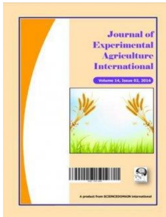

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Production of Papaya Seedlings Using *Spirulina platensis* as a Biostimulant Applied on Leaf and Root

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Authors' contributions

This work was carried out in collaboration between all authors. The authors GAD, AMFO, RFL and LMO performed the field and laboratory research, statistical analysis and preliminary draft. While the authors WAG and JLAR collaborated in the rewriting of the manuscript, improving the bibliographic review based on the data obtained. Authors RHCRA and JFL collaborated in the development of the study and performed corrections of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study evaluates the influence of *Spirulina platensis* concentrations on the growth and quality of papaya (Formosa Group) 'Tainung-1'.
Study Design: The experimental design was completely randomised, with plots subdivided over time.

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Place and Duration of Study: From April to June 2017, two greenhouse experiments were carried out at the Agro-Food Science and Technology Center of the Federal University of Campina Grande, Pombal-PB, Brazil.
Methodology: We carried out two greenhouse experiments in completely randomised designs, one with the application of *S. platensis* on the leaf and other with an application on the root. In both experiments, the treatments consisted of six biomass concentrations of *S. platensis* (0.0, 0.4, 0.8, 1.2, 1.6, 2.0%).
Results: At 52 days after sowing, we measured the following variables: growth, production of dry and fresh mass of roots and shoot, and the seedlings quality index. The modelling functions predict that the suspensions with 1.08% of *S. platensis* applied on root may provide the best growth and biomass production of the seedlings. However, suspensions of *S. platensis* applied on leaf were inefficient in the improvement of seedling quality.
Conclusion: When applied to the roots, a suspension of 1.08% *S. platensis* increases the growth and biomass production of the seedlings; and when used in the concentration of 0.88% improves the quality of the 'Formosa' papaya seedlings. Suspensions of *S. platensis* applied on leaves do not affect the growth and quality of seedlings of papaya.

Keywords: Carica papaya L.; seedling production; biofertilizer.

1. INTRODUCTION

The papaya (*Carica papaya* L.) has great importance in Brazilian agribusiness, with the main production occurring in Bahia and Espírito Santo states, comprising 794,565 and 399,790 t, respectively, which is 71% of national yield [1]. It is necessary to renovate the orchard in a period of two to four years to guarantee high productivity, depending on the cultivar and the growing conditions. Such a need for renovation generates a great demand for the production of good quality seedlings [2].

The search for ecologically sustainable technologies, such as biostimulants, aiming to enhance plant growth and productivity has increased in recent years [3,4]. In this sense, the use of microalgae extracts or biomass in agriculture as a biostimulant has been extensively studied, especially in annual crops [5,6,7,8,9,10].

Most research confirms the positive effect of microalgae on plant growth when supplied upon leaves or root suspensions. The bioactive compounds in the microalgae biomass can act through gene expression, signalling, and hormonal interactions [4]. For example, [9] demonstrated that *Arthrospira platensis* increases the polyamide contents in lettuce seedlings and raises the levels of chlorophyll, proteins, sugars, and free amino acids in beets.

The *Spirulina platensis*, specially produced under Brazilian climatic conditions, is a microalga with high potential for use as a biostimulant since it has high levels of free amino acids, proteins, carbohydrates, lipids, hormones, and some mineral nutrients [11]. Dias et al. [7] observed that the foliar application of *S. platensis* in eggplant at the concentration of 1% (m/v) increased the fruit yield without influencing the N, P, K, and Na leaf contents, but the concentrations above 1% favoured the vegetative growth of plants to the detriment of production. However, research involving seedling production is still scarce [8]. Shakila and Krishnapriya [10] observed an increase in growth, protein, and lipid contents in *Trigonella foenum-graecum* seedlings under root supply of *S. platensis*.

In the present work, we aimed to evaluate the influence of *S. platensis* concentrations, applied upon leaves and root, on the growth and quality of *papaya* (Formosa Group) ‘Tainung-1’.

2. MATERIALS AND METHODS

From April to June 2017, two greenhouse experiments were carried out at the Agro-Food Science and Technology Center of the Federal University of Campina Grande, Pombal-PB, Brazil. The first experiment comprised the application of *Spirulina platensis* on leaves and the second the application on root.

