BIOSCIENCE JOURNAL

ORGANIC ONION GROWTH, YIELD AND STORAGE IMPROVED BY FOLIAR SPRAYS OF MICROALGAE AND FULVIC ACID AS A NATURAL BIOFERTILIZER

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How to cite: GEMIN, L.G. et al. Organic onion growth, yield and storage improved by foliar sprays of microalgae and fulvic acid as a natural biofertilizer. *Bioscience Journal*. 2022, **38**, e38045. https://doi.org/10.14393/BJ-v38n0a2022-58854

Abstract

The use of microalgae as natural biofertilizer in horticulture has recently been reported, while the use of humic substances is widespread. However, the combined use of microalgae with humic substances applied to plant leaves is still unexploited. Thus, the objective of this work was to combine fulvic acid (FA) with the *Scenedesmus subspicatus* microalga biomass (SC) as a natural biofertilizer applied via leaf in two onion cultivars in organic system. Four experiments were conducted: i) bioassays to verify the bioactive effect of FA, SC and combinations using the *Vigna radiata* model plant; ii) greenhouse pot experiment with foliar applications of FA, SC and combination in two onion cultivars; iii) field experiment in organic system with foliar applications of FA, SC and combination in two onion cultivars; and iv) onion bulb storage experiment. The bioactive effect of SC, FA and their combinations was identified by promoting changes in root growth of *V. radiata*. In pots, treatments containing FA, SC and combination promoted increase in fresh and dry leaf mass. The foliar application of FA, SC and combination promoted an increase in field bulb productivity, reduced mass loss in stored bulbs and increased carbohydrate, amino acid and protein contents in onion bulbs.

Keywords: *Allium cepa* L. Humic substances. Organic farming. *Scenedesmus subspicatus*.

1. Introduction

The production of foods free of synthetic substances has been valued by consumers, causing farmers to migrate to alternative production systems such as organic farming (Madail et al. 2011). As a result, the development of sustainable technologies, suitable for the organic system and providing productivity and storage gains has become strategic.

As a sustainable technology Biofertilizers can be composed of natural substances or microorganisms which can promote plants growth and productivity (Du Jardin 2015). Furthermore, Kelp extract and humic substances (HS) are reported as biofertilizer sources, and recently, the use of microalgae in horticulture showed effect on promoting plant growth as well as increasing productivity (Mógor et al. 2018b).

Estimates indicate the existence of 800,000 microalgae species, and only about 6.25% have been described (Suganya et al. 2016). Recent studies approach the potential of using microalgae, as these unicellular organisms present compounds of interest to the biofuel, pharmaceutical, animal and human

nutrition industries (Ishaq et al. 2016). Additionally, phytohormones such as auxins and cytokines were found in microalgae *Arthrospira platensis* and *Scenedesmus almeriens*. These hormones when applied to plants can provide significant increase in root growth (Plaza et al. 2018).

Studies using *Arthrospira platensis* in lettuce (Mógor et al. 2018a), *Acutodesmus dimorphus* in tomato plants (Garcia-Gonzalez and Sommerfeld 2016), *Scenedesmus subspicatus* in onion (Gemin et al. 2019) and in beet (Barone et al. 2017) identified the growth promoting effect of these microalgae.

In addition to microalgae, the most studied fractions of HS, humic and fulvic acids also influence promoting plant growth and development (Canellas et al. 2015). When studying the foliar application of HS in onion cultivation, Bettoni et al. (2016) observed the increment in total solids concentration, as well as a gain of fresh and dry mass in bulbs. This effect on plant growth and development can be partly explained by the positive effect of humic substances on plant photosynthetic metabolism and by the stimulation of the enzyme H+ATPase, an effect like phytohormones auxin (Canellas and Olivares 2014).

The use of humic acid and microalgae applied via leaves results in the promotion of plant growth with productivity gains, greater accumulation of sugars in plants and reduction in mass loss over storage (Bettoni et al. 2016; Shehata et al. 2017; Mógor et al. 2018a). The combination of humic acid and microalgae was recently reported in a study of onion seedlings immersion with significant yield gains in the organic system (Gemin et al. 2019). Yet, the foliar use of fulvic acid combined with microalgae is still unexplored.

Therefore, the objective of this work was to evaluate the effect of foliar applications of fulvic acid and *Scenedesmus subspicatus* microalga biomass, alone or in combination through the implementation of four experiments: (i) bioassay using *Vigna radiata* L. model plant to identify bioactivity of microalga biomass (SC), fulvic acid (FA) and their combinations (FS) in promoting root growth; (ii) initial growth of onion plants grown in pots in a protected environment submitted to foliar applications of FA, SC and FS; (iii) field experiment in organic system evaluating the effect of FA, SC and FS foliar applications on yield and biochemical alterations in bulbs of two cultivars; and (iv) influence of foliar application of FA, SC and FS on onion plants on mass loss and biochemical alterations of bulbs stored for 60 days.

2. Material and Methods

The fulvic acid (Fulvital® wsp80) Humin tech (GmbH - Germany) used in the experiment is a dry powder extracted from the mineral Leonardite with high water solubility and suitable for use in organic agriculture (EU Reg.EC 834/2007 and 889 / 2008).

The microalga *Scenedesmus subspicatus* Chodat (synonym: *Desmodesmus subspicatus*) biomass was obtained in an autotrophic axenic cultivation performed in semi-continuous photobioreactor, using WC culture medium maintained at constant temperature (20–22°C) and light (5500 lx), at the Crop Sciences and Plant Protection Department of the Federal University of Paraná. After 25-day cultivation period, the biomass was separated from the culture medium through centrifugation, attaining 0.95 g L^{-1} DW and then it was lyophilized. The strain was provided by the "Elizabeth Aidar" Microalgae Collection from the Fluminense Federal University, Niteroi, Rio de Janeiro - Brazil.

Bioassays

Bioassays were conducted with *Vigna radiata* L. using growth chambers with controlled temperature and light (22ºC; photon flux intensity: 0.52–0.56 mmol/m^2 /sec) for 15 days (Mógor et al. 2018a). The objective was to evaluate the possible bioactivity of SC (first step) and FA and of combinations of SC (second step) in root growth.

