Journal of Agricultural Science; Vol. 11, No. 6; 2019
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Biomass of Microalgae via Root Under the Production of Yellow Passionfruit Seedlings

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Received: December 7, 2018 Accepted: March 17, 2019 Online Published: May 15, 2019

doi:10.5539/jas.v11n6p105 URL: https://doi.org/10.5539/jas.v11n6p105

Abstract

Seedlings production is one of the most important stages of the production system and directly influences the performance of the plant in the field. In this sense, the present work aims to evaluate the use of biomass doses of *Spirulina platensis* and *Scenedesmus* sp. via root system on the production of yellow passion fruit seedlings. A greenhouse experiment was carried out using a randomized block design in a 2 × 5 factorial scheme (*Spirulina platensis* and *Scenedesmus* sp., at the doses 0.0%, 0.2%, 0.4%, 0.8 % and 1% m/v), with four blocks and two experimental units per plot. At 60 days, leaf number, total seedling length, shoot diameter, root system length, shoot length, shoot and root fresh mass, dry mass, root dry mass and total chlorophyll were measured. *Spirulina platensis* showed superior performance compared to Scenedesmus sp., for the variables stem diameter, shoot length, fresh shoot mass, fresh root mass, dry shoot mass and root dry mass. The doses influenced the number of leaves, root fresh mass, root dry mass, shoot length and fresh shoot mass significantly. The best dose of microalga applied was 0.8% in passion fruit seedlings at 60 days.

Keywords: innovation, *Passiflora edulis Sims*, *Scenedesmus* sp., *Spirulina platensis*

1. Introduction

Brazil is the world's leading producer and consumer of passion fruit, occupying a prominent position in the world market. According to IBGE (2016) in the year 2016, the passion fruit yield in Brazil was 14.10 tons/ha with a production of more than 703 thousand tons in an area of more than 50 thousand ha, thus revealing the importance of the crop in the market. The fruits of the passion fruit tree have pleasant taste and aroma, excellent nutritional qualities being rich in minerals and vitamins, mainly A and C (Aguiar, Sperry, & Junqueira, 2001).

Passionfruit consumption in Brazil is mainly in the form of juice, pulps, jellies, nectars, refreshments, ice cream and other culinary products. The bark is rich in soluble fibers, mainly pectin, and the flour can help in the reduction of cholesterol and blood glucose and slimming diets. The seeds are good sources of essential fatty acids and have vermifuge properties (Carvalho, 2015).

However, seedling production and crop establishment have problems, evidencing the need for planning and the adoption of technologies in the production. To obtain good quality seedlings, it is necessary to adopt techniques that improve the establishment, development and precocity in the seedling production. Echer et al. (2006) report that the production of passion fruit seedlings is one of the most important stages of the production system, directly influencing plant performance.

Currently, there is a greater interest in natural products that stimulate the growth of plants. Microalgae are examples of natural products studied both as a source of food, as well as in agriculture, in the stages of seedling formation, development and production of plants (Mallmann & Jahno, 2015; Marafon & Simonett, 2016).

Spirulina platensis and *Scenedesmus* sp. have a high concentration of organic and inorganic substances in their commercial form that characterize it as a product with the potential to be used as a biofertilizer (Priyadarshani & Rath, 2012). Therefore, the present work aimed to evaluate doses of *Spirulina platensis* and *Scenedesmus* sp., via root system on the production of yellow passion fruit seedlings.

2. Material and Methods

The experiment was conducted in a greenhouse at the Center for Science and Technology Agro-Food (CCTA) of the Federal University of Campina Grande (UFCG), Campus Pombal-PB, from September to December 2017. The municipality of Pombal is located at geographical coordinates 6°46′13″ south latitude and 37°48′06″ longitude west of Greenwich and altitude of 144 m. The climate is classified as semi-arid (“AW” hot and humid) with annual average rainfall of 431.8 mm and temperature of 28 °C, according to Köppen.