The experiments were set up in a completely randomised design, with five replicates, each replicate consisting of three plants. In both experiments, the treatments consisted of six concentrations of *S. platensis* [0.0; 0.4; 0.8; 1.2; 1.6; 2.0% (m/v)]. We used the powder of *S.*

platensis from Tamanduá® (Table 1 shows the composition) to prepare the concentrations.

Seedlings of the hybrid papaya (Formosa Group) 'Tainung-1' were produced from commercial seeds (ISLA ®) in black polyethene bags (14 x 20 cm). The bags were filled with 1.0 dm³ of a substrate comprising a mixture of soil, bovine manure, and sand, in a ratio of 3:1:1 (Table 1). Before filling the bags, the substrate was autoclaved for one hour at 127°C under a pressure of 1.5 atm. Three seeds were sown in each sack, at a depth of 1.0 cm. Sixteen days after sowing (DAS), we performed the thinning of seedlings, leaving only one per bag.

The plants were grown in a greenhouse covered with shade cloth (50% of sunblock), at an average temperature of 29.2°C and a relative humidity of 65%. Manually irrigations were performed daily in the early morning and late afternoon, based on the water requirement of the plants to maintain the substrate close to the field capacity.

During the growing period, five applications of *S. platensis* suspensions were carried out (16, 23, 30, 37, and 42 DAS). In each period, the foliar supply was carried out with a hand sprayer until the point of drainage of the suspensions over the leaves. The root application was performed by the addition of 100 mL of the solution in each bag.

At 20, 28, 36, 44, and 52 DAS, the number of leaves (NL), the plants height (PH), stem diameter (SD), and leaf area (LA) were measured. The leaf area in cm² was estimated by the sum of the individual areas, using the equation proposed by Alves and Santos [12] and cited by Santos et al. [13], from the measurement of the midrib (M), in cm, of each leaf, according to the following expression:

$$\mathrm{LA} = 0.0947\ \mathrm{M}^{2.7352}$$

At the end of experiments (52 DAS), we measured the length of the primary root (LR), and the fresh mass of shoot (FMS), roots (FMR), and the total (FMT). Subsequently, the plant material was dried in a forced air circulation oven at 60 to 65°C to obtain the dry mass of the shoot (DMS), roots (DMR), and the total (DMT). We calculated the root:shoot ratio (DMR/DMS) and the Dickson Quality Index (IQD) [14], according to the expression:

$$\mathrm{DQI} = \frac{\mathrm{DMT}}{(PH/SD) + (DMS/DMR)}$$

At the end of the experiment with the application of *S. platensis* on roots, we carried out the substrate characterization (Table 2) according to Embrapa [15].

Table 1. Composition of the *Spirulina platensis* biomass used in the experiments

Physical-chemical characterisation	Value	Organic composition (g/100 g)	Value
Humidity and volatiles (g/100 g)*	12.10	Aspartic acid	4.85
Ashes (g/100 g)	9.00	Glutamic acid	7.99
Proteins (g/100 g)	51.82	Serina	2.38
Nitrogen (g/100 g)	8.29	Glycine	2.63
Carbohydrates (g/100 g)	14.20	Histidine	0.75
Arsenic (mg/100 g)	0.044	Arginine	3.45
Cadmium (mg/100 g)	0.004	Threonine	2.63
Calcium (g/100 g)	0.198	Alanine	3.72
Copper (mg/100 g)	0.111	Proline	1.85
Chromium (mg/100 g)	0.052	Tyrosine	2.50
Iron (mg/100 g)	20.10	Valine	3.23
Phosphorus (g/100 g)	1.58	Methionine	0.90
Magnesium (g/100 g)	0.39	Cystine	0.21
Manganese (mg/100 g)	3.26	Isoleucine	3.60
Potassium (mg/100 g)	2.29	Leucine	4.47
Sodium (mg/100 g)	1.297	Phenylalanine	2.29
Zinc (mg/100 g)	0.84	Lysine	2.43
pH (2% suspension m/v)	5.85	Tryptophan	0.67
EC (2% suspension, dS/m)	1.95	Total lipids (g)	6.90
-		Carbohydrates (g/100 g)	14.2

