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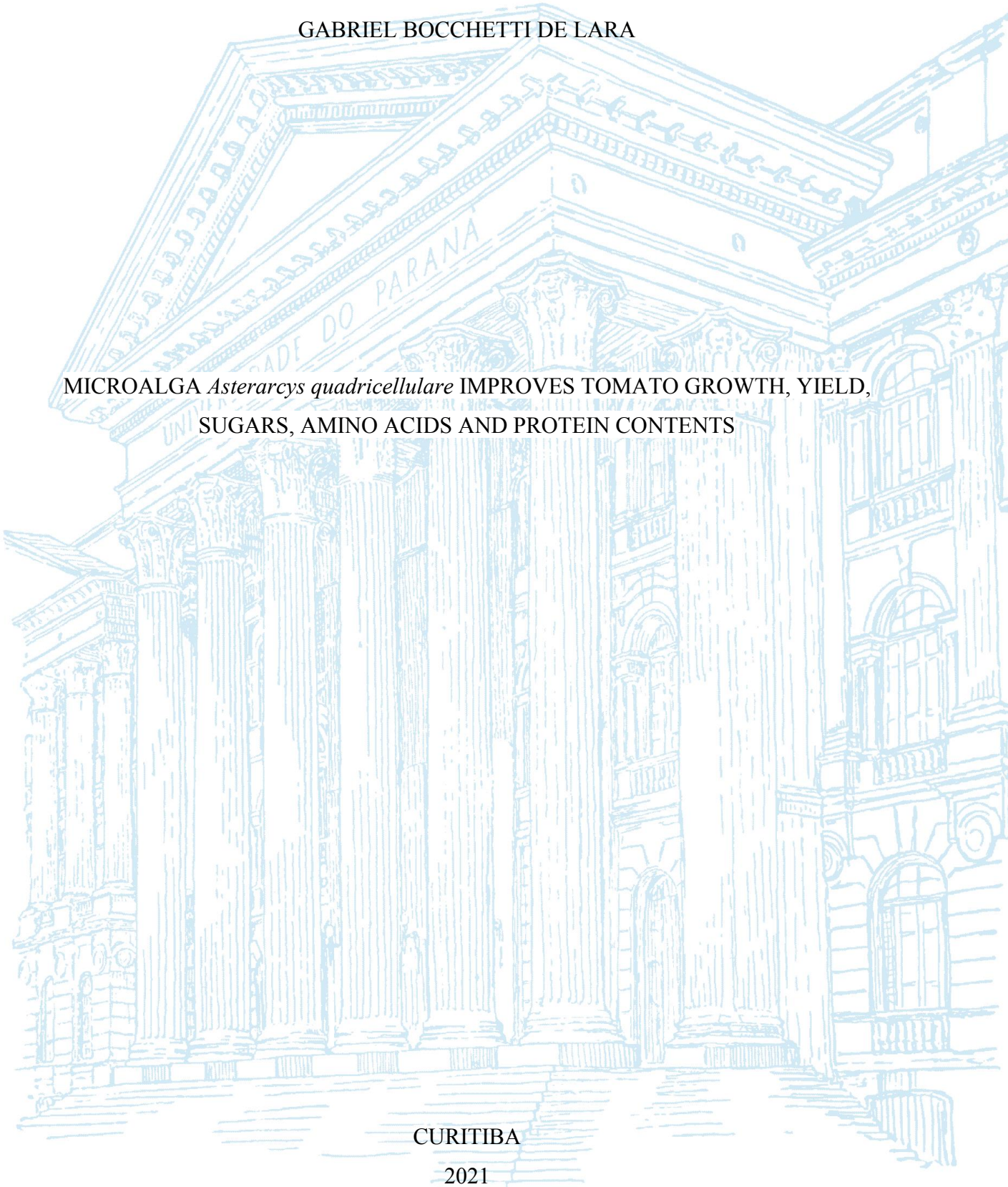

MICROALGA *Asterarcys quadricellulare* IMPROVES TOMATO GROWTH, YIELD, SUGARS, AMINO ACIDS AND PROTEIN CONTENTS

CURITIBA

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Dissertação apresentada ao Programa de Pós-Graduação em Agronomia, Área de Concentração em Produção Vegetal, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito à obtenção do título de Mestre em Ciências.

Orientador: Prof. Dr. Átila Francisco Mógor

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em AGRONOMIA (PRODUÇÃO VEGETAL) da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **GABRIEL BOCCHETTI DE LARA** intitulada: **MICROALGA *Asterarcys quadricellulare* IMPROVES TOMATO GROWTH, YIELD, SUGARS, AMINO ACIDS AND PROTEIN CONTENTS** , sob orientação do Prof. Dr. ÁTILA FRANCISCO MÓGOR, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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“Those who contemplate the beauty of the earth find reserves of strength that will endure as long as life lasts. There is something infinitely healing in the repeated refrains of nature - the assurance that dawn comes after night, and spring after winter.”

— Rachel Carson, *Silent Spring* (1962)

RESUMO

Visando atender à crescente demanda por alimentos, o desenvolvimento de soluções sustentáveis para os sistemas de produção é crucial. Para isso, fontes para biofertilizantes estão sendo investigadas pela sua capacidade de promover maior produtividade das lavouras. Essas substâncias naturais envolvem uma grande variedade de ingredientes ativos ou substâncias orgânicas, as quais podem promover, direta ou indiretamente, alterações metabólicas em plantas cultivadas. Nesse contexto, destacam-se as biomassas de microalgas. As microalgas são recursos naturais de baixo custo, produzidas em grande quantidade em pouco tempo, e ricas em moléculas bioativas. De tal modo, estudar aplicações de biomassas que ainda carecem de informações sobre seu potencial biotecnológico, como a microalga *Asterarcys quadricellulare* (*AQ*), pode auxiliar tanto na descrição de sua composição, como seu potencial agronômico. Com isso, teve-se como hipótese que esta biomassa, semelhante a de outras Chlorophytas, pode ser fonte de aminoácidos livres capazes de aumentar o crescimento e produtividade do tomateiro. Portanto, visando investigar seu potencial como biofertilizante foi realizado um trabalho de dois anos, aplicando a biomassa de *AQ* através de pulverização foliar em tomateiro sob cultivo protegido e orgânico. No primeiro ano foi utilizada a cultivar Giuliana®, na qual aplicações semanais ocorreram ao longo de seu ciclo. Foram testadas as soluções com concentração: 0.05; 0.15; 0.25; 0.40 g L^{-1}; além de um controle (somente com água). Assim, a concentração de máxima eficiência (*Mec*) foi investigada por meio de análise de regressão, avaliando a produtividade. No segundo ano, na mesma estufa agrícola, a *Mec* (0.25 g L^{-1}) foi testada em duas cultivares de tomateiro (Giuliana® e Netuno®) através de pulverizações semanais e quinzenais, além de um tratamento controle para cada cultivar. Nesse experimento foi avaliado, além produtividade, o tamanho dos frutos, número de frutos por planta, e área foliar; bem como os conteúdos livres de aminoácidos, proteínas, e açúcares em folhas e frutos. Em adição, analisou-se os teores de pigmentos, compostos fenólicos, e nitrato redutase nas folhas. Como resultado, observou-se expansão foliar, frutos mais pesados e maior produtividade, independentemente da frequência usada da concentração 0.25 g L^{-1} de *AQ* para ambas as cultivares. Além disso, foi observada elevação dos níveis de aminoácidos livres, proteínas e açúcares totais nas folhas e frutos. Dessa forma, demonstrando o efeito biofertilizante da biomassa de *AQ* aplicada mesmo quinzenalmente na concentração 0.25 g L^{-1} em tomateiro sob cultivo orgânico.

Palavras-chave: Biofertilizante. Microalgas. *Solanum lycopersicum* L. Agricultura sustentável.

ABSTRACT

To meet the growing demand for food the development of sustainable solutions for production systems is crucial. For this, biofertilizers sources are being investigated for their ability to promote greater crop productivity. These natural substances involve a wide variety of active molecules or organic substances, which can directly or indirectly promote metabolic changes in cultivated plants. In this context, microalgae biomasses stand out. Microalgae are low-cost natural resources, rich in bioactive molecules, produced in large quantities in a short time. In this sense, studying biomass applications that still lack information about their biotechnological potential, such as the microalgae *Asterarcys quadricellulare* (*AQ*), can help both in the description of its composition and its agronomic potential. Thus, it was hypothesized that this biomass, like other Chlorophytes, could be a source of free amino acids capable of increasing tomato growth and productivity. Therefore, to investigate its potential as a biofertilizer, a two-year work was carried out, applying *AQ* biomass through foliar spraying on tomato plants under protected and organic cultivation. In the first year, the cultivar Giuliana® was used, in which weekly applications occurred throughout its cycle. Solutions with concentration 0.05; 0.15; 0.25; 0.40 g L^{-1}; plus a Control (only with water) were tested. Thus, the maximum efficiency concentration (*Mec*) was investigated through regression analysis, evaluating crop productivity. In the second year, in the same agricultural greenhouse, the *Mec* (0.25 g L^{-1}) was tested on two tomato cultivars (Giuliana® and Netuno®) through weekly and biweekly spraying, also with a Control treatment for each cultivar. In this experiment, additionally to productivity, the fruit size, number of fruits per plant, and leaf area were evaluated; as well as the free contents of amino acids, proteins, and sugars in leaves and fruits. In addition, the contents of pigments, phenolic compounds, and nitrate reductase in the leaves were analyzed. As a result, leaf expansion, heavier fruits and higher yield were observed, regardless of the frequency used of the 0.25 g L^{-1} *AQ* concentration for both cultivars. Furthermore, an increase in the levels of free amino acids, proteins and total sugars in leaves and fruits was observed. Thus, demonstrating the biofertilizing effect of *AQ* biomass applied even biweekly at a concentration of 0.25 g L^{-1} in organic tomato.

Keywords: Biofertilizer. Microalgae. *Solanum lycopersicum* L. Sustainable agriculture.

FIGURES LIST

TABLES LIST

LIST OF ABBREVIATIONS OR ACRONYMS

Aa - Amino acids
AaC - Amino acids content
AQ - *Asterarcys quadricellulare*
BfT - Biweekly frequency treatment
BSA - Bovine serum albumin
cv. - Cultivar
DAS - Days after sowing
G' - Giuliana® Tomato
GOGAT - Glutamate synthase
GS - Glutamine synthetase
HPLC - High performance liquid chromatography
L-*Aa* - Levogyre Amino acids
Mec - Maximum efficiency concentration
N - Nitrogen
N' - Netuno® Tomato
nRs - Non-Reducing Sugars
PITC - Phenylisothiocyanate
PMSF - Phenylmethylsulfonyl fluoride
PTC - Phenylthiocarbamyl
Pl - Plant
PVP - Polyvinylpyrrolidone
Pt - Protein
PtC - Protein content
Rs - Reducing sugars
SD - Standard deviation
Ts - Total sugars
TsC - Total sugars content
WfT - Weekly frequency treatments

SUMMARY

1 GENERAL INTRODUCTION

Chemical fertilizers are still the most used practice for increasing crop production, however they are known to impact the environment (RAO, 2014). Since the Green Revolution this is a problem related to the conventional agriculture that needs to be solved. The strong dependency of synthetic pesticides and chemical inputs applications are practices that can degrade the soil, reduce water and food quality, and compromise producer's safety (CASTRO et al., 2020). Concurrently, the increasing awareness of consumers about healthy food coupled with their concern of conventional agriculture environmental impacts have stimulated organic farming over the years (ABOU CHEHADE et al., 2017).