We used a randomized complete blocks design (RCB), in a factorial scheme 2 × 5 (*Spirulina platensis* and *Scenedesmus* sp. at the doses 0.0%, 0.2%, 0.4%, 0.8% and 1.0%) constituting ten treatments, with four blocks and two plants per experimental unit. The yellow passion fruit seedlings were produced in a greenhouse in a 16 × 20 cm container filled with a substrate formulated with soil, sand and cured bovine manure in the proportions of 3:2:1, pre-sterilized in an autoclave for one hour at 127 °C and 1.5 atmosphere of pressure. The physical and chemical characteristics of the substrate are shown in (Table 1).

Table 1. Chemical and physical analysis of the substrate used to produce yellow passion fruit seedlings.

Chemical	pH	CEas	Ca	Mg	K	Sa	N	P	M.O
	H_2O	dS m^{-1}	----------------- meq/100 g -----------------				%	mg/100 g	%
	7.5	0.99	3.07	3.95	2.21	0.32	0.21	37.20	3.4
Physical	Sand	Silt	Clam	Class	Density		Porosity	Moisture	
	----------------- % ----------------				g/cm^3		--------------------- % -------------------		
	67.09	27.40	5.51	Sandy	1.4		47.95	0.45	

Note. Soil and plant nutrition laboratory-LSNP, Pombal-PB. pH_{H_2O} in water, KCl and $CaCl_2{\cdot}2H_2O$-Ratio 1:2.5; P, K, Na: Mehlich-1 extractor; Al, Ca, Mg: extractor KCl-mol/L; EC in water-ratio 1:2.5; CTC-Cation exchange capacity at pH 7.0; V: base saturation. * Granulometry: by the Bouyoucos method, apparent density: 100 mL beaker method; real density: balloon method.

We used ISLA® brand seeds, sowing two seeds per container. The emergence occurred at 12 days after sowing, when the plants emitted the first pair of definitive leaves, we removed one seedling leaving only one plant per container. Sixteen days after emergence, we started the microalgae applications at seven day intervals, totaling five applications.

To obtain the solutions, we used biomass powders of microalgae purchased commercially, at Fazenda Tamanduá, in the municipality of Santa Terezinha-PB. The concentrations proposed in the treatments were weighed on analytical scale and diluted separately, the following amounts: 0%-0 g, 0.2%-1.6 g, 0.4%-3.2 g, 0.8%-6, 4 g and 1%-8 g *Spirulina platensis* biomass, and 0%-0 g, 0.2%-1.6 g, 0.4%-3.2 g, 0.8%-6.4 g 1%-8 g of *Scenedesmus* sp. biomass, diluted in 800 ml of tap water, under constant stirring until complete homogenization of the solution. Afterwards, 100 mL of the solution per plant was applied at the end of the afternoon, via fertirrigation. At 60 days after sowing (DAS), we conducted the morphological evaluations of plant growth. We estimated leaf numbers (LN), total length of seedling (TLS), plant height (cm) (PH), stem diameter (mm) (SD), shoot length (cm) (SL), root system length (RSL), shoot fresh mass and root fresh mass (g/plant) (SFM and RFM, respectively), shoot dry mass (g/plant) (SDM), root dry mass (g/plant) (RSM) and total chlorophyll (g/m^2) (TCL).

The data were evaluated through analysis of variance by the F test at 1 and 5% probability levels, and the regression analysis for the doses and interaction between the microalgae and the doses studied with the aid of the SISVAR statistical software version 5.6 (Ferreira, 2011).

3. Results and Discussion

According to Guimarães et al. (2015) microalgae are photosynthetic organisms that, with the combination of water and carbon dioxide from the atmospheric air, and sunlight produce diverse forms of energy, thus producing biomass, which is composed of polysaccharides, proteins, lipids and hydrocarbons. The composition of microalgae is highly variable, being affected by factors such as species, nutrients, temperature, photoperiod, salinity, carbon source and light intensity (Ramirez, 2013).

