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Microalga improve the growth, yield, and contents of sugar, amino acid, and protein of tomato

Microalga melhora o crescimento, rendimento, e teores de açúcares, aminoácidos e proteínas do tomateiro

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ABSTRACT

The development of sustainable ways to increase crop productivity is essential to meet the growing demand for food. Microalgae are rich in bioactive molecules and can be produced on a large scale and at a low cost. Therefore, we hypothesized that the microalga *Asterarcys quadricellulare* (CCAP 294/1), a rich source of free L-amino acids, can increase the growth and yield of tomatoes. To determine the potential of *A. quadricellulare*, we performed a two-year study by applying *A. quadricellulare* biomass using a foliar spray on tomato plants. In the first season, weekly applications were performed during the full cycle of tomatoes. The effect of *A. quadricellulare* biomass of 0.05, 0.15, 0.25, and 0.40 g L^{-1} on tomato yield was determined through regression analysis. In the second season, the solution of 0.25 g L^{-1}, which showed the best results, was tested on two tomato cultivars using a weekly and a biweekly frequency of application. Both cultivars were positively affected by the application of biomass, which promoted the increase in leaf area and yield, along with higher contents of sugar, free amino acid, and protein. Thus, we determined the role of *A. quadricellulare* as an effective biofertilizer in tomatoes.

Index terms: Biofertilizer; *Asterarcys quadricellulare*; *Solanum lycopersicum* L.; Sustainable agriculture.

RESUMO

O desenvolvimento de soluções sustentáveis visando aumentar a produtividade agrícola é crucial para atender à crescente demanda por alimentos. As microalgas são organismos cuja composição é rica em moléculas bioativas e podem ser produzidas em escala, com baixo custo. Dessa forma, a hipótese desse trabalho é de que a microalga *Asterarcys quadricellulare* (CCAP 291/1), uma fonte de L-aminoácidos livres, pode aumentar o crescimento e o rendimento de plantas de tomate. Para investigar seu potencial como biofertilizante, foi realizado um trabalho de dois anos, aplicando biomassa *A. quadricellulare* através de pulverização foliar em plantas de tomate. No primeiro experimento, foram realizadas aplicações semanais durante o ciclo completo da cultura. O efeito das soluções contendo 0.05; 0.15; 0.25; e 0.40 g L^{-1} de biomassa de *A. quadricellulare* sobre o rendimento do tomate foi medido através de análise de regressão. No segundo experimento, a concentração com melhor performance (0.25 g L^{-1}), foi testada em duas cultivares de tomate com pulverizações de frequência semanal e quinzenal. Como resultado, para ambas as cultivares, as aplicações aumentaram a área foliar, o rendimento e o conteúdo de açúcares, aminoácidos e proteínas livres. Assim, mostrando efeito biofertilizante em tomate.

Termos para indexação: Biofertilizante; *Asterarcys quadricellulare*; *Solanum lycopersicum* L.; Agricultura sustentável.

INTRODUCTION

Algae biomasses are cost-effective, renewable sources that are considered as alternative inputs for conventional crop production and can help to achieve sustainable agriculture (Rouphael; Colla, 2018). Among these organisms are eukaryotic photosynthetic microalgae and prokaryotic cyanobacteria (Ortiz-Moreno; Sandoval-Parra; Solarte-Murillo, 2019).

The green algae (Chlorophyta) and blue-green algae (Cyanophyta) are often considered biofertilizers (Mógor; Mógor, 2022) or biostimulants (Bumandalai; Tserennadmid, 2019). Moreover, the Chlorophyta microalgae contain various compounds, such as amino acid, polyamines, and polysaccharides, which can be applied to plants as signaling molecules for stimulating plant growth (Nardi et al., 2016; Mógor et al., 2018b; Gemin et al., 2019).