The treatments used to evaluate the microalga SC lyophilized biomass were (first step) the following: control with distilled water, 0.25, 0.50, 0.75 and 1.00 g L^{-1} SC. The treatments using FA and combinations with SC were (second step) the following: distilled water control, 0.05 g L^{-1} FA; 0.10 g L^{-1} FA; 0.15 g L^{-1} FA; 0.2 g L^{-1} ; FA; 0.05 g L^{-1} FA + 0.5 g L^{-1} ^{1} SC (0.05 FS); 0.10 g L^{-1} FA + 0.5 g L^{-1}SC (0.1 FS); 0.15 g L^{-1} FA + 0.5 g L^{-1} SC (0.15 FS) and 0.20 g L^{-1} FA + 0.5 g L^{-1} SC (0.2 FS).

Average root length (sum of the length of all roots) and root average volume were determined using the Winrhizo Pro® software (Regent Instr.® Canada) coupled with an Epson® double lens scanner (V700 PHOTO model).

A completely randomized design was used in the first step; five treatments were used and in the second, nine treatments, both with 4 repetitions containing 8 plants per repetition. Data were submitted to test of Shapiro-Wilk, Bartlett, and ANOVA tests. When significant, the mean test adopted was Scott-Knott ($p < 0.01$) using the Assistat 7.7 beta statistical software.

Pot experiment

Based on the results obtained in the bioassay, concentrations of FA, SC and combination (FS) were defined to conduct the experiment in a greenhouse. Direct sowing was conducted in plastic pots (3 L) of two onion cultivars, one open pollinated ('B' - cv. BR-29) and the other hybrid ('P' - cv. Perfecta F1) both from Topseed ®. The pots were filled with soil (0-40 cm depth Latosol red-yellow alico) suitable for onion cultivation, with: 5.84 pH (H_2O); 26.31 $g.dm^{-3}$ organic matter; 49 $mg.dm^{-3}$ P; 1.32 $cmol_c.dm^{-3}$ K; 5.28 $cmol_c.dm^{-3}$ Ca; 3.05 $cmol_c.dm^{-3}$ Mg; 0 $cmol_c.dm^{-3}$ Al; 2.93 $cmol_c.dm^{-3}$ Al+H; 12.58 $cmol_c.dm^{-3}$ CEC and 76.7% of base saturation.

The treatments consisted of solutions containing the control with distilled water, 0.2 g L^{-1} FA, 0.5 g L^{-1} SC and combination with 0.2 g L^{-1} FA + 0.5 g L^{-1} SC (FS), applied weekly totaling 6 applications. A Kawashima electric sprayer (model Kws Pem-P20) was used, maintaining constant pressure (2 bar) in the applications, resulting in a spray volume of 60 mL per repetition.

To identify the effect of the applications on the initial growth, the aerial part of the onion plants was collected after 60 days of sowing (DAS). Data of fresh mass, dry mass, and fresh/dry mass ratio of the aerial part of both cultivars were obtained using a two-digit digital scale after the dots.

The experimental design was completely randomized in a factorial scheme (n = 4) (factor A was the cultivars; factor B the treatments), each repetition consisting of two pots containing three plants per pot. The average data per pot were submitted to Shapiro-Wilk, Bartlett and ANOVA tests. The mean test adopted when significant was Scott-Knott ($p < 0.05$) using the Assistat 7.7 beta statistical software.

Field experiment

The experiment was conducted in the organic garden area of the Federal University of Paraná (UFPR), where the organic production system has been adopted for over a decade. The climate of the region according to the Köppen classification is Cfb. The soil was a Latosol red yellow alico with clay texture and chemical composition of the soil shows the following average values: 6.30 pH (H_2O), 33.30 $g.dm^{-3}$ organic matter; 133.10 $mg.dm^{-3}$ P; 1.44 $cmol_c.dm^{-3}$ K; 9.30 $cmol_c.dm^{-3}$ Ca, 4.30 $cmol_c.dm^{-3}$ Mg; 0 $cmol_c.dm^{-3}$ Al; 3.7 $cmol_c.dm^{-3}$ Al+H; 18.34 $cmol_c.dm^{-3}$ CEC and 80% base saturation. One week prior to onion seedling transplantation, soil preparation was conducted with the incorporation of organic compost at a dose of 8 t ha^{-1}, whose chemical characteristics were the following: C = 30.3 g kg^{-1}; N = 30.3 g kg^{-1}; P = 8.5 g kg^{-1}; K = 6.6 g kg^{-1}; Ca = 8.1 g kg^{-1} and Mg = 4.1 g kg^{-1}.

Seedling production was conducted in beds protected by a polyethylene tunnel, where the same onion cultivars ('B' and 'P') of the pot experiment were sown. After 45 sowing (DAS) the seedlings were ready for transplanting, with an average of 5 leaves, with average pseudo stem diameter of 10.94mm and 13.62mm for 'B' and 'P', respectively. The onion seedlings were transplanted into beds with a size of 1.20 x 48 m, with a spacing of 30 cm between rows and 10 cm between plants, distributed in 4 planting rows, equivalent to a plant population of 230,000 per hectare.

The treatments were the same as those used in the pot experiment. Applications began simultaneously with the beginning of bulb development (90 DAT). Seven foliar applications were performed at weekly intervals, ending at 140 DAT. An electric sprayer (Kawashima – model Kws Pem-P20) with a constant pressure of 3 bar was used, and a spray volume equivalent to 600 $L.ha^{-1}$ per application was used for each treatment.

Biometric and yield analysis

At 150 days after transplanting, about 80% of the plants showed collapse of the pseudo stem, thus indicating the proper harvest time. Eight onion plants were collected from each repetition, separating the leaves from the bulbs. Following this, the fresh mass was measured using a digital scale. For the dry mass, the bulbs were cut into 8 parts, placed in paper bags, and placed in an oven at 60 °C with forced ventilation until they reached constant weight. Then, the bulb dry mass was measured, allowing the calculation of the fresh and dry mass ratio. Yield data were calculated according to the average fresh bulb mass per treatment extrapolated per hectare for a population of 230,000 plants.

Bulb calibers were classified according to the Brazilian commercial classification (MAPA - Ministry of Agriculture, Livestock and Supply), which classifies the bulbs into the following types: type 1 (<35mm), type 2 (35 to 50 mm), type 3 (50 to 70 mm), type 4 (70 to 90 mm) and type 5 (> 90mm).