Balancing food production to achieve higher healthy food demands while maintaining environmental sustainability is challenging. Nevertheless, it can be surpassed with new studies in crop science, associated with organic agriculture, and the investment support from research companies and the government. Thus, the development of alternative techniques and new studies with renewable resources capable to raise productivity have great importance.

In this scenario, biofertilizers have emerged as cost-effective, eco-friendly inputs which are beneficial for any crop production system. Additionally, they can be more proficient, productive, and easily accessible, even for small farmers (MAHAJAN et al., 2003). In organic production, these natural compounds are fundamental tools, since in Brazil this planting technique is regulated, and synthetic inputs cannot be used (BRASIL, 2004). However, in a broader view, it can be said that these products are not only intended to reach the increasing food demand, but also to assist all agronomic systems to achieve profitability, sustainability, workers safety, and healthier food.

Biofertilizer applications have been documented to aid plant growth and yield, which could help to reduce the need for chemical fertilizer. It is also reported that these applications, depending on its biomass source (e.g., microalgae), can promote vegetable metabolic changes. Microalgae are a large group of diverse single-celled autotrophic organisms, mainly photosynthetic. In recent decades, research of this biomass has been explored for its potential as alternative sources for several sectors, including agronomic. Among these microalgae species, the agricultural potential of Chlorophyta stands out (ECKARDT, 2010). Many of them have already been studied for their biofertilizer capacity, demonstrating potential as sustainable sources that promote plant growth (MÓGOR et al., 2018; AMATUSSI et al., 2020). In this context, is noteworthy that *Asterarcys quadricellulare* (*AQ*) studies are scarce. The few

information that exists is about its composition and its similarity with other Chlorophyta microalgae. Their biomass, rich in free amino acids (*Aa*), are the target of research and development of products with agronomic focus. Hence, new studies are needed to unveil *AQ* full potential.

Several vegetables of economic and social importance demonstrate good results of growth and production when applied with microalgae. Among them tomato (*Solanum lycopersicum* L.) receives great attention due to its globally economic importance. Tomato fruit is from the Solanaceae family, normally eaten *in natura* or in the form of extracts, has an enormous dietary impact related to its nutrients (BOITEUX et al., 2008), such as vitamin C, potassium, folic acid, and carotenoids (PARVEEN et al., 2015).

Tomato is a vegetable with national and world prominence, being the most produced in several countries due to its high consumption. In 2019, in Brazil, about 63 thousand ha of tomato were cultivated, with a production of 4.11 million tons (FAO, 2021). In 2019 Paraná was ranked the fifth state of Brazil in tomato production (229.966 tons), the first ones being Goiás, São Paulo, Minas Gerais, and Bahia, all those being responsible for 78% of Brazil total production (IBGE, 2021).

Under organic cultivation, studies indicate how the quality of the fruit can improve with higher content of total sugars, organic acids, vitamin C and phenolic compounds when compared with conventional agriculture vegetables (HALLMANN, 2012). Considering its importance and the complexity of its cultivation (FILGUEIRA, 2008), tomatoes are among the most studied vegetables, but still lack agronomic research in more sustainable production systems. Therefore, it is deeply relevant that research use different cultivars of *Solanum lycopersicum* as test plants for studies with plant growth promoters. This way results may show possible interactions between plant cultivars and microalgae applications.

In that scenario, it is essential that the production of healthy foods meets the population's ever-increasing food demand. Still, conventional forms of production can cause damage to the environment. Therefore, as the space for agronomic fields are finite, the solution for this problem is to produce more in the same space, while using a sustainable way. Furthermore, producers and society depend on new effective techniques and products (e.g., biofertilizers) that strive for sustainability. This way, concomitantly, higher productivities could be reached, while offering food safety and lesser environment impact when compared to conventional methods.

For this, new research of natural sources, such as microalgae, are needed. Since their use is recent, these studies can produce more knowledge about their biotechnological potential.

Hence, microalgae biomasses require more studies to find its effective concentration to promote plant growth and higher yield.

However, as research with *AQ* are scarce, many questions about its biotechnological potential need to be answered. Studies describe its composition as like other Chlorophyta species. Those biomasses are effectively used for biofertilizer experiments, being considered viable solutions to help solve the problems of meeting the global increasing food demand. Thus, the hypothesis that *AQ* biomass, as a source of free amino acids, can promote plant growth and higher yield when applied as foliar spray in tomato plants, was tested in this work.

Therefore, in the first year, this work aimed to test different concentrations of the *AQ* biomass as a potential source for biofertilizer through leaf applications in tomato plants, examining its effect on productivity. Later, in the second experiment, the objective was to analyze the Maximum efficiency concentration (*Mec*) effect on two cultivars and frequencies, evaluating plant growth, yield, and biochemical variables.

2 LITERATURE REVIEW

2.1 TOMATO

The tomato (*Solanum lycopersicum* L.) is an herbaceous Solanaceae. Despite being classified as a fruit, tomatoes are studied within the group of vegetables. They are part of the Brazilian diet, together with other species, normally eaten *in natura* as part of salads. Among all vegetables, tomatoes stand out among the most consumed, after lettuce, they have grown in importance after the expanding of fast-food companies (DOSSA and FUCHS, 2017).

Originated from the west coast of South America, where moderate temperatures between 15 to 19°C prevail, it can bloom and bears fruit in the most variable climatic conditions (TREICHEL et al., 2016). In tropical countries, such as Brazil, there can be one to two harvests per year. Thus, it can grow well in tropical climates of high altitude, subtropical and temperate, allowing its cultivation in different regions of the globe (BECKER, 2016).

Italian type tomato is known for their elongated shape, intense red color, and sweet flavor. According to Sakata (2021) the cultivar Giuliana® is distinguished for its big and firm fruits (210 g average), great performance under protected environment, and indeterminate stem growth. Another Italian indeterminate tomato cultivar is Netuno®. According to Blueseeds (2021) it also has a long cycle, and diseases tolerance (*Verticillium* race 1, *Fusarium* races 1 and 2, Nematodes Galls and Tobacco Mosaic Virus), however have a higher productivity of fruits from medium size (170 g average).

Olericulture is one of the activities that generates the highest income in the field for each hectare cultivated, with high use of labor for the various stages of the production process, from sowing to marketing (MARTA, 2018). In Brazil, tomato production generates many jobs annually, during its cycle that can occur twice a year, employing many workers since the sowing until its final commercialization (TREICHEL et al., 2016).

The largest producer of tomato is China with a cultivated area of more than one million hectares and an annual production of more than 61 million tons (FAO, 2021). Brazil produced 4,110,242 tons of tomato, reaching an average productivity of 65.14 tons per hectare, occupying the 10th position in tomato production worldwide (2%), led by China, India, and the United States, accounting for approximately 34%, 11% and 7%, respectively (FAO, 2021).

According to IBGE (2021), in Brazil around 35% of the tomato produced was destined for industrial tomatoes, and the remainder for fresh consumption. Most of this production takes

place in small areas. Hence, it is characterized as a family farming activity, where many adopt the organic system, they can reach productivity between 60 to 80 ton ha^{-1}, considered high (DOSSA and FUCHS, 2017).

2.2 ORGANIC PRODUCTION

Organic farming is an alternative form of production, based on the sustainability of the ecosystem, the constant worry for consumer health, and the development of natural and effective techniques to increase productivity (SUCIU et al., 2018).

In Brazil, Law N°. 10,831, of December 2003, regulated by Decree N°. 6,323, of December 27, 2007, provides for organic agriculture:

> Organic farming systems are considered to be those in which specific techniques are adopted, through the optimization of the use of available natural and socioeconomic resources and respect for the cultural integrity of rural communities, with the objective of economic and ecological sustainability, the maximization of benefits minimizing dependence on non-renewable energy, employing, whenever possible, cultural, biological and mechanical methods, as opposed to the use of synthetic materials, the elimination of the use of genetically modified organisms and ionizing radiation, at any stage of the process. production, processing, storage, distribution and marketing, and the protection of the environment. (BRAZIL, 2003).

Many crops have their agronomic studies based on this system. Therefore, one of the biggest challenges when seeking greater productivity, in organic systems, is the search for synthetic inputs alternatives. Since these are still greatly used in conventional agriculture, which can cause environmental impact, contamination of water and food, in addition to risks to workers (SAVCI, 2012).

In this context, it is noted that in the north of Paraná, in tomato plantations, up to 36 applications of pesticides are made in a single harvest, due to the attack of the whitefly (LUZ et al., 2007). Consequently, as tomatoes are widely consumed *in natura*, they have the possibility of contamination by pesticide residues (MADAIL et al., 2011). Gravel et al. (2010) adds that the salad consumption of this fruit, when organically produced, promotes human health, and enhances food safety. Thus, consumer demand for organic tomatoes is increasing over the last decades.

In that scenario, implementing sustainable farming systems is advisable for ensuring future food and ecosystem security (REGANOLD and WACHTER, 2016). The same authors comments that organic producer, using natural resources as plant growth promoters, are observing efficient results to their productivity problems, a solution that contributes to the wellbeing of farmers and their communities. In comparison with the production of organic and conventional tomatoes, it was found that organic tomatoes in protected cultivation are agronomically viable in terms of their economic aspect (REDDY, 2013). Authors indicate that their productivity is equivalent, even providing benefits for organic cultivation, such as lowering its production cost by 17.2%, and raising profitability by 59.9% (LUZ et al., 2007).

Olivares et al. (2014) studying field-grown organic tomato observed enhancements in the plant performance when applied with humic substances through foliar sprays, even when cultivated in high fertility soil. This plant metabolism changes were related with plant growth and increased fruit production (OLIVARES et al., 2014). Therefore, showing that vegetables obtained from sustainable agriculture systems aggregate benefits for human consumption and environment preservation.

2.3 BIOFERTILIZERS

The use of biofertilizers in agriculture is an important strategy, not only in organic production systems, when natural inputs are essential. They are crucial in all ways of seeking to increase productivity, with sustainability, to meet the growing demand for food. It is seen in the literature how this theme has been the subject of research over the last decades (MÓGOR et al., 2008; VASCONCELOS and GONÇALVES, 2013).