The results of the analysis of variance showed a significant interaction between the microalgae factors (A) and doses (D) for the SL variable. There was no significant interaction for the other variables. By smoothing the microalgae factor alone, a significant effect was observed for the SL, RFM and RDM, DC and SDM variables. For isolated factor doses, there was a significant effect for NF, RFM RDM, SL and SFM (Table 2).

Table 2. Summary of variance analysis for leaf numbers (LN), total length of seedling (TLS), plant height (PH), stem diameter (SD), shoot length (SL), root system length (RSL), shoot fresh mass (SFM), root fresh mass (RFM), shoot dry mass (SDM), root dry mass (RSM) and total chlorophyll (TCL) of yellow passion fruit seedlings produced with microalgae at different doses of application at 60 days of age

Source of variation	DF	Mean Square				
		LN	TLS	SD	RSL	SL
Microalgae (A)	1	2.50^{ns}	6.04^{ns}	0.75^{*}	21.46^{ns}	700.06^{**}
Dose (D)	4	4.83*	494.12^{ns}	0.16^{ns}	6.75^{ns}	403.76^{**}
A×D	4	0.32^{ns}	90.89^{ns}	0.01^{ns}	6.36^{ns}	216.48^{**}
Block	3	1.44^{ns}	159.29^{ns}	0.40^{ns}	4.36^{ns}	51.44^{ns}
Error	27	1.21	284.74	0.16	10.48	51.74
CV (%)		9.4	25.0	8.5	12.4	19.1
General mean		11.6	67.3	4.6	26.03	37.6
	GL	SFM	RSM	SDM	RDM	TCL
Microalgae(A)	1	63.35^{ns}	117.40^{**}	2.53^{*}	3.11^{**}	0.16^{ns}
Dose (D)	4	69.20^{**}	13.65^{*}	0.88^{ns}	0.78^{*}	0.83^{ns}
A×D	4	4.65^{ns}	6.91^{ns}	0.37^{ns}	0.08^{ns}	1.11^{ns}
Block	3	10.99^{ns}	12.78^{ns}	1.16^{ns}	0.64^{ns}	0.23^{ns}
Error	27	15.23	4.77	0.48	0.28	0.41
CV (%)		24.5	20.6	23.6	27.02	27.9
General mean		15.8	10.5	2.9	1.9	2.2

Note. ** significant at 1%; * significant at 5%; ^{ns} not significant at 5%; CV: coefficient of variation.

The variable SD was statistically superior in the seedlings produced with *Spirulina platensis* via irrigation water than with *Scenedesmus*, whose averages were 4.82 and 4.55 mm, respectively (Table 3). Greater viability was observed in the use of *Spirulina platensis*, due to the higher SD. We believe that *S. platensis* provided greater supply of nutrients for yellow passion fruit seedlings due to its composition of macronutrients, such as Ca, K, P, N and micronutrients such as Fe, Cu, Zn, B, Mn, Co (Marich et al., 2014).

We also verified similar behavior for the variable SL, RFM, SDM and RDM, where the best results occurred in seedlings produced with *S. platensis*, whose averages were 41.84 cm, 12.27 g, 3.19 g and 2.25 g, respectively. There was no significant difference for shoot fresh mass between the prepared solutions based on microalgae (Table 3). The extracts based on algae may act as chelants, soil conditioners, improving the use of mineral nutrients by plants, soil structure and aeration, stimulating organ growth in the seedlings (Calvo, Nelson, & Kloepper, 2014; Tarraf, Talaat, El-Sayed, & Balbaa, 2015).

According to Tarraf et al. (2015), the use of *Spirulina platensis* in fenugreek plants provided significant increase in plant height, number of leaves, number of branches, fresh weight and dry weight of plants at the stage of vegetative growth and stage of flowering of plants, especially in plants treated with 5 g/L of algae extracts in sandy and clayey soils.