The bioactivity of microalga *Asterarcys quadricellulare* (*AQ*), which is related to its free L-amino acid (L-*Aa*) content, was reported to promote the growth and yield of potatoes, and it also increased the contents of chlorophyll, amino acid, and sugar (Cordeiro et al., 2022). Microalgae with high protein content could be a source of L-*Aa*. The isomers of L-*Aa* are known to be biologically active in vegetable metabolism (Mógor et al., 2018a).

Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops in the world and the most preferred species grown in greenhouses (Coban et al., 2020). Furthermore, a study by Garcia-Gonzalez and Sommerfeld (2016) reported that some vegetables from the Solanaceae family, such as tomato and pepper, are positively affected by the application of microalgae, which promoted plant growth.

Therefore, in the present study that spanned for two years, we aimed to first evaluate the maximum efficiency concentration (*Mec*) of *AQ* biomass on the production of organic tomatoes. We also determined the effect of *AQ* biomass on the biochemical changes and yield in two tomato cultivars by using different application intervals.

MATERIAL AND METHODS

Microalgae source and analysis

The microalga *Asterarcys quadricellulare* (CCAP 294/1) biomass, supplied by Alltech® Crop Sciences-Brazil, was obtained from mixotrophic culture and atomized through the spray drying method that produced a fine greenish colored powder. After the cell disruption (Stirk et al., 2020), the free amino acids were extracted (Magné; Larher, 1992; Winters et al., 2002) with a concentration of 90.94 mg g^{-1} (w/v), which corresponded to 9% of the total microalga biomass.

Experiment I

The first experiment was performed in December 2018 that determined the *Mec* of the microalga *Asterarcys quadricellulare* (CCAP 294/1) biomass on the productivity of the cultivar (CV) Giuliana-Sakata®. The study was performed inside a protected environment at the Organic Horticulture Research Area of the Federal University of Paraná, located at the municipality of Pinhais-PR, Brazil at 25° 23' 30'' S and 49° 07' 30'' W, at an average altitude of 920 m, with a Cfb type temperate climate according to the Köppen classification.

The sowing was performed in a plastic modular trays (200 cells) filled with an organic substrate (Provaso®) and was kept in a nursery with a timed micro-sprinkler irrigation until 40 days after sowing (DAS). The plants were transplanted at the growth stage of the second true leaf pair and adequate root development to a protected environment, in an arc-type polyethylene tunnel. Based on (Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA, 2013), the soil was a medium texture Alic Yellow-Red Oxisol. The chemical analysis showed the following average values from the 0–20 cm layer: pH ($CaCl_2$) = 5.84; pH (H_2O) = 6.71; 0 Al^{+3}; 2.93 cmolc dm^{-3} H + Al^{+3}; 5.28 cmolc dm^{-3} Ca^{2+}; 3.05 cmolc dm^{-3} Mg^{2+}; 1.32 cmolc dm^{-3} K^{+}; 49.0 mg dm P (Mehlich); 33.49 mg dm^{-3} S; 26 g dm^{-3} C; 76.7 V%; and 12.58 cmolc dm^{-3} CTC.

A total of 15 days before transplanting the seedlings, 10 ton ha^{-1} organic compost with the following composition was added to previously opened 0.30-m deep furrows: 31.3 g kg^{-1} C; 26.3 g kg^{-1} N; 8.2 g kg^{-1} P; 7.2 g kg^{-1} K; 8.0 g kg^{-1} Ca; and 4.2 g kg^{-1} Mg.

The spacing was 1.3 m within the rows and 0.5 m between the plants. Two drip tapes per planting row were used for the irrigation system, with daily irrigation aiming to maintain field water capacity at 80%. The inspection was performed using a tensiometer. Two stems per plant were analyzed, each having their heights limited by the removal of the apical meristem that was present two leaves above the 10th rachis.

Treatments were arranged in a completely randomized design, with six replications of *AQ* suspensions in sugarcane molasses along with microalga biomass of concentrations 50, 150, 250, and 400 g L^{-1} obtained from the spray dry method. From each suspension, 1 mL L^{-1} was diluted in water to obtain foliar sprays, which produced treatments solutions equivalent to 0.050 (*AQ*05), 0.150 (*AQ*15), 0.250 (*AQ*25), and 0.400 g L^{-1} (*AQ*40), along with control with water.