**Analyses carried out at the Institute of Food Technology-ITAL; EC: Electrical conductivity.*

Table 2. Characterization of the substrate used in the root experiment as a function of the *S. platensis* concentrations

S. platensis concentration (%)	pH	pH_{H2O}	ECes	Ca	Mg	Ca + Mg	N	P	O.M.
			dS m^{-1}	-----$cmol_c$ dm^{-3}------			%	mg dm^{-3}	g kg^{-1}
0.0	7.96	8.63	0.32	6.20	5.70	11.90	0.7	280.77	8.68
0.4	6.85	8.43	0.25	6.30	5.00	11.30	0.7	388.52	7.57
0.8	6.32	8.87	0.15	5.50	5.40	10.90	0.6	237.79	8.28
1.2	6.29	8.66	0.24	6.50	4.60	11.10	0.7	301.48	8.38
1.6	5.92	8.59	0.20	6.90	5.50	12.40	0.8	444.21	8.50
2.0	5.85	8.95	0.18	5.90	4.90	10.80	0.6	301.48	7.17

Considering the high content of amino acids in the *S. platensis* biomass, at the end of experiments, the total nitrogen contents were determined according to Malavolta [16].

The data was analysed through response surfaces adjustments for the variables plant height, stem diameter, and number of leaves over the experimental period, using SigmaPlot 11.0 software. For the variables obtained at the end of the experiments, we carried out analyses of variance and polynomial regressions, at 5% of probability, using the SISVAR statistical software [17].

3. RESULTS AND DISCUSSION

The plants showed a linear growth over time, but the concentrations of *S. platensis* applied on leaves did not influence the growth. These relationships are shown in surface models for number of leaves (Fig. 1A), stem diameter (Fig. 1B), plant height (Fig. 1C), and leaf area (Fig. 1D).

The application of *S. platensis* on leaves also did not affect the fresh mass production (Fig. 2A), dry mass (Fig. 2B), primary root length (Fig. 3A), root:shoot ratio (Fig. 3B), N-total (Fig. 3C), and IQD values (Fig. 3D). In papaya seedlings cultivated for 60 days, [18] applied weekly suspensions of a product based on *S. platensis* on leaves in concentrations ranging from 0 to 4%. The authors also did not observe any effect on growth or the gas exchange of plants.

The failure of *S. platensis* applied on leaves in promoting the growth and quality of papaya seedlings suggests that the plants did not absorb the organic and mineral components of the suspension in sufficient quantity to affect their metabolism. It may be associated with the small leaf area of the plants in the seedling stage. The magnitude of the values of the variables of plant growth is consistent with those obtained in other studies with papaya under similar conditions [19,20,21].

The application of *S. platensis* on root positively affected all variables (Fig. 4, 5, and 6). The number of leaves per plant (Fig. 4A), stem diameter (Fig. 4B), plant height (Fig. 4C), and leaf area (Fig. 4D) showed a quadratic response as a function of time and concentrations of *S. platensis*.

The concentrations of *S. platensis* influenced the fresh mass (Fig. 5A), dry mass (Fig. 5B), primary root length (Fig. 6A), root:shoot ratio (Fig. 6B), and Dickson quality index (Fig. 6D), all of which fitted to quadratic models. The N-total leaf contents adjusted to the increasing linear model (Fig. 6C). The root dry mass was not affected by the use of *S. platensis* (Fig. 5B).

Using the model functions to estimate the ideal concentration of *S. platensis*, we found that the maximum total fresh mass and total dry mass was obtained at a concentration of 1.08%. Taking the 0.0% treatment as a reference, the ideal concentration promotes a gain of 34% and 50%, in the productions of total fresh mass and total dry mass, respectively. The maximum value of IQD was obtained with the concentration estimated at 0.88%, which is 0.2% lower than the estimated concentration for the maximum phytomass production. According to Eloy et al. [22] the IQD values, regardless of the treatments 0.3 and 0.6 being considered above the ideal minimum that is 0.2. According to these authors, this index is among the best indicators of plant quality because it associates robustness parameter (PH/SD) and biomass production (DMS/DMR), avoiding, in the case of seedlings, the wrong choice of etiolated plants in detriment of shorter plants but with better potential for survival and sound development in the field.