The concept of biofertilizers is defined by the Ministry of Agriculture, Livestock, and Supply, in Normative Instruction 61 of July 8, 2020:

> Biofertilizer are product that contains an active ingredient or organic agent, free from pesticides, capable of acting, directly, or indirectly, on all or part of the cultivated plants, increasing their productivity, without considering their hormonal or stimulating value. (BRAZIL, 2004).

Therefore, its noteworthy that although there are divergences between biofertilizer or biostimulant nomenclature in the classification of these products, this is due to the rules and regulations of each country (MORAES and AZEVEDO, 2016; MÓGOR, 2017).

Within this context, biofertilizers have been gaining prominence for their ability to generate productivity gains, while minimizing the impacts of agriculture (SINGH et al., 2016; RENUKA et al., 2018), and may also assist with nutritional quality of food (DU JARDIN, 2015).

The composition of a biofertilizer is vast, and may include algae biomass, humic substances, protein hydrolysates, and beneficial microorganisms, that when applied to plants can produce metabolic changes (DU JARDIN, 2015), also improving the chemical and biological quality of the soil or stimulating plant growth (ABDEL-RAOUF et al., 2012). Yakhin et al. (2017) adds that even in small concentrations they can provide great results, as it would improve physiological and biochemical processes, in search of the maximum genetic and productive potential of plants.

Therefore, the way they can stimulate physiological responses when applied to plants is being associated to it signaling action (MÓGOR et al., 2017; STADINIK et al., 2017). Results show they can biometrically increase roots and leaves, assisting in vegetable physiology, or even stimulate responses to biotic and abiotic stresses (NARDI et al., 2016; STADINIK et al., 2017).

Other studies shows that due to the diversity of compositions, the use of these substances and organisms as a biofertilizer can bring several advantages to cultures (ALVAREZ et al., 2021). Like L-amino acids, protein hydrolysates, and microalgae biomasses, in the plant growth promotion (MÓGOR et al., 2008); polysaccharides, stimulating growth, greater yield, nutrient absorption, and resistance to stress (TARRAF et al., 2015; EL ARROUSSI et al., 2016). In addition, the supply of amino acids in leaf solutions provides plants with the necessary elements for the development of structures, saving metabolic energy (GARCIA et al., 2012; PLAZA et al., 2018), being related to a series of metabolic processes that will be covered in a topic ahead.

Among these sources, emphasis is placed on the biochemical composition of microalgae as a raw material to develop new products to improve plant growth (GARCIA-GONZALEZ and SOMMERFELD, 2016; EL ARROUSSI et al., 2018; MÓGOR et al., 2018), offering a viable alternative to reduce the dependence of chemical fertilizers.

The potential of these substances has been explored and is being considered a harmless, eco-friendly, and renewable source, which for agronomic purposes is essential to increase promotion with sustainability (MAHAJAN et al., 2003).

2.4 FOLIAR ABSORPTION

The foliar application of biofertilizers has been an excellent strategy to complement mineral fertilization (SINGH et al., 2016). The absorption of these molecules depends on the leaf anatomy, as cutin and waxes are the most hydrophobic components that prevent the absorption of water solutions (TAIZ and ZEIGER, 2017). However, pectins and cellulose are more hydrophilic forming absorption paths for foliar sprays. In addition, the stomata guard cells have a more permeable cuticle with less cutin and wax deposition (TAIZ and ZEIGER, 2017), which makes them an important absorption route.

Furthermore, in relation to amino acid absorption, such as those highly presented in microalgae composition, some studies indicate that there are glutamate receptors in plants (PRICE et al., 2012; FORDE and ROBERTS, 2014). These receptors can be activated by levogyre amino acids like glutamate, serine, and alanine (VINCILL et al., 2012; FORDE and ROBERTS, 2014). This way glutamate receptors can mediate plant responses involving plant stress signaling and immunity, stomatal movements, photosynthesis, and plant growth (WEILAND et al., 2015; TEIXEIRA et al., 2018).

After entering in the leaf mesophyll cells the amino acids can be loaded into phloem via a symplastic or apoplastic transport path, a route that depends on the absence or number of plasmodesmata between phloem parenchyma and companion cells (TEGEDER, 2014). This way, amino acids released from phloem can move to terminal sink cells via symplastic and apoplastic routes, varying from different sink tissues, developmental phase, and plant species (TEGEDER and HAMMES., 2018). Furthermore, its noted that in roots and sink leaves, the post-phloem translocation of amino acids occurs symplastically (TEGEDER and HAMMES., 2018). Thus, dedicated transport proteins are necessary in the mediation of intracellular and intercellular translocation, likewise participating in long-distance transport of amino acids in plants (YANG et al., 2020).

2.5 AMINO ACIDS AS BIOFERTILIZERS

Plants need many amino acids to achieve optimal development. Although they can synthesize their own compounds, the process is complex, and requires a lot of carbon and nitrogen, consuming a lot of energy. Thus, studies indicate that applications providing amino acids can improve the development of the plants in different phases. Hence, supplying *Aa* promptly to plants can reduce the need to its synthesis, saving essential energy and productivity, particularly during the growth stages (REICHLING, 2018). Furthermore, as seen previously, the amount of biomolecules supplied are so low, that these biomasses applications are better associated to signaling events, which then produce metabolic changes (MÓGOR et al., 2018).

Researchers add that amino acids act through gene expression signaling, which result in protein synthesis. Consequently, leading to hormonal levels alteration, entailing higher enzymatic activity, causing biochemical and fundamental structural changes to plant development (CASTRO et al., 2019). Furthermore, influencing a series of physiological processes (HILDEBRANDT et al., 2015).

In this context, it is known that amino acids are very important in the process of assimilation of nitrogen by plants. After $NO3^-$ assimilation, it undergoes the transformation processes to $NH4^+$, being converted into glutamate through the action of the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT). Later, being incorporated to other amino acids through transamination reactions (TAIZ and ZEIGER, 2017).

In the composition of green microalgae extract (e.g., *Asterarcys quadricellulare*) the amount of macro and micronutrients, oligosaccharides, amino acids, and plant hormones may vary. Among the amino acids, the highest percentage is glutamic acid (CASTRO et al., 2017; LU et al., 2019). This amino acid participates in vegetables metabolic pathways, such as nitrogen synthesis. They are considered key in plant growth and development, as they are precursor to other amino acids that are produced through transamination (FORDE and LEA, 2007). Glutamic acid can promote energy savings for plants, hence contributing for the culture development furtherly favoring productivity (RÖDER et al., 2018).

Additionally, in plant metabolism, L-glutamic acid participates in the process of photosynthesis and in the synthesis and activation of chlorophyll (YARONSKAYA et al., 2006). Research using L-glutamic acid and protein hydrolysate in Solanaceae plants promotes growth, with positive impacts on productivity and increased concentration of biomolecules such as chlorophylls and enzymes related to the N cycle (COLLA et al., 2014; RÖDER et al., 2018).

In this scenario, it is seen that some microalgae biomass rich in amino acids are being studied for their ability to serve as a source of these biomolecules for biofertilizers. Studies with applications of these substances in plants point to results of agronomic importance, such as: improving the total content of soluble proteins in plants, promoting better nitrogen assimilation, and stimulating the metabolism of amino acids (NARDI et al., 2016). Other effects involve: their performance as stress-reducing agents, a source of nitrogen and hormonal precursors (ZHAO, 2010). Corroborating with Nardi et al. (2016) and Zhao (2010), other authors report that the use of microalgae benefits the development of plants by producing growth-promoting molecules, vitamins, amino acids, polypeptides, and polymers such as exopolysaccharides that improve plant growth and productivity (SAFI et al., 2014). Thus, studying the Chlorophyta microalgae species, it can be seen a great potential for crop science research related to plant growth promotion.

2.6 POTENTIAL OF ALGAE IN AGRICULTURE

Algae constitute a large group of photosynthetic organisms, including eukaryotic microalgae, macroalgae, and prokaryotic cyanobacteria (ANDERSEN et al., 2013), with growing environmental and economic importance (RENUKA et al., 2018). In natural conditions, microalgae are preferably autotrophic and able to use carbon dioxide and nutrients such as nitrogen, phosphorus, and potassium, from water environments for its metabolism (BRENNAN and OWENDE, 2010).

Most algae, both micro and macro, play an important role in carbon sequestration and are responsible for 50% of total photosynthesis on earth (MORONEY et al., 2009). They play a key role in maintaining aquatic ecosystems and cycling resources, improving nutrient availability through cycles and transformations (MORONEY et al., 2009).

They have evolved to be practically ubiquitous throughout the world (FEHLING et al., 2007), whether in aquatic or terrestrial environments they have extremely diverse metabolic capacities between species (ANDERSEN et al., 2013). Algae are pioneers in several habitats even in adverse conditions, including saline or contaminated soils, showing natural resilience due to their composition (PANDEY et al., 2005). The nature of these organisms reveals their extreme adaptability, with high growth rates and resistance to environmental stresses, their

composition contains molecules that give these characteristics both to it and to its products (AZAMAN et al., 2017).

In this context, it is seen that historically algae extracts are one of the oldest sources to be used in agriculture, although the effects of application in plants have been recently identified (DU JARDIN, 2015). It is estimated that there are around 800 thousand species of microalgae in the world, of which approximately 6% are described (SUGANYA et al., 2016). In relation to green microalgae and cyanobacteria, studies show its action in the mineralization, incorporation, and mobilization of organic and inorganic nutrients, and in the production of many bioactive compounds (PRASANNA et al., 2014).

Therefore, each microalgae potential is dependent on the composition of its biomass. These diverse metabolisms produce a range of compounds of high interest, including nutraceuticals and bioactive compounds such as carotenoids (BOROWITZKA, 2013), polyunsaturated fatty acids (RATLEDGE, 2004), polysaccharides (ISHAQ et al., 2016), polyamines (STIRK et al., 2013), carbohydrates (KHAN et al., 2005), proteins of high value (ISHAQ et al., 2016), and free amino acids (RENUKA et al., 2018), such as L-amino acids (MÓGOR et al., 2008).

Their rich compositions can induce metabolic changes when applied to plants. As examples, researchers cite the microalga *Chlorella vulgaris* and *Spirulina platensis* as promoters of these changes by raising levels of total sugars, amino acids, and phenolic compounds in onion plants (DINESHKUMAR et al., 2020). In addition, other research cites applications with microalga *Dunaliella salina* as capable of mitigating the effects of oxidative stress in tomato under the effect of salinity (EL ARROUSSI et al., 2018).

The performance in the primary metabolism, through the increase of cellular division and expansion in plants, as well as in the secondary metabolism has been studied (EL NAGGAR et al., 2020). These molecules can cause changes in the levels of reducing sugars, such as glucose and fructose, which are little mobile in phloem, in addition to non-reducing sugars, such as sucrose, which are more mobile (TAIZ and ZEIGER, 2017). Thus, in addition to allowing greater plant growth, they are also related to the quality of the products produced.