The weekly foliar sprays (*Wf*) were started at 54 DAS and ended a week before the last rachis (10th rachis) was harvested. The *Wf* were performed between 9:00 a.m. and 10:00 a.m., using an electronic sprayer (Kawashima®), at constant pressure (40 psi), by applying a volume based on the development of the plants (from 30 to 100 mL per plant).

The first experiment determined the effect of biomass on tomato yield (ton ha^{-1}). The harvesting point was considered when the fruits reached 50% of red coloration. The weight of all fruits from the six representative plants per repetition was measured using a precision scale.

Experiment II

The effect of *AQ Mec* on tomato biometrical and biochemical changes was determined in the second experiment, which started in November 2019. Therefore, one year after the start of experiment I, the experiment was performed at the same place, following the same methodology for plant management (sowing, transplantation, spacing, and foliar spray technique). In the second experiment, a factorial scheme (2 × 3) was established, applying *AQ*25 on two CVs (Netuno-Blueseeds® and Giuliana-Sakata® = Factor 1), under two frequencies (weekly and biweekly = Factor 2), along with a control, for six treatments with four replications each. Again, a full-cycle was performed with two stems per plant, each having their heights limited by the removal of the apical meristem that was present two leaves above the 14th rachis.

Three leaves were collected for fresh mass measurement (g) from the middle of the third section of each six representative plants per repetition, and their respective dry mass (g) was measured after two days on a drying oven, both quantified on a precision scale. From the same section, the leaf area (cm^2) was represented by an average of three leaves in each plot and was measured using WinRhizo®. The first fruit from the 6th rachis and its respective leaf above were collected for biochemical analysis. The relative chlorophyll of the leaf was evaluated biweekly, during the month of the 6th rachis harvest, which resulted in an average of three measurements, from 30 leaflets of each plot, using a portable meter (N-Tester®)

Fruit mass (g) was quantified from all fruits of the representative plants using a precision scale. The length (mm) and width (mm) of fruits were measured using a pachymeter. The number of fruits per plant and the total yield (ton ha^{-1}) were also calculated.

Biochemical analysis

The leaf and fruit samples were washed and then macerated using liquid nitrogen until a fine powder was obtained. The total free amino acid (*Aa*) was extracted from the leaves and fruits based on a method given by Magné and Larher (1992). The soluble proteins (*Pt*) were extracted from the leaves and fruits based on a method given by Du et al. (2010). The contents of total sugar (*Ts*), reducing sugar (*Rs*), and non-reducing-sugar (*nRs*) of the leaves and fruits were measured based on a method given by Maldonade, Carvalho and Ferreira (2013).

The evaluation of the content of chlorophylls (*Chl*) and carotenoids in the leaves was performed based on a method given by Lichtenthaler (1987) with modifications, using the formulas described by Lichtenthaler and Buschmann (2001). Furthermore, the enzyme *Nr* activity of the leaves was determined based on the methodology given by Jaworski (1971).

Statistical analysis

For the first experiment, all data were evaluated for their homogeneity of variances by the Bartlett test and then submitted to variance and regression analysis. The *AQ Mec* of productivity was evaluated by the first derivative of the regression equation, which was equal to zero. For the second experiment, after the data homogeneity was confirmed, it was analyzed as a 2 × 3 factorial experiment, with averages compared by the Tukey's test ($p < 0.05$). Statistical analysis was performed using the Assistat 7.7 Beta software (Silva; Azevedo, 2016).

RESULTS AND DISCUSSION

Experiment I

The regression analysis (Figure 1) of the *AQ* foliar spray on *Solanum lycopersicum* L. (CV Giuliana®) yield showed a *Mec* of 0.247 g L^{-1} at *Wf*. Thus, it can be seen that the biofertilizer potential of microalga is inherent in its biomass. Similar results were obtained in a previous study on other Chlorophyta that could promote higher plant yield until the plant reached its maximum potential, and then led to diminishing returns because of the metabolism cost (Cordeiro et al., 2022).