Regarding quality (IQD), the application of the concentration of *S. platensis* at 0.88% represents a gain of 32%. If one considers half of this dose (0.44%), the increase in IQD, concerning the dose of 0.0%, will be 24%, which would represent a total of 2.2 g of *S. platensis* per plant, taking in to account the five applications. The economic viability of the application of these doses will depend on the acquisition price of the *S. platensis* biomass combined with the sale price of the seedlings produced.

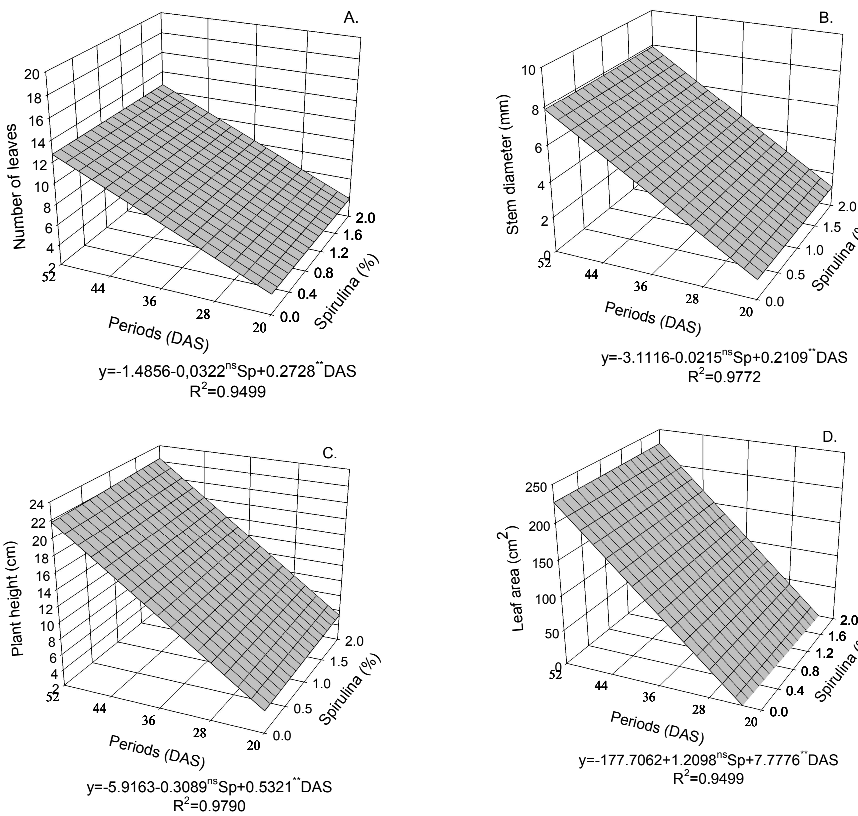

Fig. 1. Response surface for number of leaves (A), stem diameter (B), plant height (C), and leaf area (D) of 'Formosa' papaya seedlings as a function of different concentrations of *Spirulina platensis* (Sp) and evaluation periods (DAS). **significant at 1% probability; (ns) not significant

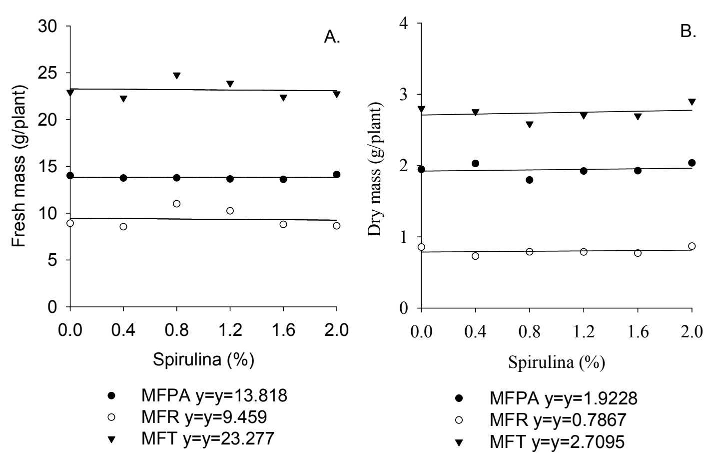

Fig. 2. Fresh mass (A), and dry mass (B) of 'Formosa' papaya seedlings as a function of different doses of *Spirulina platensis* and periods of evaluation