Other examples of good results with the use of different algae, its noteworthy that these effects can be stimulated on seedlings and adult plants (GEMIN et al., 2018). Authors confirm the biofertilizer effect of the microalga *Arthrospira platensis* on beet plants, by increasing the concentration of chlorophyll and amino acids in seedling leaves (MÓGOR et al., 2018). Research also shows promising results in the metabolic modifications of plants by promoting

productivity gains concomitantly with the increase in the concentration of biomolecules, such as carbohydrates and proteins (DINESHKUMAR et al., 2020), providing healthier foods, since they are considered harmless and eco-friendly (ALVAREZ et al., 2021).

In this sense, in relation to fruit quality, Coppens et al. (2016) conducted research with tomatoes grown in a hydroponic medium testing the biomass of *Nannochloropsis* as a biofertilizer, noting an increase in the levels of sugars and carotenoids in the fruits. Regarding changes in the root part, Barone et al. (2018) observed biometric increases in the roots and increased expression of genes related to the acquisition of nutrients by the roots of beet plants treated with the microalgae of the genus *Scenedesmus* and *Chlorella*.

Another form of interaction between the biomass of microalgae and plants is through a phytohormonal like effect since algae present these molecules in their composition (AMATUSSI et al., 2020). In this same study with biofertilizer based on calcareous algae, rich in humic substances, an auxin like effect was observed when observing growth promotion in plants, improving the quality of agricultural crops (AMATUSSI et al., 2020).

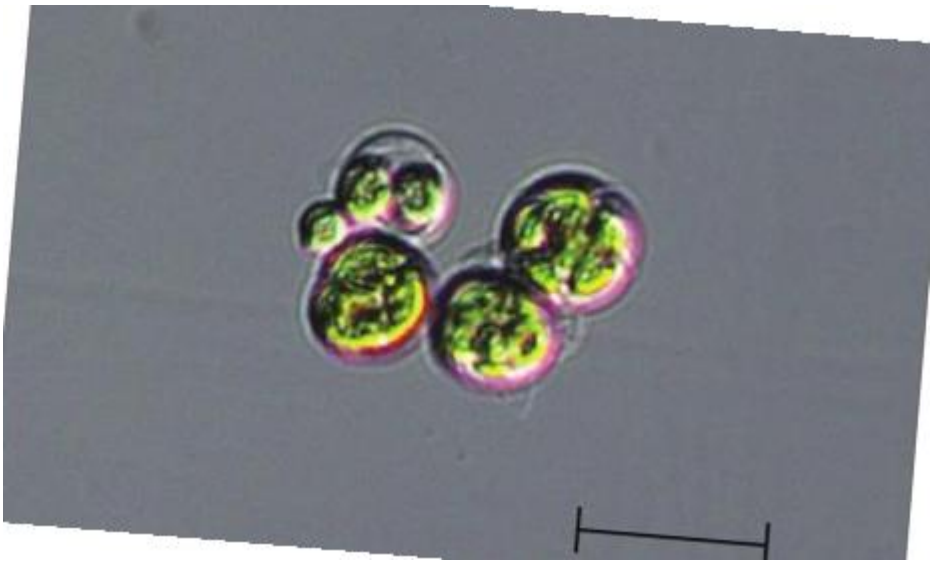
Additionally, on a phytohormone like effect, Plaza et al. (2018), in research with *Scenedesmus* spp. and *Arthrospira* spp. hydrolyzed, observed these effects after solutions foliar applications, which promoted an increase in the dry masses of the aerial and root parts. Also, increasing the number of flowers and concentration of macronutrients in plant tissue. In this scenario, studies with different microalgae are concurrently indicating that its biomass are capable to act as plant growth promoters (HUSSAIN and HASNAIN, 2011), likewise inducing resistance to pathogens and diseases (GEMIN et al., 2018).

As seen in this section, the agriculture potential of microalgae is related to their ability to provide a wide range of bioactive and effective molecules for biofertilization. Furthermore, it is concluded that the mechanisms induced by microalgae in plants are very complex and have not yet been fully understood. Most likely, its action involves the interaction of several molecules (BARONE et al., 2019), which not necessary are linked to the supply of nutrients, but rather with an intricate signaling network (MÓGOR et al., 2018).

2.7 MICROALGA *Asterarcys quadricellulare*

The microalga *Asterarcys quadricellulare* belongs to the phylum Chlorophyta, which is composed of single-celled green microalgae (Fig. 1) found in fresh and marine water, varying from 2 to 10 μm in diameter and are dependent on light for their autotrophic growth.

FIGURE 1 - LIGHT MICROSCOPE IMAGE OF *Asterarcys quadricellulare* KNUA020



Bars represent 20 μm. Source: Adapted from Hong et al., (2012).

To better understand this microorganism, it is valid to observe other organisms of Chlorophyta, since in the *Asterarcys* genus there is only one species (HEGEWALD et al., 2010). Some Chlorophyta genus (e.g., *Scenedesmus* and *Chlorella*) have potential related to their bioactive molecules that may include amino acids and proteins, which are studied for their plant growth promoting capacity. Some microalgae species present 50-56% of their dry weight in proteins (ISHAQ et al., 2016).

The few information that has been described about *AQ* reveals its composition and forms of cultivation (HONG et al., 2012). Under controlled cultivation conditions, this microalga may have a high content of proteins, amino acids, lipids, polysaccharides, and pigments (VARSHNEY et al., 2018; SINGH et al., 2019). Thus, regarding their potential use, authors indicate them as a potential source to produce bioethanol and biodiesel due to their lipid content (OLIVEIRA et al., 2017; CHAUDHARY and KHATTAR, 2019).

Furthermore, authors indicate that its protein content is in the range of 39-45% of its biomass, which is rich in different amino acids (GHOSH et al., 2017). Corroborating, authors

describe that in most species of microalgae, aspartate and glutamate generally constitute a large proportion of the total amino acid content (XUPENG et al., 2017). Additionally, these molecules in the levogyre form would provide bioactivity causing an effect in improving plant growth (MÓGOR et al., 2008). This potential still needs to be verified in new species, such as *AQ*, when applied to vegetables. In this sense, the ability to act on plant metabolism, causing higher yield, while producing quality food is sustainably remarkable, and calls for research attention. Furthermore, comparing Chlorophyta microalgae, it is assumed that the biomass of new species, such as *AQ*, can also serve as a source of amino acids for biofertilizers.

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3 SUBMITTED ARTICLE

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Title: MICROALGA *Asterarcys quadricellulare* IMPROVES TOMATO GROWTH, YIELD, SUGARS, AMINO ACIDS AND PROTEIN CONTENTS

ABSTRACT

The ever-growing food demand calls for sustainable approaches as the conventional fertilizers may harm the environment. Chlorophyta microalgae biomasses are known to promote plant growth, hence serving as strategic alternative to increase crop productivity. Considering the global needs for use of renewable inputs in agriculture, new studies with microalgae able to increase yield are needed. The hypothesis that the Chlorophyta microalgae *Asterarcys quadricellulare* biomass could be a source of bioactive free L-amino acids capable to promote plant growth and higher yield, when applied as foliar spray on tomato plants, was tested in this work over two years. First, the Maximum efficiency concentration (*Mec*) of this biomass was investigated through regression analysis, evaluating tomato fruit mass, caliber, number per plant, and yield. At second, the microalga *Mec* was tested on two tomato cultivars by weekly and biweekly frequency application. As a result, the *Asterarcys quadricellulare* biomass induced leaf expansion, heavier fruits, and higher yield regardless of the frequency used for both cultivars. Furthermore, it raised the levels of free amino acids, protein and total sugars in the leaves and fruits of organically grown tomato plants.

Keywords: Biofertilizer, Biostimulant, Chlorophyta, Organic production, *Solanum lycopersicum* L.

3.1 INTRODUCTION

As the global demographic expanse pressures agricultural production for food, the need for novel and sustainable approaches toward satisfying the ever-growing demand for yield becomes critical (Chiaiese et al. 2018). In this regard, environmental impact and food quality awareness become growing subjects, especially among horticultural practices (Rieder et al. 2018).

As an alternative for conventional crop production inputs, algae biomasses are eco-friendly, cost effective renewable sources that can help to achieve sustainable agriculture (Tuteja and Gill 2013; du Jardin 2015; Rouphael and Colla 2018). Among them are microalgae photosynthetic microscopic eukaryotic organisms and prokaryotic cyanobacteria (Ortiz-

Moreno et al. 2019), being a biomass source that have great advantages involving adaptability to different environments, easy cultivation, and growth rates at low cost (Wang et al. 2014).

The microalgae often reported as biofertilizers or biostimulants primarily are blue-green algae (Cyanophyta) and green algae (Chlorophyta) (Bumandalai and Tserennadmid 2019). A wide range of substances have been identified in their composition, including amino acids (*Aa*), polyamines, polysaccharides, and hormone-like compounds that when applied to plants may act as signaling molecules stimulating plant growth (Nardi et al. 2016; Mógor et al. 2017; Gemin et al. 2019).

The Chlorophyta *Asterarcys quadricellulare* (*AQ*) was first described by Hegewald and Schmidt (1992). Hong et al. (2012) researching this microalga composition found that the most abundant fatty acid was α-linolenic acid, which is nutritionally important, also identifying a significant amount of hexadecenol, a long-chain fatty alcohol used for cosmetics and biofuel industry. Additionally, its potential for carotenoid production (Singh et al. 2019) and other industrial applications, due to its high protein (*Pt*) and carbohydrate content are reported (Ghosh et al. 2017), but its agronomic potential is yet to be discovered.

Nonetheless, it is noteworthy that high protein content microalgae could be a source of L-*Aa*, whose isomerism is known to be biologically active to promote plant growth (Mógor et al. 2018). For some species of Chlorophyta, their potential as plant growth promoters is associated to their complex composition including L-*Aa* (Dineshkumar et al. 2020).

Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crop around the world and the most preferred species grown in greenhouses (Coban et al. 2020). El Arroussi et al. (2018) in concordance with Garcia-Gonzalez and Sommerfeld (2016) described that some vegetables from Solanaceae family, like tomato and pepper, are positively affected by microalgal application in relation to plant growth promotion. Furthermore, ÖzdemİR et al. (2016) used *Chlorella* sp. biomass as biofertilizer on organically grown tomato production in greenhouses obtaining an increase on development, yield, and total soluble solids, demonstrating some physiological changes promoted by microalgae.

Thus, new works using microalgae biomass as biofertilizers or biostimulants can aid toward the standardization of its raw material, helping to develop knowledge about how they act over different crops (Barone et al. 2018). Considering tomato plant importance and the complexity of its cultivation, this is one of the most studied vegetables, nonetheless, its sustainable production methods require further agronomic research, mainly under organic production system (Amatussi et al. 2020).