The control plants produced 16-ton ha^{-1} less than the *AQ Mec*. *AQ25* was chosen for experiment II because its concentration of microalga biomass led to a greater yield of tomatoes.

Experiment II

The leaf area (Table 1) of Giuliana® (G′) was higher than that of Netuno® (N′). Moreover, at biweekly sprays (*Bf*), the leaf area was 107.27 cm² higher in N′ and 76.48 cm² higher in G′ than that in control, which corresponded to an increase of 21.46% and 19.52%, respectively. This indicated that *AQ*25 promoted leaf expansion equally for both CVs.

Even though the results of the leaf fresh and dry mass showed no differences between the CVs, *Bf* led to higher masses than that of the control. As a result, the leaf fresh mass on *Bf* increased 27.09% in N′ and 34.42% in G′. Furthermore, N′ leaf dry mass on *Bf* had an increase of 26.50%, whereas G′ had an increase of 39.86%. No statistical difference was observed in the leaf fresh/dry mass ratio (Table 1).

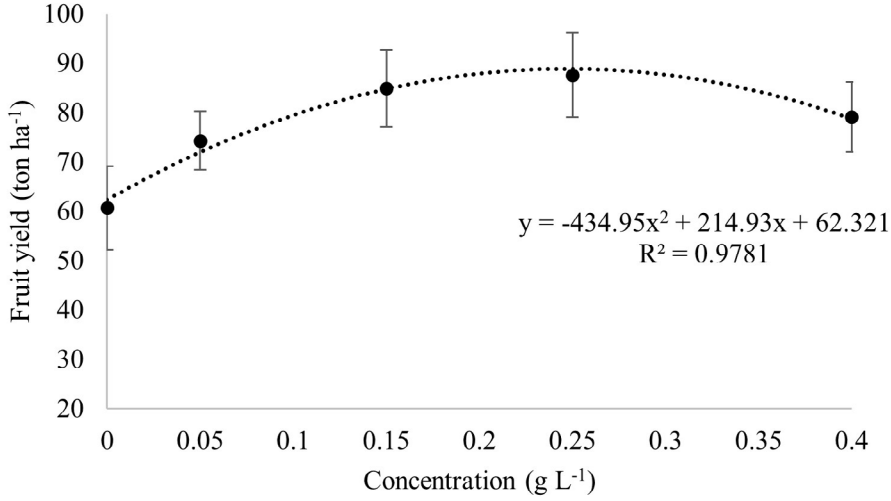

Figure 1: Fruit yield (ton ha^{-1}) of *Solanum lycopersicum* L. cultivar. Giuliana treated with different concentrations of the microalga *Asterarcys quadricellulare* applied weekly using a foliar spray.

Table 1: Leaf area (cm²), leaf fresh mass (g), leaf dry mass (g), and leaf fresh/dry mass ratio of *Solanum lycopersicum* L. cultivars Netuno (N') and Giuliana (G') treated with 0.25 g L^{-1} *Asterarcys quadricellulare* biomass (*AQ*25) applied weekly (*Wf*) and biweekly (*Bf*) using a foliar spray.

	Leaf area	Leaf fresh mass	Leaf dry mass	Leaf fresh/dry ratio
N	564.7 a"			
G	454.3 b			
Control	445.8 b	24.86 b	3.3 b	
Wf	545.1 a	30.88 a	3.8 b	
Bf	537.6 a	33.05 a	4.4 a	
N x Control	499.8	26.6	3.5	7.7
N x *Wf*	587.2	29.6	3.5	8.6
N x *Bf*	607.1	33.8	4.4	7.8
G x Control	391.7	23.1	3.0	7.6
G x *Wf*	503.0	32.1	4.2	7.7
G x *Bf*	468.2	32.3	4.4	7.4

"Different letters in the same column indicate significant differences (p<0.05).