Table 3. Steam diameter (SD), shoot length (SL), shoot fresh mass (SFM), root fresh mass (RFM), shoot dry mass (SDM) and root dry mass (RDM) of yellow passion fruit seedlings produced with microalgae biomass at different application rates at 60 days after sowing

Algae species	SD	SL	SFM	RFM	SDM	RDM
Spirulina platensis	4.82 a	41.84 a	17.13 a	12.27 a	3.19 a	2.25 a
Scenedesmus sp.	4.55 b	33.48 b	14.61 a	8.85 b	2.68 b	1.69 b

Note. Means followed by the same letter in the column do not differ from each other, by the Tukey test at the 5% probability level.

The highest leaf numbers (12.41) occurred using the highest dose of *Spirulina platensis* (Figure 1), with a mean of 14.34% higher than the control. The higher LN may be related to the higher concentration of the solution applied. These solutions present in their constitution nutrients such as nitrogen, phosphorus and potassium, essentials for plant growth, thus providing adequate nutrient levels for yellow passion fruit seedlings (Marich et al., 2014; Tarraf et al., 2015). According to Oliveira, Góes, Costa, and Silva (2011), the use of extracts based on *Ascophyllum nodosum* in the production of yellow passion fruit seedlings provided a significant increase in the number of leaves as the doses were increased up to the estimated value of 4.05 ml/L of the compound, above this value there was a decrease.

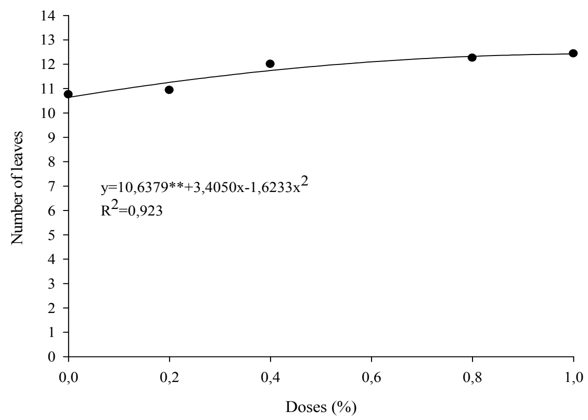

Figure 1. Number of leaves of yellow passion fruit, produced with microalgae biomass at different doses of application, at 60 days after sowing

Silva et al. (2015) observed in *Annona glabra* seedlings that the largest leaf numbers were obtained with the 2.0 ml/L dose of *Ascophyllum nodosum* extract, and the use of larger doses showed a reduction in the expression of the growth variables evaluated. Authors associate the reduction of the phytotechnical characteristics to the condition of saline stress caused by the solutions when applied in higher doses.

The dose of 0.8% of the solution based on *Scenedesmus* increased 43.33% of SL concerning the control (Table 3). However, applications above 0.8%, decreased 10.19% in SL. Possibly, the presence of high amounts of nutrients and the increase of EC in the *Scenedesmus*-based solution provided an inhibitory effect when concentrations above 0.8% were used.

Using increasing doses of *Spirulina platensis* we found that the 1.0% solution provided an increase in SL over the control of 43.17%. The behavior observed in the seedlings through the effect of the applied doses showed that solutions prepared up to 1% of *Spirulina platensis* could be suitable for the cultivation of yellow passion fruit and promote positive changes in plant metabolism and physiology, improving their morphological characteristics uniformly.

Our result corroborates those of Rocha et al. (2017), that found that the use of *Spirulina platensis* at low concentrations (1%) provides an improvement in the productive performance and influence on the water use efficiency (E/A) of papaya seedlings. According to Dias, Rocha, Araújo, Lima, and Guedes (2016) studying the application of Spirufert®, a product based on *Spirulina platensis*, 10 g L^{-1} applications provide a higher yield of eggplant fruits without influencing foliar N, P, K and Na. However, superior applications of the product favored the vegetative growth of the plants rather than the production.