Therefore, in this work the aim was to evaluate *AQ* biomass as a biofertilizer for organic tomato production, determining yield over two years and biochemical changes related to this microalga bioactivity.

3.2 MATERIAL AND METHODS

3.2.1 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 spray drying method producing a fine greenish colored powder. After cell disruption (Show et al. 2015) the free amino acids were extracted (Magné and Larher 1992; Winters et al. 2002), indicating a concentration of 90.94 mg g^{-1} (w/v), which corresponds to 9% of the microalga biomass.

The analysis of the *Aa* profile present in *AQ* biomass (Tab. 1) was determined in three stages, the first, adapted from CBAA (2013), through the sample analysis for the measurement of nitrogen and *Pt* using the combustion method. The second stage, by high performance liquid chromatography (HPLC) using the Pico-Tag methodology (White et al. 1986). This method is an integrated technique, derivatized with phenylisothiocyanate (PITC), followed by the separation of phenylthiocarbamyl (PTC) *Aa* by HPLC. PTC *Aa* have a strong ultraviolet absorbance; hence the detection is done by measuring this absorbance at 254 nm. Lastly, at the third stage, occurred the determination of tryptophan through *Pt* hydrolysis (Lucas and Sotelo 1980), performed with 5 N of NaOH containing 5% SnCl2 with 4 N of LiOH, at 145°C, triggering tryptophan maximum values after 4-8 hours of hydrolysis. The aminogram was carried out at the CBO Analysis Laboratory - Valinhos, São Paulo, Brazil.

TABLE 1 - FREE L-AMINO ACIDS (L-*Aa*) COMPOSITION OF *Asterarcys quadricellulare* (CCAP 294/1) BIOMASS

Amino acids	Percentage (%)	Amino acids	Percentage (%)
Glutamic acid	4.27	Glycine	1.54
Aspartic acid	3.32	Threonine	1.45
Alanine	2.41	Isoleucine	1.41
Leucine	2.36	Phenylalanine	1.37
Arginine	2.17	Tyrosine	0.95
Lysine	2.11	Histidine	0.71
Valine	1.81	Methionine	0.51
Serine	1.68	Tryptophan	0.37
Proline	1.60	Cysteine	0.29

3.2.2 Experiment I

To determine the Maximum efficiency concentration (*Mec*) of the microalga *Asterarcys quadricellulare* (CCAP 294/1) biomass over fruits mass and tomato yield, an experiment was conducted applying different concentration weekly (*WfT*) through foliar spray on cultivar (cv.) Giuliana-Sakata®. This experiment started in December of 2018 and was conducted at the Organic Horticulture Research Area of 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 Cfb type temperate climate according to the Köppen classification.

The sowing was done in plastic modular trays (200 cells) filled with organic substrate (Provaso®) and kept in a nursery with a timed microsprinkler irrigation until 40 days after sowing (DAS). 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. According to EMBRAPA (2013) the soil is a medium texture Alic Yellow-Red Oxisol. The chemical analysis resulted in the following average values from the 0–20 cm layer: pH ($CaCl^2$) = 5.84; pH H_2O = 6.71; Al^{+3} = 0; $H+Al^{+3}$ = 2.93 cmolc dm^{-3}; Ca^{2+} = 5.28 cmolc dm^{-3}; Mg^{2+} = 3.05 cmolc dm^{-3}; K^+ = 1.32 cmolc dm^{-3}; P (Mehlich) = 49.0 mg dm; S = 33.49 mg dm^{-3}; C = 26 g dm^{-3}; V% = 76.7 and CTC = 12.58 cmolc dm^{-3}.

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

The spacing was 1.3 m within rows and 0.5 m between plants. Two drip tape per planting row were utilized for irrigation system, employing a daily frequency irrigation, aiming to maintain soil moisture at 80%, using a tensiometer for inspection. Two stems per plant were conducted and analyzed, each having the height limited by the apical meristem remotion two leaves above the 10^{th} rachis.

Treatments were arranged in a completely randomized design, with six replications (each with 6 representative plants) of *WfT* of *AQ* suspensions in sugarcane molasses with de concentrations of 50, 150, 250 and 400 g L^{-1} of the microalga biomass obtained from spray dry method. From each suspension 1 mL L^{-1} was diluted into water for foliar sprays, producing solutions equivalent to 0.050 (*AQ*05); 0.150 (*AQ*15); 0.250 (*AQ*25); and 0.400 g L^{-1} (*AQ*40) as treatments, plus a Control with water.

The leaf sprays started at 54 days after sowing (DAS) and ended a week before the last rachis (10th rachis) was harvested. The sprays were done between 9:00 a.m. to 10:00 a.m., using an electronic sprayer (Kawashima®) at constant pressure (40 psi), applying a spray volume according to plants development (from 30 to 100 mL plant^{-1}).

The plants were managed according to Brazilian regulation of organic agriculture. Thus, only products allowed by this regulation were used to control pests: *Bacillus thuringiensis*, *Azadirachta indica* and *Beauveria bassiana*.

The harvesting point was defined when the fruits reached 50% of red coloration. The first experiment focused on evaluating production variables. Thus, the fruit mass average was chosen to demonstrate this biomass effect on tomato plants. For this, all fruits mass from 2 plants per replications were computed using a precision balance.

3.2.3 Experiment II

For the second experiment, which started in November of 2019, a larger analysis was conducted, examining the *AQ Mec* effects over biometrical and biochemical variables. Therefore, after one year from the start of experiment I, at the same greenhouse, following the identical methodology for plant management (sowing, transplantation, spacing and foliar spray technique) the experiment was conducted. This time, a factorial scheme (2x3) was established, applying *AQ*25, *Mec* identified at experiment I, in two cv. (Netuno-Blueseeds® and Giuliana-Sakata® = Factor 1) under two frequencies plus a Control (weekly and biweekly = Factor 2), composing six treatments with four replications each (6 plants per replication). Again, a full cycle was carried, conducing two stems per plant, each having the heigh limited by the apical meristem remotion two leaves above the 14th rachis.

From the plants middle third section, leaf samples were collected for fresh mass measurement (g), and its respective dry mass (g) obtained after two days on a drying oven, both quantified on a precision scale. From the same section the leaf area (cm^2) values were composed by a three leaves average in each plot, and measures using WinRhizo®. The first fruit from the 6th rachis, and its respective leaf above, were collected for biochemical analysis. The leaf relative chlorophyll was measured biweekly, during the month of the 6th rachis harvest, resulting in an average of three reading in time, from thirty leaflets from each plot, using a portable meter (N-Tester®) (Mógor et al. 2013).

Fruit mass (g) was measured, from three representative plants per repetition, using a precision scale, as its length (mm) and width (mm) were measured using a pachymeter. Fruits number per plant and total yield (ton ha^{-1}) were also calculated.

3.2.4 Biochemical analysis

Leaf and fruit samples were washed, then macerated using liquid nitrogen until a fine powder was obtained. For leaves, values were expressed as μg of metabolite g^{-1} fresh mass for total free amino acids (*Aa*), total soluble proteins (*Pt*), total sugar (*Ts*), reducing sugar (*Rs*) and non-reducing-sugars (*nRs*), chlorophyll *a* and *b*, carotenoids, phenolic compounds, and nitrate reductase enzyme activity. Likewise, for fruit samples analysis *Aa*, *Pt*, *Ts*, *Rs*, and *nRs* content were analyzed following the same methodology.

Total free *Aa* were extracted from leaves and fruits, and the colorimetric reaction was performed with 1 mL of the sample plus 0.5 mL of 0.2 M pH 4.6 citrate buffer and 1 mL with ninhydrin solution (1% ninhydrin, 3% ascorbic acid in 2-methoxy ethanol). Readings were made at 570 nm. A standard curve was made with glutamine and asparagine (2 mM) with values ranging between 1.832 and 6.036 μg g^{-1} (Magné and Larher 1992).

Soluble *Pt* were extracted from leaves and fruits by Du et al. (2010) method with adaptations: using phosphate buffer pH 7.5 and 100 mM, adding 1 mM EDTA, 3 mM 1,4-dithiothreitol (DTT), 4% polyvinylpyrrolidone (PVP) (w/v), and 1 mM phenylmethylsulfonyl fluoride (PMSF). The solution was than homogenized by vortex for 10 s at low speed and then centrifuged at 9000×g for 15 min. The supernatant was collected for measuring at 595 nm (Bradford 1976). The standard curve was built using bovine serum albumin (BSA) at 0.2% (w/v) with values ranging between 613 and 1.567 μg g^{-1}.

The *Ts*, *Rs*, and *nRs* of leaves and fruits were determined with a standard curve obtained using glucose at 1 mg mL^{-1} (5.5 mM), with values expressed in μg of sugars per g of fresh plant material. Readings were performed at 540 nm (Maldonade et al. 2013).

The determination of leaves chlorophylls and carotenoids was performed according to Lichtenthaler (1987) with modifications, using the formulas described by Lichtenthaler and Buschmann (2001) and values expressed in mg per g of fresh plant material.

Following the methodology proposed by Jaworski (1971) the leaves enzyme nitrate reductase activity was determined, with modifications. Readings were performed at 540 nm and the values expressed in μ mol of NO2 h^{-1} g^{-1} of plant material.

Determination of leaves phenolic compounds followed the Prussian Blue method by Price and Butler (1977) with modifications. Readings were performed at 700 nm and the values expressed in μg per g of fresh plant material.

3.2.5 Statistical analysis

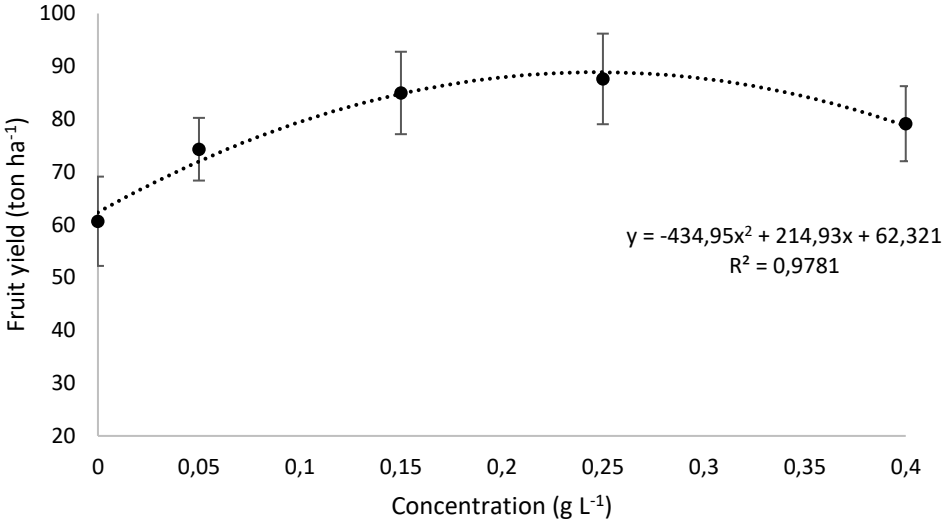
For the first experiment all data were tested for their homogeneity of variances by Bartlett and then submitted to variance and regression analysis. The *Mec* of the evaluated variables was determined by the first derivative of the regression equations, equaled to zero. For the second experiment, after the data homogeneity confirmation, it was analyzed as a 2x3 factorial experiment, with averages compared by Tukey's test ($p < 0.01$). Statistical analysis was performed using Assistat 7.7 Beta software (Silva and Azevedo 2016).