Storage of bulbs for 60 days

To verify the weight loss (WL) of the bulbs over storage, 8 bulbs of each repetition per treatment (control, FA, SC and FS) of each cultivar ('B' and 'P') were stored for 60 days. So, plastic boxes were used to pack the bulbs in a cool and ventilated place, with average temperature of 23.5°C and relative humidity of 74% ± 5. The weight loss was calculated using equation 1:

$$WL(\%) = \frac{(W_i - W_f)}{W_i} \cdot 100 \quad (1)$$

Where:

W_i= initial weight; and

W_f= final weight.

Biochemical analysis

For the quantification of total sugars, reducing sugar, total free amino acids and soluble proteins of the bulbs, four bulbs of each repetition per treatment of the field experiment and four bulbs of each repetition per treatment after 60 days of storage were selected.

For the quantification of total sugars, 1 ml of homogenized sample was collected and then added with 1 ml of HCl for acid hydrolysis in a water bath (100°C) for 10 minutes. Then, 1 ml of NaOH solution was added into it and allowed to rest for 5 minutes. After this procedure, it was added to the 3,5-Dinitrosalicylic acid (DNS) solution in the sample of total sugars and reducing sugar and reading was performed in a spectrophotometer. The standard curve for reducing and total sugars was made with 1 mg/mL (5.5 mM) glucose with ranging from 50 to 800 µg/mL (Maldonade et al. 2013).

Total free amino acids were extracted according to Winters et al. (2002), and the colorimetric reaction was performed according to Magné and Larher (1992) using glutamine for the standard curve. Therefore, in a test tube containing the sample and distilled water, they were conditioned in the water bath 100°C for 15 minutes, which allowed sample cooling and decanting for 20 minutes in an ice bath. After that, the samples were collected and centrifuged at 3000 rpm for 10 minutes. One millimeter of the sample, 0.5 ml of citrate buffer, 1 ml of ninhydrin were added into the colorimetric reaction and shaken for 2 seconds. The water bath was also used for 15 minutes at 100°C, and then it was allowed to cool by adding 60° alcohol and spectrophotometer readings were conducted.

Soluble proteins were determined by adopting the methodology described by Bradford (1976) using BSA for the construction of the standard curve. For extraction of the proteins in the sample (0.5 ml), 1.5 ml of phosphate buffer was added as described by Du et al. (2010). Then, the sample was centrifuged at 10,000 rpm for 15 minutes. After that, 70 µl of the supernatant plus 2 ml of the Bradford reagent was then added and left for rest for 15 minutes, so, the sample could have been read in a spectrophotometer.

Statistical analysis

The experimental design used for the variables fresh mass, dry mass, fresh and dry mass ratio, yield, storage and biochemical losses was a completely randomized in a factorial scheme (n = 4) (factor A the cultivars, factor B the treatments), totaling 4 repetitions per treatment. Data were submitted to Shapiro-Wilk, Bartlett and ANOVA tests. The mean test adopted when significant was Scott-Knott ($p < 0.05$). For the bulb caliber classification variable, the Kruskall-Wallis non-parametric test ($p < 0.05$) was used, and all statistical data were processed with the aid of Assistat 7.7 beta software.

3. Results

Bioassays

The results of root length and volume of *V. radiata* plants showed that the treatments containing the microalga SC (Figure 1A and B) positively influenced rooting promotion. For both variables, the concentration 0.5 $g.L^{-1}$ SC increased the average length by 45% and root volume by 66% compared to the control. Root length promotion was also observed among treatments containing FA (Figure 1C), on average showing 28% longer roots when compared to control. Nonetheless, the combination of SC (0.5 $g.L^{-1}$) with the different FA (FS) concentrations increased the root length by 36% when compared to the control (Figure 1C).

For root volume (Figure 1D), the lowest dose of FA (0.05 $g.L^{-1}$) did not differ statistically from the control. However, 0.10 and 0.15 $g.L^{-1}$ of FA showed significant gain in root volume when compared to control. Moreover, 0.2 gL^{-1} FA treatment and combinations containing concentrations of 0.05 FS, 0.1 FS, 0.15 FS and 0.20 FS showed better effect, increasing root volume by 16.5% on average when compared to control.