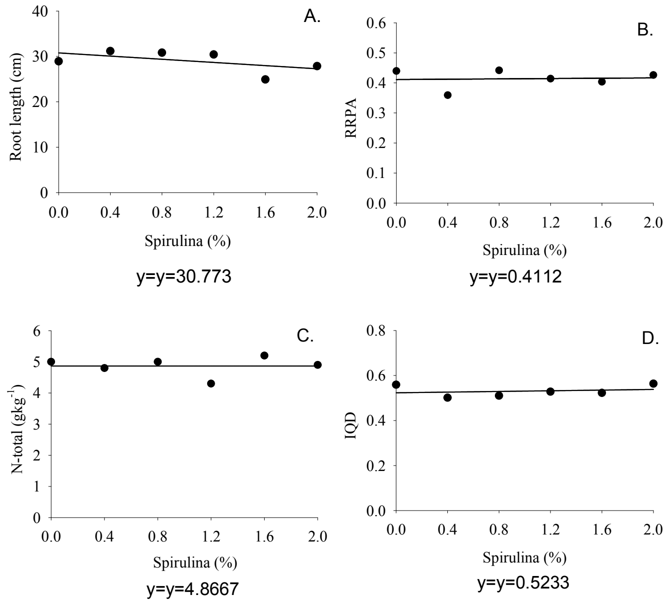

Fig. 3. Length of primary root (A), root:shoot ratio (B), N-total (C), and Dickson quality index (D) of 'Formosa' papaya seedlings as a function of different doses of *Spirulina platensis* and periods of evaluation

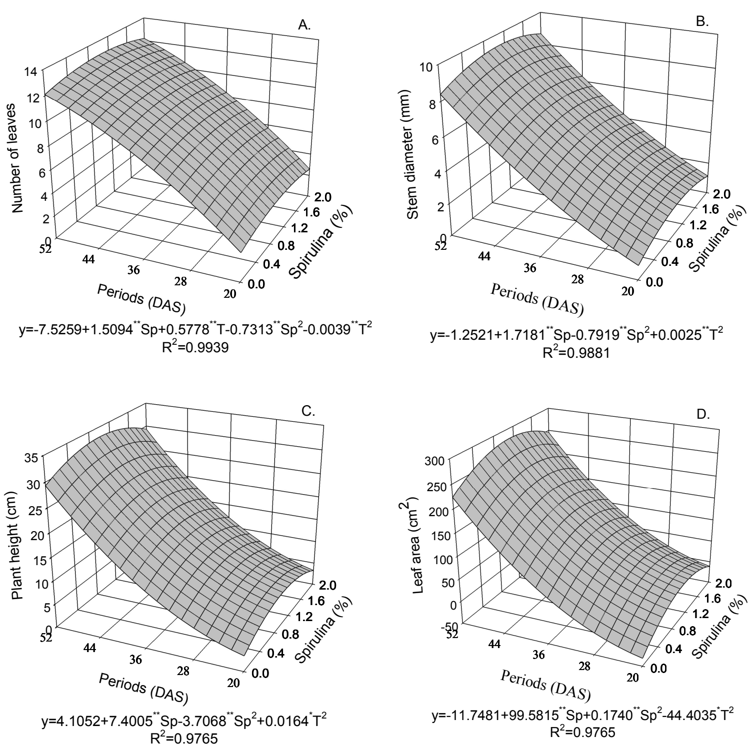

Fig. 4. Response surface for of leaves (A), stem diameter (B), plant height (C), and leaf area (D) of 'Formosa' papaya seedlings as a function of different doses of *Spirulina platensis* and periods evaluation