3.3 RESULTS

3.3.1 Experiment I

The regression analysis (Fig. 2) of *AQ* applied in a *WfT* through foliar spray on *Solanum lycopersicum* L. cv. Giuliana, indicated the *Mec* of 0.247 g L^{-1} for fruit yield. Thus, showing that weekly applications of this Chlorophyta microalga can lead to a greater production of tomato.

FIGURE 2 - FRUIT YIELD (ton ha^{-1}) OF *Solanum lycopersicum* L. cv. GIULIANA TREATED WITH DIFFERENT CONCENTRATIONS OF THE MICROALGA *Asterarcys quadricellulare* APPLIED WEEKLY (*WfT*) THROUGH FOLIAR SPRAY



All *AQ* treatments showed better yield results compared to the Control, however demonstrating diminishing returns around 0.25 g L^{-1}. When only applied with water tomato plants produced 16 ton ha^{-1} less than the *AQ Mec*. Consequently, the 0.25 g L^{-1} *AQ* concentration (*AQ*25) was chosen for the experiment II.

3.3.2 Experiment II

Comparing cultivars, the leaf area (Tab. 2) of Giuliana (G') were higher than Netuno (N'), while comparing treatments, the leaf area on a biweekly frequency treatment (*BfT*) was 107.27 cm² higher in N' and 76.48 cm² higher in G' over Control, which correspond to an increase of 21.46% and 19.52%, respectively. This indicate that the *AQ*25 promoted leaf expansion equally for both cv. regardless of the spray frequency used.

Although the leaf fresh mass results showed no different effects among cultivars, both *AQ* frequencies presented higher fresh mass than control. For dry mass, only the *BfT* showed increment. The leaf fresh mass on a *BfT* increased 27.09% in N' and 34.42% in G'. Furthermore, N' leaf dry mass on a *BfT* had an increase of 26.50%, while G' had an increase of 39.86%. For leaf fresh/dry mass ratio no statistical difference was found (Tab. 2).

TABLE 2 - (a) LEAF AREA (cm²), (b) LEAF FRESH MASS (g), (c) LEAF DRY MASS (g), AND (d) LEAF FRESH/DRY MASS RATIO OF *Solanum lycopersicum* L. cv. NETUNO (N') AND GIULIANA (G') TREATED WITH 0.25 g L^{-1} OF *Asterarcys quadricellulare* BIOMASS (*AQ*25) APPLIED WEEKLY (*WfT*) AND BIWEEKLY (*BfT*) THROUGH FOLIAR SPRAY

(a) Leaf area (cm²)

	Control	*WfT*	*BfT*	$\overline{X}^1$
N'	499.8 ± 78.6	587.23 ± 68.5	607.1 ± 144.1	564.7 a
G'	391.7 ± 33.1	503.0 ± 41.2	468.2 ± 59.2	454.3 b
$\overline{X}^2$	445.8 b	545.1 a	537.6 a	
C	**			
T	*			
CxT	ns			

(b) Leaf fresh mass (g)

	Control	*WfT*	*BfT*
N'	26.62 ± 3.38	29.62 ± 3.86	33.84 ± 4.31
G'	23.10 ± 3.68	32.14 ± 5.45	32.26 ± 6.57
$\overline{X}^2$	24.86 b	30.88 a	33.05 a
C	ns		
T	**		
CxT	ns		

(c) Leaf dry mass (g)

	Control	*WfT*	*BfT*
N'	3.47 ± 0.53	3.47 ± 0.49	4.39 ± 0.72
G'	3.05 ± 0.52	4.19 ± 0.84	4.43 ± 1.05
$\overline{X}^2$	3.26 b	3.83 b	4.41 a
C	ns		
T	*		
CxT	ns		

(d) Leaf fresh/dry ratio

	Control	*WfT*	*BfT*
N'	7.71 ± 0.55	8.55 ± 0.54	7.77 ± 0.62
G'	7.61 ± 0.63	7.73 ± 0.72	7.35 ± 0.55
C	ns		
T	ns		
CxT	ns		

Means within rows and columns followed by different letters are significantly different by Tukey's test at $p < 0.01$ (**), $p < 0.05$ (*), and if not significant (ns). C = Cultivars, T = Frequencies, and CxT = Interaction. $\overline{X}^1$ = C averages, $\overline{X}^2$ = T averages, (± SD, $n = 4$).

Fruit mass results (Tab. 3) demonstrated a factor interaction, indicating that this variable is frequency dependent. While for N' both frequencies showed higher masses than the Control, for G' only *BfT* promoted fruit mass gains. Accordingly, when comparing Control to *BfT,* it is noted that *AQ*25 promoted heavier fruits, with 5.65% and a 5.93% fruit mass increases for N' and G', respectively.

TABLE 3 - (a) FRUIT MASS (g), (b) LENGTH (mm), (c) WIDTH (mm), AND (d) NUMBER PER PLANT OF *Solanum lycopersicum* L. cv. NETUNO (N') AND GIULIANA (G') TREATED WITH 0.25 g L^{-1} OF *Asterarcys quadricellulare* BIOMASS (*AQ*25) APPLIED WEEKLY (*WfT*) AND BIWEEKLY (*BfT*) THROUGH FOLIAR SPRAY

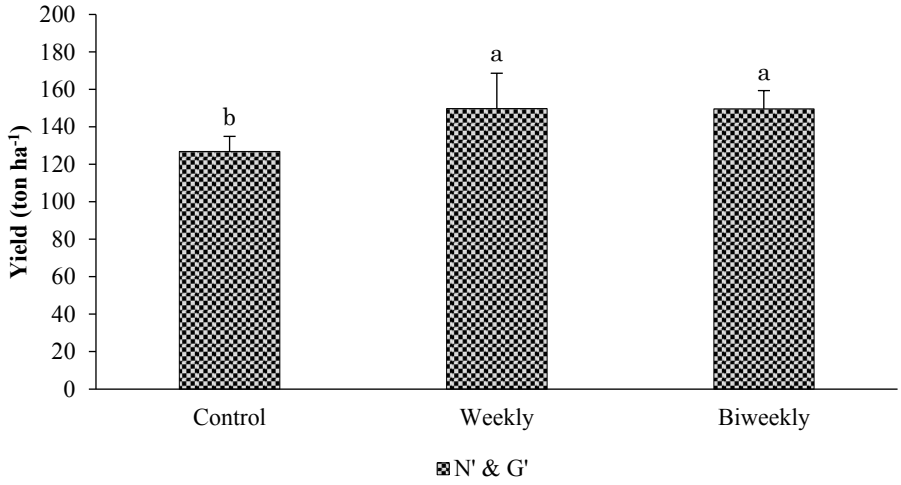
(a) Fruit mass (g)				
	Control	*WfT*	*BfT*	
N'	91.6 ± 1.8 bB	97.3 ± 4.5 bA	96.8 ± 2.3 bA	
G'	118.7 ± 1.4 aB	118.2 ± 1.0 aB	125.8 ± 2.8 aA	
C	**			
T	**			
CxT	*			
(b) Fruit length (mm)				
	Control	*WfT*	*BfT*	
N'	75.0 ± 0.5 bB	78.2 ± 3.4 bA	74.7 ± 1.0 bB	
G'	84.4 ± 2.9 aB	83.1 ± 1.7 aB	87.7 ± 1.0 aA	
C	**			
T	ns			
CxT	**			
(c) Fruit width (mm)				
	Control	*WfT*	*BfT*	
N'	48.0 ± 1.4 bA	49.7 ± 1.1 bA	48.6 ± 0.7 bA	
G'	53.1 ± 0.6 aA	52.1 ± 1.8 aA	54.1 ± 0.3 aA	
C	**			
T	ns			
CxT	*			
(d) Fruit number per plant				
	Control	*WfT*	*BfT*	$\overline{X}^1$
N'	94.8 ± 5.0	99.5 ± 8.8	96.6 ± 5.3	97.0 a
G'	70.6 ± 4.9	77.5 ± 7.8	78.5 ± 1.5	75.5 b
C	**			
T	ns			
CxT	ns			

Means within rows (capital letters) and columns (lowercase) followed by different letters are significantly different by Tukey's test at $p < 0.01$ (**), $p < 0.05$ (*), and if not significant (ns). C = Cultivars, T = Frequencies, and CxT = Interaction. $\overline{X}^1$ = C averages, $\overline{X}^2$ = T averages, (± SD, $n = 4$).

Although none of the *AQ*25 applications frequency promoted statistical differences of fruit width and number per plant, it should be considered that for its length (Tab. 3) there was a factor interaction that led control fruits to have lesser length when compared to *WfT* for N' and *BfT* for G'. Also, is noteworthy that N' naturally produces a higher number of fruits than G', however G' produces heavier fruits, compensating the yield (Fig.3).

Consequently, this biometric changes influenced by *AQ*25 aided the higher fruit mass, which than boosted fruit yield, showing that both cultivars are responsive to *AQ*25 foliar spray, even when applied biweekly (Fig. 3). Thus, when comparing *BfT* to the control, N' produced in average 13.52 ton ha^{-1} more, while G' was 31.76 ton ha^{-1} higher, corresponding a 10.19% and 26.20% greater output, respectively.

FIGURE 3 - FRUIT YIELD (ton ha^{-1}) AVERAGES OF TWO CULTIVARS OF *Solanum lycopersicum* L. cv. NETUNO (N') AND GIULIANA (G') TREATED WITH 0.25 g L^{-1} OF *Asterarcys quadricellulare* BIOMASS (*AQ*25) APPLIED WEEKLY (*WfT*) AND BIWEEKLY (*BfT*) THROUGH FOLIAR SPRAY



Vertical bars indicate standard deviation, (n=4). Columns with the same letter do not differ statistically. ANOVA: Cultivar: ns; Frequencies: **; and Interaction: ns; by Tukey's test at $p < 0.01$ (**).

Relative chlorophyll values (Tab. 4) in corroboration to chlorophyll biochemical data (Tab. 5) showed a natural difference between cv., indicating darker green leaves for N'. Furthermore, considering *AQ*25 application frequencies, relative chlorophyll results demonstrated better gains for N' through *WfT*, whereas for G' both frequencies were superior to the control.