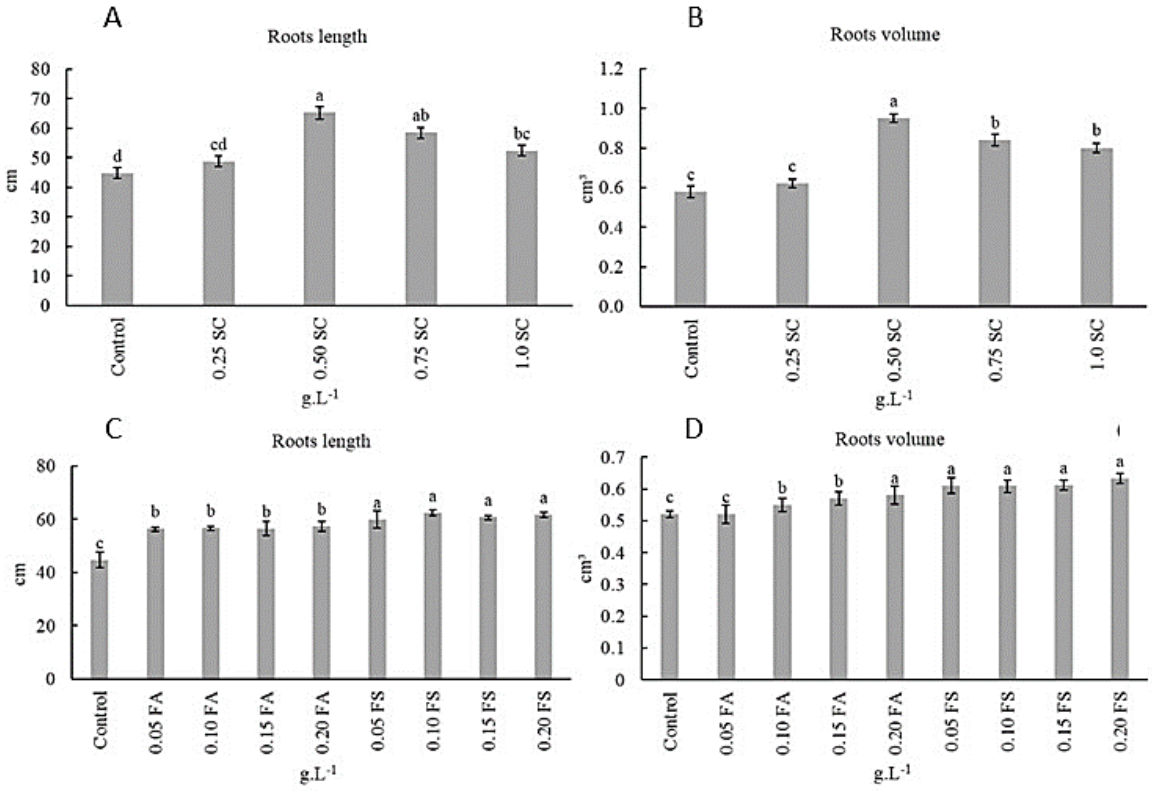

Figure 1. Average values of roots length (a) and volume (b) of *Vigna radiata* plants under treatments with: control and microalga *Scenedesmus subspicatus* (SC) lyophilized biomass aqueous solutions; Average values of roots length (c) and volume (d) of *Vigna radiata* plants under treatments with: control, fulvic acid (FA) and fulvic acid plus *Scenedesmus subspicatus* (FS) lyophilized biomass aqueous solutions. CV% (1a) = 3,49; CV% (1b) = 3,67; CV% (1c) = 2,61 and CV% (1d) = 2,88.

Pots experiment

Among cultivars, 'P' plants presented on average higher fresh and dry mass when compared to 'B' plants. The treatments showed significant differences in fresh and dry weight. Plants receiving FA (0.2 $g.L^{-1}$), SC (0.5 $g.L^{-1}$) and FS (0.2 $g.L^{-1}$ FA + 0.5 $g.L^{-1}$ SC) in foliar application showed an increase by 19% in fresh mass and 32% in leaf dry mass when compared to the control (Table 1).

The ratio between fresh and dry weight of the leaves among the cultivars did not present significant differences. However, it was observed that the mean value of the ratio was higher in the control when compared to the other treatments.

Table 1. Effect of foliar spray with fulvic acid (FA), *Scenedesmus subspicatus* (SC) and combination (FS) treatment on fresh and dry weight and fresh/dry weight ratio of aerial parts of two onion cultivars conducted in the organic system. Values represent the means and ± the standard deviation.

Fresh Weight					
	Control	FA	SC	FS	$\bar{x}$
'B'	8.03 ± 0.58	9.24 ± 0.92	9.45 ± 0.68	9.86 ± 1.55	9.15[b]
'P'	13.72 ± 1.97	17.30 ± 2.15	15.53 ± 1.78	16.85 ± 1.15	15.85[a]
$\bar{x}$	10.88[b]	13.27[a]	12.49[a]	13.35[a]	
ANOVA					
C	**				
T	**				
C x T	ns				
Dry Weight					
	Control	FA	SC	FS	$\bar{x}$
'B'	0.92 ± 0.09	1.17 ± 0.12	1.21 ± 0.93	1.26 ± 0.14	1.14[b]
'P'	1.63 ± 0.34	2.21 ± 0.26	1.98 ± 0.20	2.29 ± 0.16	2.03[a]
$\bar{x}$	1.27[b]	1.69[a]	1.59[a]	1.78[a]	
ANOVA					
C	**				
T	**				
C x T	ns				
Ratio Fresh/Dry Weight					
	Control	FA	SC	FS	$\bar{x}$
'B'	8.78 ± 0.29	7.84 ± 0.36	7.8 ± 0.47	7.81 ± 0.60	8.06[a]
'P'	8.5 ± 0.59	7.82 ± 0.50	7.84 ± 0.19	7.33 ± 0.20	7.87[a]
$\bar{x}$	8.64[a]	7.83[b]	7.82[b]	7.57[b]	
ANOVA					
C	ns				
T	**				
C x T	ns				

Different letters show significance ($p < 0.05$) by the Scott-Knott test between treatments. Anova: ns = not significant; * and ** = significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. c = cultivars; t = treatments and c x t = interactions. CV% (Fresh weight) = 11,71; CV% (Dry weight) = 12,38; CV% (Ratio Fresh and dry weight) = 7,47.

Field experiment

Significant differences were observed between treatments. Furthermore, an interaction was found between cultivar factors and treatments for fresh bulb weight (Table 2). The 'P' bulbs presented higher fresh weight when compared to 'B' bulbs. In addition, 'P' bulbs submitted to treatments with FA, SC and FS provided, on average, an increase in fresh weight (48%) when compared to the control. In 'B', the treatments also positively influenced the gain of fresh weight when compared to the control, where the largest difference was observed in plants that received SC and FS via leaf (gains of 50% and 47%, respectively).

Similarly, dry weight data (Table 2) also showed interaction between cultivars and treatments. 'P' bulbs showed higher dry weight values compared to 'B' bulbs. The application of FS in 'P' provided higher values of dry weight when compared to the other treatments, twice as much as the control. The FA and SC

treatments in 'P' promoted gains by 45% and 85% in dry mass compared to control. For 'B' bulbs, the treatments SC and FS increased the dry weight of the bulbs by 33% and 36% respectively when compared to the control. The FA treatment in 'B' bulbs presented statistically dry mass equal to the control.

The values of fresh and dry weight ratio (Table 2) in the bulbs showed interaction between cultivars and treatments. In 'B', there was not enough evidence that the treatments influence the ratio values, nonetheless, the control, FA and SC treatments had lower ratio values when compared to the same treatments used in 'P'. For the FS treatment, in both cultivars, they presented equal ratio values. Moreover, in 'P' bulbs, the SC and FS treatments provided lower ratio values when compared to the control and FA treatments.

As observed in the results in onion bulb weight, yield data also showed significant differences between the cultivars and the treatments (Figure 2A). The cultivar 'P' showed on average 39 t ha^{-1} of bulbs, while 'B' presented 17 t ha^{-1}. The treatments influenced positively both cultivars by increasing the bulb production.

In relation to 'P', the foliar applications containing FA, SC and FS promoted an average yield increase (42 Mg/ha) of about 48.5% when compared to the control, which produced 28.5 Mg/ha. In 'B', the treatments that stood out were SC and FS, producing on average 20 Mg/ha, with an increase of 47.8% compared to the control (13.7 Mg/ha). The FA treatment increased the productivity when compared to the control in 27%; yet it was not superior to the SC and FS treatments.