The expansion of leaf induced by *A. quadricellulare* (CCAP 291/1) can be due to its biomass composition because some Chlorophyta species have bioactive molecules capable of inducing metabolic changes in plants, thereby promoting leaf growth (Ronga et al., 2019). Among these bioactive molecules, L-*Aa* has maximum relevance (Mógor et al., 2018a). Similar results are reported for potato plants with the application of *A. quadricellulare* (CCAP 291/1), which increased the leaf area (Cordeiro et al., 2022).

Aspartate and glutamate (glutamic acid) generally constitute a large proportion of the L-*Aa* in many microalgae species (Xupeng; Song; Xuran, 2017). Glutamic acid is the most abundant *Aa* in the *AQ* biomass (Cordeiro et al., 2022). Previous studies have identified glutamate receptors in plants (Forde; Roberts, 2014) that can play a role in plant regulation involving plant growth, photosynthesis, and stress signaling (Weiland et al., 2015), which also shed light on the effect of microalga on plant responses.

However, the results may be dependent on plant genetics or spray interval, as observed in the results of fruit mass (Table 2). The results showed a factorial interaction which indicated that this variable is dependent on the time of application. For N′, both frequencies promoted higher masses than those in the control. For G′, higher masses were only observed at *Bf*. Accordingly, when comparing control with *Bf, AQ*25 promoted heavier fruits, with a 5.65% and 5.93% increase in fruit mass for N′ and G′, respectively.

The application of *AQ*25 had no effect on the width and number of fruits per plant for both CVs, which had a naturally elongated shape. However, an increase in the fruit length (Table 2) of N′ *Wf* and G′ *Bf* was observed, compared with that of the control. Also, the natural differences between CVs are noteworthy. N′ produced a higher number of fruits and G′ produced heavier fruits, which led to similar yields.

Consequently, the changes in the biometric due to the application of *AQ*25 increased the fruit mass, which boosted fruit yield. This result showed that both CVs are responsive to *AQ*25 foliar spray, even at *Bf* (Figure 2). Thus, as no interaction occurred between the CVs, the increase in yield showed the average value of the CV, which was due to the increases in mass and length at *Bf*. Accordingly, comparing *Bf* with the control, N′ produced an average of 13.52-ton ha^{-1} more, whereas G′ produced 31.76-ton ha^{-1} more, which corresponded to a greater output of 10.19% and 26.20%, respectively.

A correlation between the leaf area results and yield gains was noticeable. The application of biomass may have played an important role in the nitrogen metabolism of vegetables through a signaling behavior because the foliar sprays increased the plant productivity. This signaling could be associated with the rich *Aa* composition of biomass (Häusler; Ludewig; Krueger, 2014). In addition, amino acids are associated with a series of plant metabolisms, such as assimilation of nitrogen and plant growth (Okumoto et al., 2016), and as a precursor to the polyamine synthesis, which is related to biological processes that trigger plant growth and development (Mógor et al., 2018b).

Table 2: Fruit mass (g), length (mm), width (mm), and number of fruits per plant of *Solanum lycopersicum* L. cultivars Netuno (N') and Giuliana (G') treated with 0.25 g L^{-1} *Asterarcys quadricellulare* biomass (*AQ*25) applied weekly (*Wf*) and biweekly (*Bf*) using a foliar spray.

	Fruit mass	Fruit length	Fruit width	Fruit per plant
N				97.0 a
G				75.5 b
N x Control	91.6 bB"	75.0 bB	48.0 bA	94.8
N x *Wf*	97.3 bA	78.2 bA	49.7 bA	99.5
N x *Bf*	96.8 bA	74.7 bB	48.6 bA	96.6
G x Control	118.7 aB	84.4 aB	53.1 aA	70.6
G x *Wf*	118.2 aB	83.1 aB	52.1 aA	77.5
G x *Bf*	125.8 aA	87.7 aA	54.1 aA	78.5

"Different letters in the same column indicate significant differences (p<0.05).