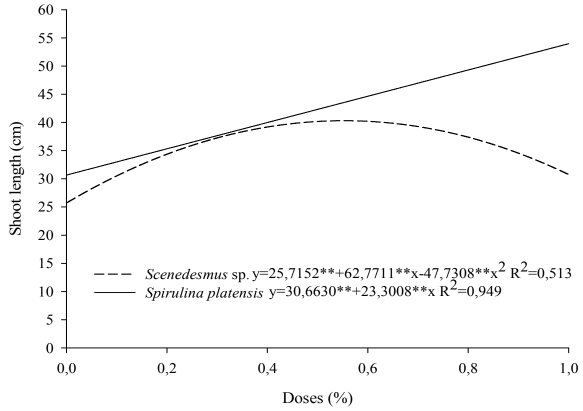

Figure 2. Interaction of the shoot length of yellow passion fruit, produced with microalgae biomass at different doses of application at 60 days after sowing

We verified at the application of 0.8% of the solutions based on microalgae an increase of 56.30% of the shoot length (43.69 cm) concerning the control. However, applications above 0.8% decreased the shoot length in 4.55% (Figure 3). It is possible that an inhibitory effect occurred when larger concentrations were used in solutions based on microalgae. Probably, this inhibitory effect was caused by the mineral nutrients, which are present in high concentration in the microalgae powder used for the research.

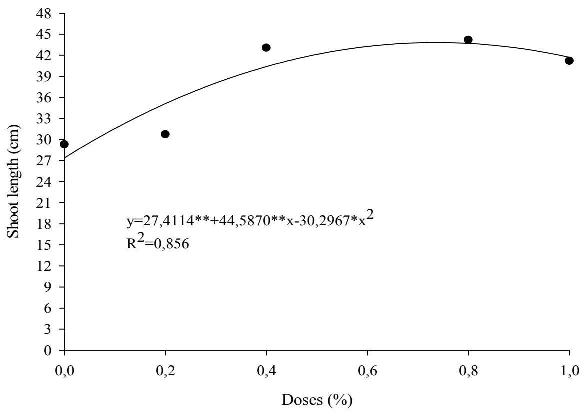

Figure 3. Shoot length of yellow passion fruit, produced with microalgae biomass at different doses of application at 60 days after sowing

Regarding to the shoot fresh mass, we found a positive effect of the organic matter supplied through the application of solutions based on microalgae. The lowest value (12.58 g) occurred in the absence of microalgae, while the increasing doses promoted a linear increase in the shoot fresh mass, an increase of 35.27% in relation to the control (Figure 4). Calvo et al. (2014) found that there was a gradual increase in plant growth, when the proportion of organic compounds in the soil was increased through algal applications. These results may be due to the high content of free amino acids of microalgae-based solutions, as well as their macronutrient and micronutrient contents, and the presence of some growth promoting substances directly absorbed by the seedlings (Babadzhanov et al., 2004; Aly & Essawy, 2008).

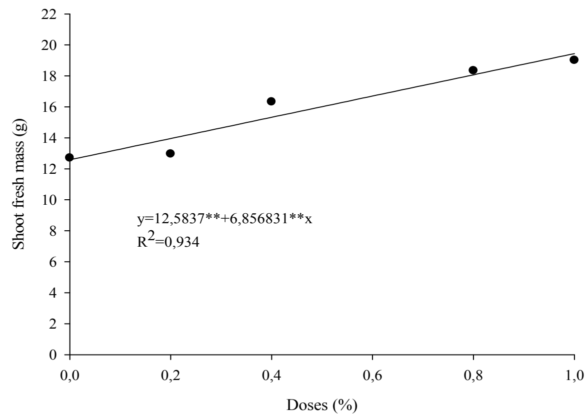

Figure 4. Shoot fresh mass of the yellow passion fruit seedlings, produced with microalgae biomass at different application rates at 60 days after sowing