Table 2. Effect of foliar spray with fulvic acid (FA), *Scenedesmus subspicatus* (SC) and combination (FS) treatment on fresh and dry weight and fresh/dry weight ratio of bulbs of two onion cultivars conducted in the organic system. Values represent the means and ± standard deviation.

Fresh Weight of Bulbs					
	Control	FA	SC	FS	$\bar{x}$
'B'	59.92 ± 3.96^{Cb}	75.95 ± 2.57^{Bb}	89.24 ± 1.61^{Ab}	87.15 ± 5,17^{Ab}	78.07
'P'	124.32 ± 5.13^{Ba}	180.29 ± 7.86^{Aa}	185.43 ± 6.10^{Aa}	189.03 ± 3.19^{Aa}	169.76
$\bar{x}$	92.12	128.12	137.34	138.09	
ANOVA					
C	**				
T	**				
C x T	**				
Dry Weight of Bulbs					
	Control	FA	SC	FS	$\bar{x}$
'B'	6.55 ± 0.65^{Bb}	7.35 ±0.34^{Bb}	8.75 ± 0.96^{Ab}	8.97 ± 1.11^{Ab}	7.90
'P'	8.46 ± 0.35^{Da}	12.34 ± 1.37^{Ca}	15.69 ± 1.32^{Ba}	17.47 ± 1.52^{Aa}	13.49
$\bar{x}$	7.51	9.85	12.22	13.22	
ANOVA					
C	**				
T	**				
C x T	**				
Ratio Fresh and Dry Weight of Bulbs					
	Control	FA	SC	FS	$\bar{x}$
'B'	9.17 ± 0.30^{Ab}	10.34 ± 0.54^{Ab}	10.29 ± 1.16^{Ab}	9.77 ± 0.70^{Aa}	9.89
'P'	14.71 ± 1.21^{Aa}	14.68 ±0.96^{Aa}	11.86 ± 0.94^{Ba}	10.81 ± 0.21^{Ba}	13.02
$\bar{x}$	11.94	12.51	11.08	10.29	
ANOVA					
C	**				
T	**				
C x T	**				

Different letters show significance ($p < 0.05$) by the Scott-Knott test between treatments. Capital letters = treatments; lowercase letters = cultivars. Anova: ns = not significant; * and ** = significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. c = cultivars; t = treatments and c x t = interactions. CV% (Fresh weight) = 3,84; CV% (Dry weight) = 8,59 and CV% (Ratio Fresh and dry weight) = 7,30.

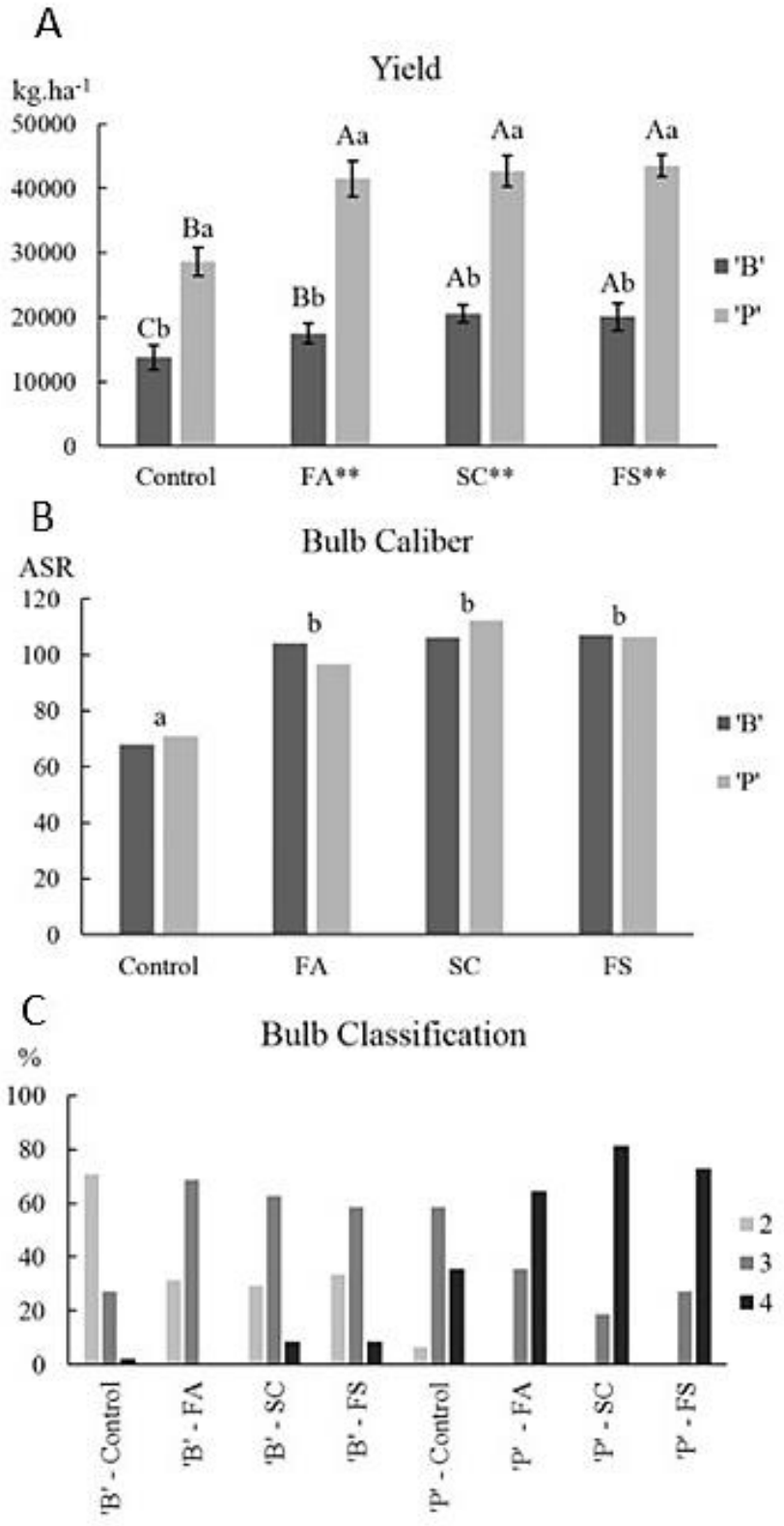

Figure 2. Effect of foliar spray with control, fulvic acid (FA), *Scenedesmus subspicatus* (SC) and combination (FS) treatment. (a) average yield of two onion cultivars conducted in the organic system. Bars indicated the standard deviation. Different letters show significance ($p < 0.05$) by the Scott-Knott test between treatments. Capital letters = treatments; lowercase letters = cultivars. Anova: ns = not significant; * and ** = significant at $p \leq 0.05$ and $p \leq 0.01$, respectively; (b) Bulb caliber and (c) Percentage of bulb per caliber of two onion cultivars conducted in the organic system. Asr = sum of the ranks of bulbs. Different letters show significance ($p < 0.05$) in (b) by the Kruskal-Wallis non-parametric test between treatments. CV% (Average yield) = 3,90; CV% (Bulb caliber) = 8,44 and CV% (Bulb classification) = 9,02.

