71 a
	20+2	4.00 ± 0.006 a	11.7 ± 0.161 a	6.13 ± 0.039 a	3.18 ± 0.016 a	4.78 ± 0.041 a	16.3 ± 0.26 b	37.3 ± 0.73 a	44.7 ± 0.80 a	22.3 ± 0.96 a	8.16 ± 0.14 ab	365 ± 4.60 bc
Method		ns	ns	**	**	ns	*	**	**	ns	*	ns
Dose		**	**	**	**	**	**	**	**	ns	*	**
Method x Dose		**	**	**	**	**	ns	**	*	ns	ns	**

Values are means ± SE (n= 3). Within each parameter data followed by the same letter indicate that values are similar ($p < 0.05$). ANOVA: ns = not significant; *, ** and *** = significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. DM = dry matter.

Table 3 Concentrations of nitrogen, carbon, carbon to nitrogen ratio and isotope ^{13}C in onion bulbs after foliar pulverization (FP) or immersion plus foliar pulverization (IM+FP) with different doses of humic substances (HS) (0, 10 and 20 mL L^{-1} HS for IM and 0, 1 and 2 mL L^{-1} HS for FP).

Treatment		**N**	**C**	**C/N**	**$\delta^{13}C$**
Method	Dose	(%)	(%)		
FP	**0**	1.83 ± 0.10 a	41.9 ± 0.46 a	23.0 ± 1.48 ab	-29.4 ± 0.04 bc
	1	2.09 ± 0.14 a	42.2 ± 0.68 a	20.3 ± 1.09 ab	-28.9 ± 0.06 a
	2	2.22 ± 0.09 a	43.0 ± 0.25 a	19.4 ± 0.87 b	-29.0 ± 0.08 ab
IM+FP	**0+0**	1.66 ± 0.12 a	43.5 ± 0.21 a	26.4 ± 1.66 a	-30.0 ± 0.08 d
	10+1	1.87 ± 0.14 a	39.9 ± 3.78 a	21.4 ± 1.62 ab	-29.3 ± 0.14 abc
	20+2	2.09 ± 0.15 a	43.0 ± 0.49 a	20.8 ± 1.68 ab	-29.7 ± 0.15 cd
Method		ns	ns	ns	**
Dose		*	ns	*	**
Method x Dose		ns	ns	ns	ns

Values are means ± SE (n= 5). Within each parameter data followed by the same letter indicate that values are similar ($p < 0.05$). ANOVA: ns = not significant; *, ** and *** = significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.