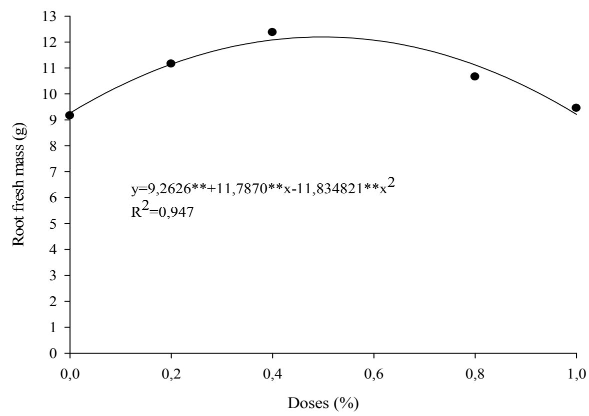

Figure 5. Root fresh mass of sour yellow passion fruit seedlings, produced with microalgae biomass at different application rates at 60 days after sowing

The application of 0.4% of solutions based on microalgae, resulted in an increase of 23.34% of root fresh mass in relation to the control. After the dose of 0.4% of microalgae there was a decrease of 23.74%, this effect was accompanied by the root dry mass, in which there was also a decrease in this same dose of microalgae (Figure 4). These microalgae present important nutrients essential for the plant growth (nitrogen, phosphorus and potassium), favoring the accumulation of phytomass through the use of mineral nutrients by the plants and improving soil structure and aeration, stimulating the growth of the root system (Calvo et al., 2014; Marich et al., 2014). According to Silva et al. (2015), studying the application of *Ascophyllum nodosum* seaweed extracts in the production of araticum-do-brejo rootstocks, the use of doses above 4 ml/L negatively influences the vegetative growth of the plants, due to the solutions.

The root dry mass showed a quadratic behavior, and the maximum value for this variable (2.22 g) occurred at a dose of 0.8%. This value is 30.17% higher than that observed in the yellow passion fruit seedlings produced without the use of the solutions. After this dose, a decrease in the root dry mass of 4.10% was observed (Figure 6). The use of algae and seaweed extracts act on soil properties as conditioners, increasing its moisture retention and favoring the growth of microorganisms in the soil (Calvo et al., 2014; Tarraf et al., 2015; Ribeiro et al., 2017). These characteristics may have contributed to the increase of the root area when using up to 0.8% of the solution based on microalgae.

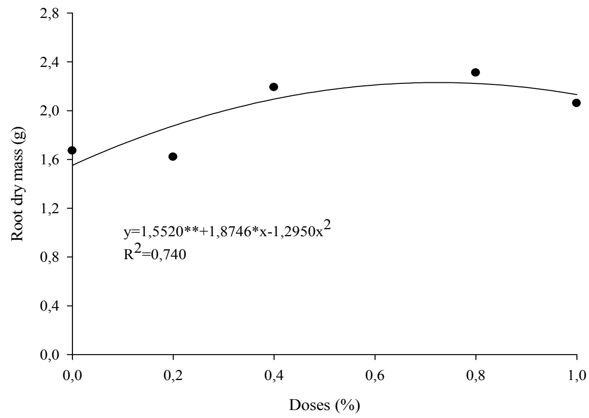

Figure 6. Root dry mass of the yellow passion fruit seedlings produced with microalgae biomass at different application rates at 60 days after sowing

The decrease in the dry mass gain of the root system may be due to the electrical conductivity of the solutions. When at the dose of 1% of the extract of *Spirulina platensis* and *Scenedesmus* sp. the electrical conductivity of the solutions were 0.88 and 1.37 dS m^{-1}, respectively (Table 4). These effects may be due to the direct contact of root system with the osmotic environment, which possibly makes the seedlings more susceptible to the adverse conditions of the culture medium, thus influencing its dry mass gain of the root system (Silva et al., 2015).

4. Conclusions

Spirulina platensis provides greater phytomass accumulation in relation to *Scenedesmos* sp. in the production management of yellow passion fruit seedlings.

The best dose of algae applied was 0.8% for seedlings of yellow passion fruit at 60 days.

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