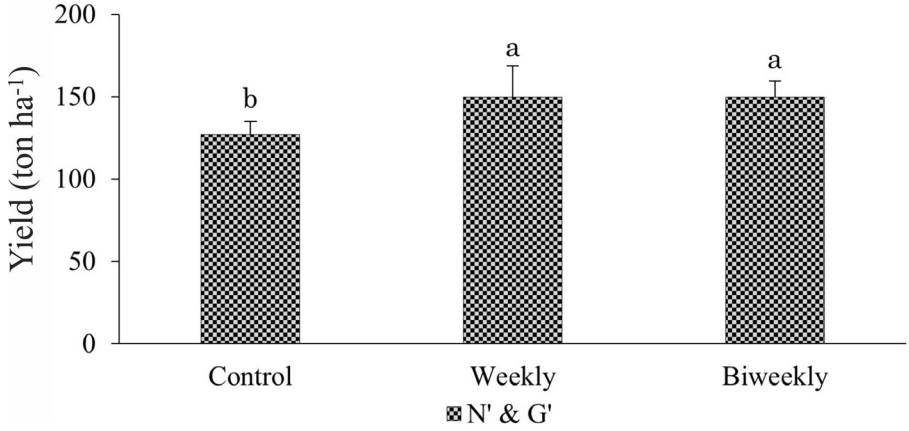

Figure 2: Fruit yield (ton ha^{-1}) averages of two cultivars of *Solanum lycopersicum* L., cultivars Netuno (N') and Giuliana (G'), treated with 0.25 g L^{-1} of *Asterarcys quadricellulare* biomass (*AQ*25), applied weekly (*Wf*) and biweekly (*Bf*) using a foliar spray.

Columns with the same letter do not differ statistically. Cultivar: n.s.; Timing: **; and Interaction: n.s.; (p<0.01).

Previous studies based on microalgae have reported improvement in the growth and development of several vegetables, such as lettuce, red beet, and tomato, which demonstrated its effect in open-field and greenhouse conditions (Faheed; Fattah, 2008; Garcia-Gonzalez; Sommerfeld, 2016). Studies on similar microalgae biochemical compositions that are rich in free *Aa* and *Pt* showed that these molecules could be delivered directly through plant leaves and produce growth responses in vegetables (Ronga et al., 2019).

Relative *Chl* values (Table 3) in corroboration with *Chl* biochemical data (Table 3) showed differences between the CVs because the noticeable darker green leaves of N′ also had more *Chl b* than G′. Furthermore, considering the frequencies of *AQ*25 application, relative *Chl* results demonstrated better gains for N′ through *Wf,* whereas for G′ both frequencies were better than that of the control.

Minor changes in the content of *Chl a* were due to the applications because G′ at *Bf* had a 5.88% higher leaf *Chl a* than that of the control. No difference in *Chl a* was observed between the frequencies of N′. Moreover, the results of *Chl b*, total *Chl*, and carotenoids (Table 3) showed no statistical difference between the treatment frequencies. A contrasting result based on the same product sprayed on potatoes (Cordeiro et al., 2022) suggested that the effectiveness of *AQ* in *Chl* is affected by different crops and CV. In the present study, even though statistically significant differences were not observed for the *Chl* ratio between the CVs, a small difference between the application timing was indicated for the time factor.

For the total sugar content (*TsC*) of leaves, N′ treatments showed no difference. For G′, *TsC* increased for both frequencies. Hence, G′ at *Bf* contained 50.5 µg g^{-1} more *TsC*, which corresponded to a 7.12% gain than that of the control.

For both CVs, the reducing sugar content (*Rs*) of leaves (Table 4) showed the same result. Concurrently, *AQ*25 applications increased *Rs* compared with that of the control at both frequencies. Therefore, selecting *Wf* for comparison showed a similar increase of *Rs* for N′ (17.72%) and G′ (19.82%). For the non-reducing sugars (*nRs*) of leaves (Table 4), *Wf* and control averages were more than that of the *Bf*, with no statistical difference between the CVs.