Regarding bulb caliber, significant differences were found between treatments (Figure 2B), where the sum of the ranks by the non-parametric test of Kruskal-Wallis indicates an increase in the number of larger caliber onion bulbs in both cultivars treated with FA, SC and FS, when compared to the control. In addition, the population of the produced bulbs fell in type 2 to 4 for both cultivars within the treatments used. The percentage of larger caliber bulbs was found in 'P' compared to 'B', which explains the superiority of 'P' in productivity (Figure 2C).

Biochemical analysis and weight loss in bulb storage

Interaction was found between cultivars and treatments for biochemical variables (Table 3). Bulbs 'B' showed higher concentration of total sugars when compared to 'P' bulbs. Moreover, in 'B', the FS treatment promoted higher concentration of total sugars when compared to the other treatments, followed by SC, FA and control treatments. In 'P', SC application provided higher accumulation of total sugars in the bulbs, followed by FS, FA and control treatments. The largest accumulation of reducing sugar was observed in 'P' bulbs in comparison to 'B'. Among the treatments, in 'B', the application of FS provided the largest accumulations of reducing sugar, followed by statistically equal SC and FA, where the control had the lowest concentration. In 'P', the application of SC and FS increased in reducing sugar accumulation in the bulbs in relation to control and FA.

There was not enough evidence that the averages differ from each in the concentration of free total amino acids in 'P' bulbs. In addition, higher free total amino acid concentrations in 'B' bulbs were quantified on average. The FA, SC and FS treatments provided significant gains in free total amino acid in 'B' bulbs when compared to the control.

For soluble proteins, there was no significant difference between treatments in 'B', which in turn presented lower protein values when compared to 'P'. In addition, the application of SC and FS raised protein concentration when compared to other treatments in 'P'.

Throughout the 60 days of evaluation on bulb loss, it was possible to identify a difference in weight loss by 29% more of cultivar 'B' compared to 'P' (Table 4). Treatments showed a 32% reduction in weight loss in bulbs treated with FA, 25% with SC and 30% with FS when compared to control.

In addition, at the end of the storage period, it was possible to verify biochemical changes in onion bulbs. Interaction was observed between cultivars and treatments in the variable total sugars. Bulbs of 'B' showed higher concentration of total sugars when compared to 'P' bulbs, a result like that found in the quantification of total sugars in bulbs collected in the field before storage. The treatments that provided the highest accumulated total sugars in 'B' bulbs were SC followed by the FS treatment. The FA and control treatments presented statistically equal values, being inferior to the other treatments. In 'P', FS treatment promoted greater accumulation of total sugars, followed by SC, FA and control treatments, the last two being equal.

Regarding reducing sugar, the cultivar with the highest concentration was 'P' in relation to 'B'. Treatments SC and FS increased the reducing sugar content in 'B' bulbs when compared to the other treatments. In 'P' bulbs, the treatments FA, SC and FS were superior to the control for reducing sugar, being statistically equal.

By analyzing the average values of free total amino acids between cultivars, the cultivar 'B' accumulated as much as twice than 'P'. In addition, the SC and FS treatments in the cultivars average promoted higher accumulated in the content of free total amino acid when compared to the FA and control treatments.

The soluble proteins in the 'B' bulbs showed higher concentration when compared to 'P'. Moreover, in 'B', the SC and FS treatments significantly increased the concentration of soluble proteins when compared to FA and control. In 'P' bulbs, the treatments showed no statistical differences.

Table 3. Effect of foliar spray with fulvic acid (FA), *Scenedesmus subspicatus* (SC) and combination (FS) treatment on total sugars, reducing sugar, free amino acids and soluble protein of two onion cultivars conducted in the organic system. Values represent the means and ± the standard deviation.

Total Sugars					
	Control	FA	SC	FS	$\bar{x}$
'B'	26.09 ± 0.64^{Ca}	25.07 ± 0.54^{Ca}	29.48 ± 1.30^{Ba}	38.55 ± 0.98^{Aa}	29.80^{a}
'P'	13.53 ± 0.23^{Db}	15.63 ± 1.04^{Cb}	21.86 ± 0.73^{Ab}	19.47 ± 0.50^{Bb}	17.62^{b}
$\bar{x}$	19.81^{c}	20.35^{c}	25.67^{b}	29.01^{a}	
ANOVA					
C	**				
T	**				
C x T	**				
Reducing Sugar					
	Control	FA	SC	FS	$\bar{x}$
'B'	23.14 ± 1.13^{Cb}	25.47 ± 0.50^{Ba}	24.79 ± 0.74^{Bb}	31.47 ± 0.36^{Ab}	26.22^{b}
'P'	25.12 ± 0.38^{Ba}	26.95 ± 1.40^{Ba}	43.96 ± 1.06^{Aa}	42.97 ± 0.23^{Aa}	34.75^{a}
$\bar{x}$	24.13^{d}	26.21^{c}	34.38^{b}	37.22^{a}	
ANOVA					
C	**				
T	**				
C x T	**				
Free Amino Acids					
	Control	FA	SC	FS	$\bar{x}$
'B'	104.46 ± 3.83^{Ba}	173.55 ± 5.06^{Aa}	150.47 ± 3.01^{Aa}	154.04 ± 2.58^{Aa}	143.65^{a}
'P'	64.76 ± 7.70^{Ab}	79.71 ± 6.81^{Ab}	90.2 ± 8.50^{Ab}	94.73 ± 2.85^{Ab}	82.35^{b}
$\bar{x}$	84.61^{b}	126.63^{a}	120.33^{a}	124.38^{a}	
ANOVA					
C	**				
T	**				
C x T	*				
Soluble Protein					
	Control	FA	SC	FS	$\bar{x}$
'B'	10.14 ± 1.33^{Aa}	11.59 ± 0.41^{Aa}	10.63 ± 0.53^{Aa}	10.62 ± 0.41^{Aa}	10.74^{a}
'P'	9.35 ± 0.30^{Ba}	9.32 ± 0.33^{Ba}	11.63 ± 1.94^{Aa}	11.91 ± 0.70^{Aa}	10.52^{a}
$\bar{x}$	9.74^{b}	10.38^{b}	11.13^{a}	11.26^{a}	
ANOVA					
C	^{ns}				
T	*				
C x T	**				