TABLE 4 - RELATIVE CHLOROPHYLL CONTENT (N-tester®) OF *Solanum lycopersicum* L. cv. NETUNO (N') AND GIULIANA (G') TREATED WITH 0.25 g L^{-1} OF *Asterarcys quadricellulare* BIOMASS (*AQ*25) APPLIED WEEKLY (*WfT*) AND BIWEEKLY (*BfT*) THROUGH FOLIAR SPRAY

	Relative chlorophyll content (N-tester®)		
	Control	*WfT*	*BfT*
N'	629.56 ± 7.60 aB	641.78 ± 11.10 aA	625.00 ± 9.90 aB
G'	588.11 ± 6.63 bB	633.33 ± 11.05 aA	634.44 ± 5.12 aA
C	**		
T	**		
CxT	**		

Means within rows (capital letters) and columns (lowercase) followed by different letters are significantly different by Tukey's test at $p < 0.01$ (**). C = Cultivars, T = Frequencies, and CxT = Interaction.

Moreover, for each cv., leaf pigments (Tab. 5) had distinct results. Chlorophyll *a* shown factor interaction, while N' always shown higher values than G', none of *AQ*25 application increase N' content. However, for G' the *BfT* improved 5.88% chlorophyll *a* over the Control.

For chlorophyll *b*, total chlorophylls, and carotenoids results (Tab. 5), no statistical difference was found between treatment frequencies and control.

TABLE 5 - (a) LEAF CHLOROPHYLL *a*, (b) CHLOROPHYLL *b*, (c) TOTAL CHLOROPHYLLS, AND (d) CAROTENOIDS (mg g^{-1}) OF *Solanum lycopersicum* L. cv. NETUNO (N') AND GIULIANA (G') TREATED WITH 0.25 g L^{-1} OF *Asterarcys quadricellulare* BIOMASS (*AQ*25) APPLIED WEEKLY (*WfT*) AND BIWEEKLY (*BfT*) THROUGH FOLIAR SPRAY

(a) Chlorophyll *a* (mg.g-¹)

	Control	*WfT*	*BfT*
N'	0.21 ± 0.02 aA	0.23 ± 0.03 aA	0.2 ± 0.02 aA
G'	0.17 ± 0.01 bA	0.15 ± 0.02 bA	0.18 ± 0.03 aA
C	**		
T	*		
CxT	*		

(b) Chlorophyll *b* (mg.g-¹)

	Control	*WfT*	*BfT*	$\overline{X}^1$
N'	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.12 a
G'	0.09 ± 0.00	0.09 ± 0.01	0.1 ± 0.02	0.09 b
C	**			
T	ns			
CxT	ns			

(c) Total chlorophylls (mg.g-¹)

	Control	*WfT*	*BfT*	$\overline{X}^1$
N'	0.32 ± 0.03	0.36 ± 0.05	0.31 ± 0.03	0.33 a
G'	0.26 ± 0.01	0.24 ± 0.04	0.29 ± 0.04	0.26 b
C	**			
T	ns			
CxT	ns			

(d) Carotenoids (mg.g-¹)

	Control	*WfT*	*BfT*	$\overline{X}^1$
N'	0.12 ± 0.01	0.14 ± 0.02	0.12 ± 0.01	0.13 a
G'	0.10 ± 0.01	0.11 ± 0.04	0.11 ± 0.04	0.11 b
C	**			
T	ns			
CxT	ns			

Means within rows (capital letters) and columns (lowercase) followed by different letters are significantly different by Tukey's test at $p < 0.01$ (**), $p < 0.05$ (*), and if not significant (ns). C = Cultivars, T = Frequencies, and CxT = Interaction. $\overline{X}^1$ = C averages, $\overline{X}^2$ = T averages, (± SD, $n = 4$).

For leaf total sugars content (*TsC*) an increase was found among frequencies (Tab. 6). For G' treatments were equal, while for N' *AQ*25 improved *TsC* on both frequencies. Hence, N' under *BfT* had 50.5 µg g^{-1} more *TsC*, corresponding to a 7.12% gain when compared to the control.

For leaf reducing sugars content (*Rs*) (Tab. 6) both cv. showed equal behavior, concurrently, *AQ*25 applications improved *Rs* over the control at both frequencies. Thus, choosing *WfT* for comparison is possible to see a similar increase of *Rs* for N' (17.72%) and G' (19.82%). Whereas, for leaf non reducing sugars (*nRs*) (Tab. 6), the *WfT* and control averages were superior when compared to the *BfT*, with no statistical difference between cv.

TABLE 6 - (a) LEAF TOTAL SUGAR CONTENT (*TsC*), (b) REDUCING SUGARS (*Rs*), AND (c) NON-REDUCING SUGARS (*nRs*) (µg g^{-1}) OF *Solanum lycopersicum* L. cv. NETUNO (N') AND GIULIANA (G') TREATED WITH 0.25 g L^{-1} OF *Asterarcys quadricellulare* BIOMASS (*AQ*25) APPLIED WEEKLY (*WfT*) AND BIWEEKLY (*BfT*) THROUGH FOLIAR SPRAY

(a) Leaf total sugar content (µg.g-¹)			
	Control	*WfT*	*BfT*
N'	751.2 ± 4.9 aA	770.2 ± 17.5 aA	743.9 ± 19.0 aA
G'	709.6 ± 27.8 bB	770.6 ± 11.4 aA	760.1 ± 36.9 aA
C	ns		
T	**		
CxT	*		
(b) Leaf reducing sugars (µg.g-¹)			
	Control	*WfT*	*BfT*
N'	311.8 ± 24.4	351.7 ± 34.6	367.0 ± 4.7
G'	314.7 ± 32.6	340.1 ± 12.7	377.0 ± 29.1
$\overline{X}^2$	313.2 b	345.9 a	372.0 a
C	ns		
T	**		
CxT	ns		
(c) Leaf non-reducing sugars (µg.g-¹)			
	Control	*WfT*	*BfT*
N'	439.3 ± 23.9	418.4 ± 27.6	376.8 ± 21.8
G'	394.8 ± 34.5	428.2 ± 4.6	383.0 ± 37.2
$\overline{X}^2$	417.1 a	423.3 a	379.9 b
C	ns		
T	*		
CxT	ns		

Means within rows (capital letters) and columns (lowercase) followed by different letters are significantly different by Tukey's test at $p < 0.01$ (**), $p < 0.05$ (*), and if not significant (ns). C = Cultivars, T = Frequencies, and CxT = Interaction. $\overline{X}^1$ = C averages, $\overline{X}^2$ = T averages, (± SD, $n = 4$).

For fruits total sugars content (*TsC*) (Tab. 7) it was observed that *WfT* showed better gains for both cv., indicating that the source-to-sink flow of photoassimilates can be stimulated under a more frequent utilization of *AQ*25. Consequently, for N', the same frequency compared to the control improved *TsC* by 2107.31 µg g^{-1} and 2413.71 µg g^{-1} for G', corresponding an increase of 17.72% and 19.82%, respectively.

While, for fruit *Rs* (Tab. 7), no difference between treatments was found, yet N' shown higher *Rs* averages. In contrast, for fruit *nRs* (Tab. 7) an inverse behavior for cv. was found, showing higher averages for G'. Additionally, following fruit *TsC* results, the *WfT* led to a greater free sugars accumulation on fruits for both cv.

TABLE 7 - (a) FRUIT TOTAL SUGAR CONTENT (*TsC*), (b) REDUCING SUGARS (*Rs*), AND (c) NON-REDUCING SUGARS (*nRs*) (µg g^{-1}) OF *Solanum lycopersicum* L. cv. NETUNO (N') AND GIULIANA (G') TREATED WITH 0.25 g L^{-1} OF *Asterarcys quadricellulare* BIOMASS (*AQ*25) APPLIED WEEKLY (*WfT*) AND BIWEEKLY (*BfT*) THROUGH FOLIAR SPRAY

(a) Fruit total sugar content (µg.g-¹)				
	Control	*WfT*	*BfT*	
N'	26662.6 ± 994.9	28812 ± 850.7	26319.1 ± 1003.3	
G'	27454.3 ± 691.4	29975.6 ± 1890.1	26101 ± 592.8	
$\overline{X}^2$	27058.5 b	29393.8 a	26210.0 b	
C	ns			
T	**			
CxT	ns			
(b) Fruit reducing sugars (µg.g-¹)				
	Control	*WfT*	*BfT*	$\overline{X}^1$
N'	25973.4 ± 1122	27076.5 ± 689.4	25164.7 ± 1015	26071.5 a
G'	25097.8 ± 468.4	23243.7 ± 2202.8	23938.3 ± 1231.4	24093.3 b
C	**			
T	ns			
CxT	ns			
(c) Fruit non-reducing sugars (µg.g-¹)				
	Control	*WfT*	*BfT*	$\overline{X}^1$
N'	689.2 ± 256.5	1735.6 ± 1315.9	1154.4 ± 712.6	1193.0 b
G'	2356.6 ± 732.2	6731.9 ± 3571.6	2162.7 ± 1179.9	3750.4 a
$\overline{X}^2$	1522.9 b	4233.7 a	1658.5 b	
C	**			
T	**			
CxT	ns			

Means within rows and columns followed by different letters are significantly different by Tukey's test at $p < 0.01$ (**), ns = not significant. C = Cultivars, T = Frequencies, and CxT = Interaction. $\overline{X}^1$ = C averages, $\overline{X}^2$ = T averages, (± SD, $n = 4$).

Results of the free amino acid content (*AaC*) showed a similar pattern for leaves and fruits (Tab. 8) from both cv., indicating that both frequencies offer higher *AaC* gains. For N' the *BfT* had 354.75 µg g^{-1} more *AaC* in the leaves, whereas for G' it was 317.64 µg g^{-1} higher, corresponding an increase over control of 19.36% and 15.91%, respectively.

In relation to *AaC* in the fruits, the *BfT* elevated values by 14.25% and 36.23% for N' and G', respectively. It was also noticed a similar outcome for factor 2 averages of each *AQ*25 frequency application in relation to leaves and fruit protein content (*PtC*) for both cv. (Tab. 8); with emphasis for N' leaves *PtC* under *BfT* showing a 122.81% raise over control.