For the total sugar content (*TsC*) of fruits (Table 4), *Wf* promoted better gains for both CVs, which indicated that the source-to-sink flow of photoassimilates can be stimulated by a more frequent application of *AQ*25. Consequently, compared with that of the control, the same frequency increased *TsC* by 2.11 mg g^{-1} and 2.41 mg g^{-1} for N′ and G′, respectively. This corresponded to an increase of 17.72% and 19.82% for N′ and G′, respectively.

No difference was observed between the treatments for fruit *Rs* (Table 4), yet N′ showed higher *Rs* averages. In contrast, for fruit *nRs* (Table 4) an opposite result was observed, with higher averages for G′. Additionally, following the *TsC* results, *Wf* increased the free sugar content of fruits in both CVs.

Table 3: Relative chlorophyll content (N-tester®), leaf chlorophyll *a* (*Chl a*), chlorophyll *b* (*Chl b*), total chlorophylls (Total *Chl*), carotenoids (mg g^{-1}), and chlorophyll *a/b* ratio (*Chl a/b*) of *Solanum lycopersicum* L. cultivar Netuno (N') and Giuliana (G') treated with 0.25 g L^{-1} *Asterarcys quadricellulare* biomass (*AQ*25) applied weekly (*Wf*) and biweekly (*Bf*) using a foliar spray.

	N-tester®	Chl a	Chl b	Total *Chl*	Carotenoids	Chl a/b
N			0.12 a	0.33 a	0.13 a	
G			0.09 b	0.26 b	0.11 b	
Control						1.82 a
Wf						1.76 ab
Bf						1.71 b
N x Control	629.6 aB"	0.21 aA	0.12	0.32	0.12	1.76
N x *Wf*	641.8 aA	0.23 aA	0.13	0.36	0.14	1.80
N x *Bf*	625.0 aB	0.20 aA	0.12	0.31	0.12	1.68
G x Control	588.1 bB	0.17 bA	0.09	0.26	0.10	1.88
G x *Wf*	633.3 aA	0.15 bA	0.09	0.24	0.11	1.72
G x *Bf*	634.4 aA	0.18 aA	0.1	0.29	0.11	1.74

"Different letters in the same column indicate significant differences ($p<0.05$).

Results of the free *Aa* content (*AaC*) from both CVs showed a similar pattern for leaves and fruits (Table 5). Both frequencies showed higher *AaC* gains. For N′, *Bf* had 354.75 µg g^{-1} more *AaC* in the leaves, whereas for G′ it was 317.64 µg g^{-1} more. Compared with that of the control, this corresponded to an increase of 19.36% and 15.91% for N′ and G′, respectively.

Bf increased *AaC* in fruits by 14.25% and 36.23% for N′ and G′, respectively. A similar result was observed for factor 2 averages of each *AQ*25 frequency application for leaves and fruit protein content (*PtC*) of both CVs. (Table 5). The N′ leaves *PtC* at *Bf* showed a 122.81% increase than that of the control.

Table 4: Leaf and fruit total sugar content (*TsC*), reducing sugars (*Rs*), and non-reducing sugars (*nRs*) of *Solanum lycopersicum* L. cultivar Netuno (N') and Giuliana (G') treated with 0.25 g L^{-1} *Asterarcys quadricellulare* biomass (*AQ*25) applied weekly (*Wf*) and biweekly (*Bf*) using a foliar spray.