Different letters show significance ($p < 0.05$) by the Scott-Knott test between treatments. Capital letters = treatments; lowercase letters = cultivars. Anova: ns = not significant; * and ** = significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. c = cultivars; t = treatments and c x t = interactions. CV% (Total sugars) = 4,24; CV% (Reducing sugar) = 3,50; CV% (Free amino acids) = 11,51 and CV% (Soluble protein) = 8,61.

Table 4. Effect of foliar spray with fulvic acid (FA), *Scenedesmus subspicatus* (SC) and combination (FS) treatment on weight loss (%) over 60 days, total sugars, reducing sugar, free amino acids and soluble protein of two onion cultivars conducted in the organic system. Values represent the means and ± the standard deviation.

Weight Loss (%) over 60 days					
	Control	FA	SC	FS	$\bar{x}$
'B'	16.06 ± 1.80	13.24 ± 2.08	14.28 ± 1.85	12.83 ± 1.88	14.10^{a}
'P'	14.13 ± 1.62	9.52 ± 2.04	9.74 ± 1.41	10.23 ± 2.55	10.90^{b}

$\bar{x}$	15.09[a]	11.38[b]	12.01[b]	11.53[b]	
			ANOVA		
C	**				
T	*				
C x T	ns				
			Total Sugars (mg.g^{-1})		
	Control	FA	SC	FS	$\bar{x}$
'B'	21.24 ± 1.28[Ca]	19.31 ± 1.79[Ca]	24.45 ± 1.47[Aa]	22.48 ± 2.25[Ba]	21.87
'P'	13.24 ± 0.66[Cb]	13.07 ± 1.09[Cb]	14.97 ± 1.04[Bb]	16.95 ± 0.93[Ab]	14.56
$\bar{x}$	17.24	16.19	19.71	19.72	
			ANOVA		
C	**				
T	**				
C x T	*				
			Reducing Sugar (mg.g^{-1})		
	Control	FA	SC	FS	$\bar{x}$
'B'	23.60 ± 2.39[Bb]	21.09 ± 0.68[Bb]	27.24 ± 3.54[Ab]	30.48 ± 0.52[Ab]	25.60
'P'	38.56 ± 1.04[Ba]	45.73 ± 0.50[Aa]	43.65 ± 0.62[Aa]	45.79 ± 3.65[Aa]	43.43
$\bar{x}$	31.08	33.41	35.44	38.14	
			ANOVA		
C	**				
T	**				
C x T	**				
			Free Amino Acids (mg.g^{-1})		
	Control	FA	SC	FS	$\bar{x}$
B'	110.87 ± 5.73	112.93 ± 10.98	130.85 ± 4.99	137.31 ± 12.49	122.99[a]
P'	50.32 ± 3.82	51.8 ± 2.97	60.02 ± 3.35	57.48 ± 4.78	54.905[b]
$\bar{x}$	80.59[b]	82.36[b]	95.43[a]	97.39[a]	
			ANOVA		
C	**				
T	**				
C x T	ns				
			Soluble Protein (mg.g^{-1})		
	Control	FA	SC	FS	$\bar{x}$
'B'	12.69 ± 1.00[Ba]	13.2 ± 0.84[Ba]	14.53 ± 0.55[Aa]	14.03 ±0.68[Aa]	13.61
'P'	8.83 ± 0.19[Ab]	8.93 ± 0.11[Ab]	8.25 ± 0.15[Ab]	8.44 ± 0.41[Ab]	8.61
$\bar{x}$	10.76	11.06	11.39	11.23	
			ANOVA		
C	**				
T	ns				
C x T	**				

Different letters show significance (p <0.5) by the Scott-Knott test between treatments. Capital letters = treatments; lowercase letters = cultivars. anova: ns = not significant; * and ** = significant at p ≤ 0.05 and p ≤0.01, respectively. c = cultivars; t = treatments and c x t = interactions. CV% (weight loss) = 19,95; CV% (Total sugars) = 6,12; CV% (Reducing sugar) = 5,99; CV% (Free amino acids) = 8,36 and CV% (Soluble protein) = 5,28.

4. Discussion

The bioactivity of the microalga *Scenedesmus subspicatus* (synonym *Desmodesmus subspicatus*) was found to promote root growth of *V. radiata* plants (Figure 1ab), a similar result found by Dineshkumar et al. (2018a) using *Chlorella vulgaris* cell extract in *Vigna mung* L. plants. Microalgae have in their composition

biomolecules such as proteins, oligosaccharides and phytohormones-like activities molecules capable of promoting plant growth (Ronga et al. 2019).

Similarly, the most reported effect of the application of humic substances on plant growth is related to the change in root architecture, influencing both increase in the length and induction in the lateral root (Canellas and Olivares 2014), an effect also observed in the bioassay (Figure 1C and D).

Application of SC, FA and FS (Figure 1) promoted root growth and development in *V. radiata*, indicating its potential use as biofertilizer. Thus, the treatments for the potted and field plants were chosen based on the best results obtained from the microalga (1st step) and fulvic acid (2nd step) bioassay, that is, 0.5 gL^{-1} of SC, 0.2 g,L^{-1} of FA, generating the combination (FS) 0.2 $g.L^{-1}$ of FA + 0.5 $g.L^{-1}$ of SC.