TABLE 8 - (a) LEAVES AND (b) FRUITS AMINO ACID CONTENT (*AaC*), (c) LEAVES AND (d) FRUITS PROTEIN CONTENT (*PtC*) (µg g^{-1}) OF *Solanum lycopersicum* L. cv. NETUNO (N') AND GIULIANA (G') TREATED WITH 0.25 g L^{-1} OF *Asterarcys quadricellulare* BIOMASS (*AQ*25) APPLIED WEEKLY (*WfT*) AND BIWEEKLY (*BfT*) THROUGH FOLIAR SPRAY

(a) Leaf free amino acids (µg.g-¹)				
	Control	*WfT*	*BfT*	
N'	183.2 ± 17.7	223.8 ± 20.6	218.7 ± 19.0	
G'	199.6 ± 18.4	200.0 ± 8.8	231.4 ± 22.1	
$\overline{X}^2$	191.4 b	211.9 a	225.0 a	
C	ns			
T	**			
CxT	ns			
(b) Fruit free amino acids (µg.g-¹)				
	Control	*WfT*	*BfT*	$\overline{X}^1$
N'	421.9 ± 82.1	522.9 ± 86.8	482.0 ± 26.7	475.6 b
G'	438.9 ± 40.8	603.6 ± 50.3	597.9 ± 79.3	546.8 a
$\overline{X}^2$	430.4 b	563.3 a	540.0 a	
C	*			
T	**			
CxT	ns			
(c) Leaf soluble protein (µg.g-¹)				
	Control	*WfT*	*BfT*	
N'	61.3 ± 21.2 aB	121.7 ± 20.6 aA	136.6 ± 29.6 aA	
G'	77.0 ± 14.5 aA	110.9 ± 20.1 aA	80.1 ± 19.8 bA	
C	ns			
T	**			
CxT	*			
(d) Fruit soluble protein (µg.g-¹)				
	Control	*WfT*	*BfT*	$\overline{X}^1$
N'	82.6 ± 13.9	115.1 ± 15.2	131.0 ± 23.0	109.6 b
G'	147.1 ± 15.4	148.4 ± 27.5	156.7 ± 25.9	150.7 a
$\overline{X}^2$	114.8 b	131.7 a	143.9 a	
C	**			
T	*			
CxT	ns			

Means within rows (capital letters) and columns (lowercase) followed by different letters are significantly different by Tukey's test at $p < 0.01$ (**), $p < 0.05$ (*), and if not significant (ns). C = Cultivars, T = Frequencies, and CxT = Interaction. $\overline{X}^1$ = C averages, $\overline{X}^2$ = T averages, (± SD, $n = 4$)

Phenolic compounds results (Tab. 9) were equal between cv., however the Control averages had higher values when compared to each *AQ*25 frequency. Biweekly applications promoted 15.45% and 24.98% lesser accumulation of phenolic compounds in N' and G', respectively. For nitrate reductase (Tab. 9), enzyme levels were 7.14% higher through *WfT* for N', whereas for G' *AQ*25 produced no statistical difference when compared to the control.

TABLE 9 - (a) PHENOLIC COMPOUNDS (µg g^{-1}) AND (b) NITRATE REDUCTASE (µg g^{-1}) OF *Solanum lycopersicum* L. cv. NETUNO (N') AND GIULIANA (G') TREATED WITH 0.25 g L^{-1} OF *Asterarcys quadricellulare* BIOMASS (*AQ*25) APPLIED WEEKLY (*WfT*) AND BIWEEKLY (*BfT*) THROUGH FOLIAR SPRAY

(a) Phenolic compounds (µg.g-¹)			
	Control	*WfT*	*BfT*
N'	285.2 ± 3.2	252.3 ± 21.6	241.1 ± 8.5
G'	287.1 ± 45.9	226.7 ± 28.5	215.4 ± 3.1
$\overline{X}^2$	286.1 a	239.5 b	228.2 b
C	ns		
T	**		
CxT	ns		
(b) Nitrate reductase (µg.g-¹)			
	Control	*WfT*	*BfT*
N'	0.56 ± 0.05 aB	0.70 ± 0.06 aA	0.60 ± 0.03 aB
G'	0.52 ± 0.04 aA	0.56 ± 0.02 bA	0.56 ± 0.02 aA
C	**		
T	**		
CxT	*		

Means within rows (capital letters) and columns (lowercase) followed by different letters are significantly different by Tukey's test at $p < 0.01$ (**), $p < 0.05$ (*), and if not significant (ns). C = Cultivars, T = Frequencies, and CxT = Interaction. $\overline{X}^1$ = C averages, $\overline{X}^2$ = T averages, (± SD, $n = 4$)

3.4 DISCUSSION

Chlorophyta microalgae composition include pigments, *Pt*, *Aa*, polysaccharides, antioxidants, and other compounds which are studied for their economic importance (Kapoore et al. 2021). Some species are known for its bioactive molecules to induce metabolic changes in plants acting as biostimulants (Ronga et al. 2019).

Among some microalgae bioactive molecules are the L-*Aa* (Mógor et al. 2018) showing action in promoting *Pt*, pigments and key phytohormones synthesis responsible for plant growth (Guedes et al. 2018). For example, the *Aa* tryptophan is a precursor of the plant hormones auxin, salicylic acid and aromatic secondary compounds deeply involved on multiple biological functions in vegetables, while arginine is a precursor of polyamines (Bulgari et al. 2019), related to many plant developmental processes.

For most microalgae species, aspartate, and glutamate (glutamic acid) generally constitute a large proportion of its L-*Aa* (Xupeng et al. 2017). Likewise, glutamic acid was ranked as the most present *Aa* in the *Asterarcys quadricellulare* (CCAP 294/1) biomass composition (Tab. 1). In this sense, as studies identified glutamate receptors in plants (Price et al. 2012; Forde and Roberts 2014), which can lead to plant regulation involving plant growth, photosynthesis, and stress signaling (Tegeder 2012; Weiland et al. 2015), it can shed a light on this microalga influence on tomato plant responses.

The regression analysis with increasing *AQ* concentrations applied on tomato plants indicated the better effectiveness for fruit yield around 0.25 g L^{-1} (Fig. 2). Thus, the rich free L-*Aa Asterarcys quadricellulare* (CCAP 294/1) biomass may have played an important role on plants metabolism, as the foliar applications induced increments in plant productivity.

Previous microalgae studies point to growth and development improvement results for several vegetables, such as lettuce, red beet, and tomato, demonstrating its effect under open-field and greenhouse conditions (Faheed 2008; Garcia-Gonzalez and Sommerfeld 2016; Mógor et al. 2017; El Arroussi et al. 2018; Mógor et al. 2018).

It is known that all plants depend on a constant flow of *Aa* to performing several functions, such as being the main nitrogen transporters and precursors of a huge number of metabolites (Häusler et al. 2014), affecting plant growth and development (Thomas et al. 2009; Roca et al. 2013; Dinkeloo et al. 2018), as those found with *AQ*25 treatments.

The results of leaf area (Tab. 2) showed increase for both cv. treated with *AQ*25, indicating a plant growth promotion bioactivity, even under a *BfT*. Studies about similar microalgae biochemical composition, rich in free *Aa* and *Pt*, shows that these molecules could be delivered directly through plant leaves and produce vegetable growth responses (Ronga et al. 2019).

The carbon skeleton of Glutamine and Glutamate (L-glutamic acid) are directly associated to the primary synthesis of energy, in which biosynthesis pathways regulation occurs at multiple levels (Okumoto et al. 2016). These triggering events can act on plant physiology, impacting the global transcriptome profile, also changing treated plants metabolome (Nair et al. 2012; Jannin et al. 2013). Lambais (2011) adds that applications with L-*Aa* can result in vegetables with higher levels of total soluble *Pt* in the leaves and higher activity of enzymes. Results which support how *AQ*25 applications could led the increase in *Aa* and *Pt* in tomato leaves and fruits (Tab. 8).

Therefore, a combination between the direct supply of L-*Aa*, and the metabolic signaling that they perform in plants can explain its action on plant regulation (Yang et al. 2020). Hence, the L-*Aa* foliar applications can improve plant performance by stimulating the activity of the enzyme nitrate reductase (Röder et al. 2018), as found for N' at *WfT* (Tab.10).

The applications of *AQ*25 promoted higher levels of soluble solids on leaves (Tab. 6) which could be related to the leaf higher relative chlorophyll content (Tab. 4 and 5), as plants with greater photosynthetic assimilation can translocate more photoassimilates from source-to-sink, as seen in this work in fruits *Ts* (Tab. 7). Consequently, improving fruit mass, size, and

yield for both N' and G' (Tab. 3) showing that these tomato cultivars with similar type and development, although with different leaf and fruit size, are still positively responsive to *AQ*25.

In general terms *AQ*25 improved both nitrogen and carbon metabolism, also the source to sink flows, resulting in highest yield and better fruits caliber in two tomato cultivars. Thus, it was evidenced the importance of *Asterarcys quadricellulare* (CCAP 294/1) biomass as a sustainable source that can induce plant metabolic changes causing significant yield increase, which could help reach higher healthy food demands, also improving their nutritional quality.

3.5 CONCLUSIONS

Foliar sprays at 0.25 g L^{-1} concentration, even at biweekly frequency, of *Asterarcys quadricellulare* (CCAP 294/1) when applied on organic tomato demonstrated the bioactivity of this microalga biomass on plant metabolism. The promotion effect over plant growth and yield can partially be attributed to the biologically active free L-*Aa* present in its composition, as it can promote leaf and fruit biometrical changes, equally stimulating plant metabolism, increasing total sugars, free amino acids, and protein levels in the leaves and fruits, for both cultivars.

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4 GENERAL CONCLUSION

Studies with the microalga *AQ* are still scarce, especially regarding its use in agriculture. Its full potential is yet to be discovered in several areas. Until now this microorganism information was only related to its composition, and cultivation techniques. Furthermore, it was believed that its use could be designated for human food, or as a biomass source for biofuels, due to its wide range of amino acids and lipids, respectively. However, as this work have shown, this microalga biomass can also improve plant growth and performance in greenhouse conditions, when used as leaf biofertilizer. Consequently, representing a promising alternative for more sustainable and eco-friendly agricultural practices to achieve higher yield and food quality.

As the effective concentration used in this work was very low, in the range of parts per million (ppm), this microalga biomass applications should be understood as a way of supplying bioactive molecules to plants. Studies explains that these molecules, when absorbed by the leaves, can start signaling events that may change plant metabolism. For example, the amino acids most present in the composition of *AQ* are glutamic acid and arginine. These are linked to a series of plant metabolisms such as: assimilation of nitrogen and plant growth; and being a precursor to the polyamine synthesis, which are related to important biological processes, respectively.

Moreover, this new information about *AQ* can partially elucidate the way in which this microalga biomass acts on vegetables, as it has promoted higher yield, and raised levels of sugars, amino acids and proteins in tomato leaves and fruits. Thus, this biomass should be considered as an effective natural source for new works with an agronomic scope. Furthermore, new studies are recommended for understanding its metabolic routes and influence on plants. Experiments should use this biomass as a viable plant growth promoter, while analyzing its influence on different crops and application ways.

Therefore, research like this helps to unveil the potential of new microalgae biomasses. This way, their compositions details, maximum efficient concentration, and ideal application frequency in plants can be described. Furthermore, with this knowledge in hands, companies may be able to produce eco-friendly products able to increase crop productivity and reach higher healthy food demands.