	Leaf *TsC*	Leaf *Rs*	Leaf *nRs*	Fruit *TsC*	Fruit *Rs*	Fruit *nRs*
		(µg g^{-1})			(mg g^{-1})	
N					26.07 a	1.19 b
G					24.09 b	3.75 a
Control		313.2 b	417.1 a	27.06 b		1.52 b
Wf		345.9 a	423.3 a	29.39 a		4.23 a
Bf		372.0 a	379.9 b	26.21 b		1.66 b
N x Control	751.2 aA"	311.8	439.3	26.66	25.97	0.69
N x *Wf*	770.2 aA	351.7	418.4	28.81	27.08	1.74
N x *Bf*	743.9 aA	367.0	376.8	26.32	25.16	1.15
G x Control	709.6 bB	314.7	394.8	27.45	25.10	2.36
G x *Wf*	770.6 aA	340.1	428.2	29.98	23.24	6.73
G x *Bf*	760.1 aA	377.0	383.0	26.10	23.94	2.16

"Different letters in the same column indicate significant differences (p<0.05).

Table 5: Leaves and fruits amino acid content (*AaC*), leaves and fruits protein content (*PtC*), and nitrate reductase (*Nr*) (µg g^{-1}) of *Solanum lycopersicum* L. cultivars Netuno (N') and Giuliana (G') treated with 0.25 g L^{-1} *Asterarcys quadricellulare* biomass (*AQ*25) applied weekly (*Wf*) and biweekly (*Bf*) using a foliar spray.

	Leaf *AaC*	Fruit *AaC*	Leaf *PtC*	Fruit *PtC*	Nr
N		475.6 b		109.6 b	
G		546.8 a		150.7 a	
Control	191.4 b"	430.4 b		114.8 b	
Wf	211.9 a	563.3 a		131.7 a	
Bf	225.0 a	540.0 a		143.9 a	
N x Control	183.2	421.9	61.3 aB	82.6	0.56 aB
N x *Wf*	223.8	522.9	121.7 aA	115.1	0.70 aA
N x *Bf*	218.7	482.0	136.6 aA	131.0	0.60 aB
G x Control	199.6	438.9	77.0 aA	147.1	0.52 aA
G x *Wf*	200.0	603.6	110.9 aA	148.4	0.56 bA
G x *Bf*	231.4	597.9	80.1 bA	156.7	0.56 aA

"Different letters in the same column indicate significant differences (p<0.05).

The carbon skeleton of glutamine and glutamate (L-glutamic acid) plays an important role in the primary synthesis of energy, wherein the regulation of biosynthesis pathways occurs at multiple levels (Okumoto et al., 2016). Therefore, the applications of L-*Aa* can produce vegetables with increased levels of total soluble *Pt* in the leaves and higher activity of enzyme *Nr* (Röder et al., 2018). These results showed that the applications of *AQ*25 could increase the contents of *Aa* and *Pt* in tomato leaves and fruits at both frequencies of application.

Furthermore, the applications of *AQ*25 increased the levels of soluble solids in leaves, which could be because of their higher area and relative *Chl* content. This result was supported by the higher fruit contents of *nRs* instead of leaves at the same time of analysis. Plants with higher photosynthetic assimilation can translocate more photoassimilates from source to sink (Rouphael; Colla, 2018). Thus, improved fruit mass, size, and yield were observed for both N′ and G′.

CONCLUSIONS

We demonstrated the bioactivity of foliar sprays of *Asterarcys quadricellulare* (CCAP 294/1) biomass on plant metabolism by applying it to organic tomatoes. The effectiveness of microalga as a biofertilizer can be partially attributed to its biologically active free L-*Aa*. Even though some variables were dependent on the application frequency or CV, *AQ*25 increased the levels of total sugar, free amino acid, and protein level in the leaves and fruits for both CVs. Moreover, *Bf* was a better and more cost-effective option for plant growth promotion and yielding results.

AUTHOR CONTRIBUTION

Conceptual Idea: Mógor, A.F.; Mógor, G.; Methodology design: Mógor, A.F.; Mógor, G.; Data collection: Lara, G.B.; Data analysis and interpretation: Amatussi, J.O.; Cordeiro, E.C.N.; Lara, G.B.; Marques, H.M.C.; Mógor, A.F.; Mógor, G.; and Writing and editing: Lara, G.B.; Mógor, A.F.

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