Humic substances (e.g., fulvic acid) can modulate positively the expression of the enzyme H+ATPase in plants, hence promoting the acidification of the plasma membrane, which leads to the cell expansion without loss of turgor, causing the cell growth (Canellas et al. 2015). Related results were found in a study on onion submitted to foliar applications of humic substances, where they promoted increments in the aerial part of the plants (Al-Fraihat et al. 2018). In addition, microalgae biomass contains phytohormones capable of promoting growth in plants such as cytokines and polyamines (Stirk et al. 2013), which stimulates cell division, increasing fresh and dry plant masses (Mógor et al. 2018a).

Furthermore, the application of humic acid in chrysanthemum and microalgae in sugar beet resulted in gains in fresh and dry weight with reduction of fresh/dry mass ratio (Fan et al. 2014; Mógor et al. 2018b). These results are like those found in potted onion plants (Table 1), where the application of FA, SC and FS promoted a reduction in the ratio, and may be related to the higher biomass accumulation of the aerial part when compared to control plant.

Humic substances have the potential to promote growth and development in a variety of plant species, including plants of the Liliaceous family, such as onions (Canellas et al. 2015). Moreover, Bettoni et al. (2016) when applying HS on onion, observed a significant increase in fresh and dry mass of the bulbs, with equal values of water content in control bulbs, indicating greater accumulation of photoassimilates in the highest priority drains (bulbs), a phenomenon that begins at 90 DAT (Vidigal et al. 2010) also coinciding with the start of field applications. This result may partly explain the equal values of the fresh/dry weight ratio in 'P', as there was an increase in the fresh and dry weight of the AF-treated bulbs compared to the control (Figure 2).

Dineshkumar et al. (2018b) report results in increasing the fresh and dry weight of onion plants treated with the microalgae *Spirulina platensis* and *Chlorella vulgaris*. El-sayed et al. (2018) when applying microalgae extract in onion plants observed an increase in fresh and dry weight of bulbs as fresh and dry mass ratio decreased when compared to control, as observed in 'P' where treatments SC and FS promoted increase in the mass, providing a reduction in the fresh/dry mass ratio values, demonstrating a higher biomass accumulation.

Mora et al. (2010) report that the application of HS in plants primarily affects the distribution of nitrate by increasing the activity of H + ATPase, causing concomitantly the distribution of phytohormones that provide plant growth. In addition, an increase in the onion yield has been reported in plants receiving HS as a treatment (Bettoni et al. 2016). Dineshkumar et al. (2018b) when applying the biomass of microalgae *Spirulina platensis* and *Chlorella vulgaris* in onion, observed a significant increase in the weight of the treated bulbs, as well as in the productivity and caliber of the bulbs. Therefore, its presumed that the increase in productivity are associated to the presence of molecules in microalgae biomass, such as auxin, cytokinin and gibberellin phytohormones that promote growth in the highest priority drains in plants (Plaza et al. 2018).

According to Oku et al. (2019), the accumulation of fructo-oligosaccharides produced in the aerial part (source) are later translocated to the leaf sheaths to form the bulbs (drains). The application of HS and microalgae promote the increase in carbohydrate production in plants (Bettoni et al. 2016; Dineshkumar et al. 2018b), which may cause the leaf translocation to bulbs, providing an increase in the caliber.

Zhang et al. (2016) observed that out of the carbohydrates found in the dry matter of the onion bulb, non-structural carbohydrates (about 80%) such as glucose, fructose, sucrose and fructo-oligosaccharides are mostly found in it. In addition, Ertani et al. (2011) found that the application of HS in maize plants increased the concentration of glucose, fructose and the activity of the enzyme rubisco, responsible for carbon fixation in vegetables. This result may be correlated to the increase in carbohydrates (total and reducing sugars) in

onions submitted to FA application (Table 3). The use of microalgae in plants shows the potential these microorganisms to promote plant growth and may positively influence carbohydrate concentrations in plants (Garcia-Gonzales and Sommerfeld 2016; El-Sayed et al. 2018).

Conselvan et al. (2017) testing humic substances from dissimilar sources of leonardite found an increase in enzymes involved in nitrogen metabolism with influences in the increment in amino acid and protein in plants. Additionally, microalgae have protein and amino acid content in their composition (Tibbetts et al. 2015). However, in this study, the application of FA and SC did not clearly show the increase of amino acids and proteins in bulbs of both cultivars.

Studies with different onion cultivars during storage indicate that lower mass losses are correlated with higher dry matter accumulation in the bulbs (Kahsay et al. 2013), as observed in this study (Table 2) with 'B' treated with SC and FS and 'P' treated with FA, SC and FS showed an increase in dry mass, which resulted in less bulb loss in storage (Table 3). In addition, the application of humic substances and microalgae extract in plants, provided the bulbs in storage lower mass losses in relation to the control, hence, this result was justified by the greater accumulation of dry matter and total soluble solids (Shehata et al. 2017; Mansour et al. 2019).

Besides the dry mass, the higher carbohydrate accumulation in the bulb contributes to the reduction of mass loss in storage (Kahsay et al. 2013), as observed, where 'B' plants treated with SC and FS and 'P' with FA, SC and FS accumulated more sugars, resulting in less mass loss in the bulbs (Tables 3 and 4). However, for free total amino acids and soluble proteins, it was not possible to clearly identify the effect of greater accumulation of these biomolecules on the bulbs with reduction in mass loss.

5. Conclusions

Positive effects on the root architecture of *V. radiata* treated with fulvic acid (FA) and *Scenedesmus subspicatus* microalga (SC) were detected in the bioassay, with the best results observed in the combination (FS). The effects of foliar applications of solutions containing FA, SC and FS promoted stimulation of growth in the aerial part in the early stage of plant development (up to 60 DAT), with increased field productivity with applications from the beginning of bulbification (90 DAT), which increases the concentration of sugar in the bulbs and proteins in 'B', therefore, reflecting in the reduction of mass losses in storage. However, the application of FS does not potentiate the results in the initial growth, yield, and mass loss in both onion cultivars, being the application of SC more efficient.

Authors' Contributions: GEMIN, L.G.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article and critical review of important intellectual content; MÓGOR, A.F.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article and critical review of important intellectual content; AMATUSSI, J.O.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article and critical review of important intellectual content; DE LARA, G.B.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article and critical review of important intellectual content; MÓGOR, G.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

Acknowledgments: The author thanks National Research Council of Brazil (CNPq) for his Doctoral scholarship (grant 142393/2016-9).

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Received: 14 January 2021 | **Accepted:** 29 September 2021 | **Published:** 12 August 2022