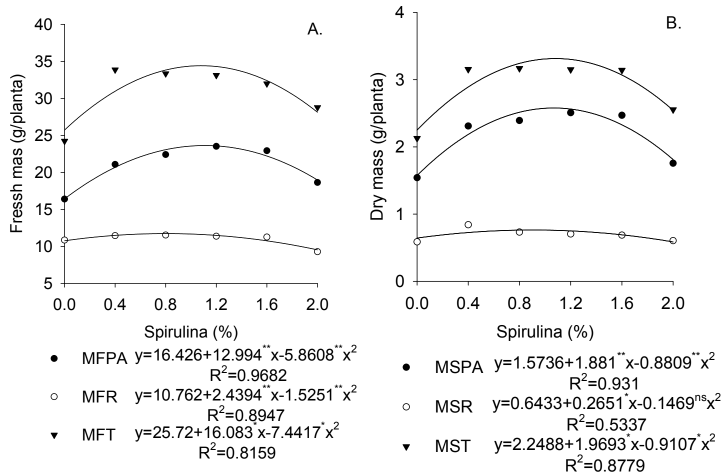

Fig. 5. Fresh mass (A), and dry mass (B) of 'Formosa' papaya seedlings as a function of different doses of *Spirulina platensis*. UFCG, Pombal-PB, 2018

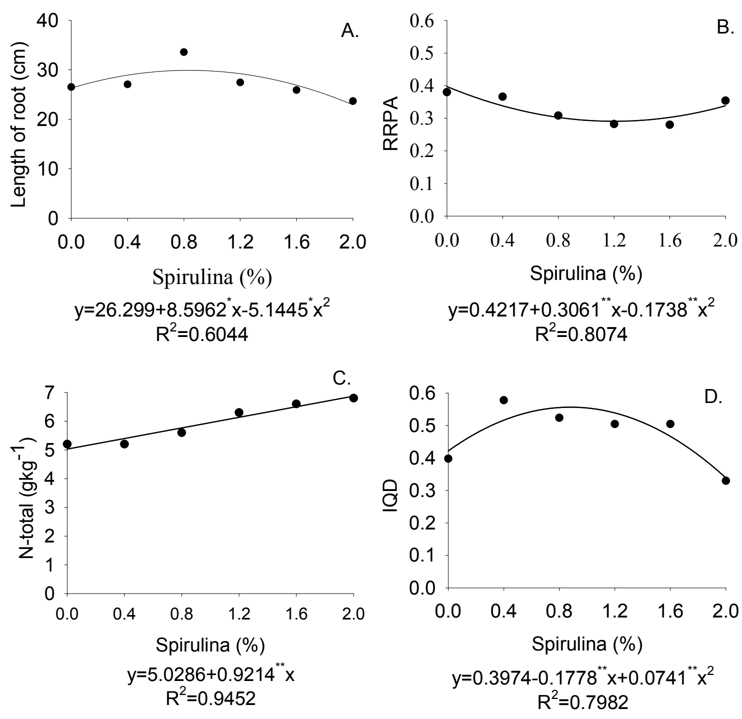

Fig. 6. Length of root (A), root:shoot ratio (B), N-total (C), and Dickson quality index (D) of 'Formosa' papaya seedlings as a function of different doses of *Spirulina platensis*. UFCG, Pombal-PB, 2018

Several studies report positive effects of suspensions of *S. platensis* or extracts of various species of algae applied on leaves [6,7,9,10]. In our study, the effects of treatments on papaya seedlings cannot be directly explained by their influence on the composition of the substrate, considering its little alteration with the concentrations of *S. platensis* (Table 2). However, in addition to nitrogen, the biomass of *S. platensis* is rich in essential amino acids, such as aspartic acid, glutamic acid, arginine, threonine, alanine, isoleucine, and leucine (Table 1).

The increase in leaf N content (Fig. 6C) may be related to the influence of the *S. platensis* composition on nitrogen metabolism, increasing the plant's ability to absorb and assimilate this nutrient, even without increasing its contents in the substrate (Table 2). Amino acids can act by stimulating plant metabolism, raising the synthesis of proteins and some hormones responsible for plant growth [9,10]. In this sense, [23] observed that the application of commercial biostimulants based on amino acids increases the productivity and quality of wheat grains. [9] report an increase in the chlorophyll, protein, sugars, and free amino acids contents in the beet grown organically by the application of *Arthrospira platensis* on leaves. Similarly, [9] observed an increase in spermine polyamide contents by the use of *Arthrospira platensis* in lettuce seedlings.

Finally, due to its richness in free amino acids, part of the positive effects of *S. platensis* could also be of indirect origin, through the activity of beneficial microorganisms in the rhizosphere [24]. The *S. platensis* may activate biochemical processes mediated by microorganisms in the substrate, stimulating the production of substances that promote plant growth.

4. CONCLUSION

When applied to the roots, a suspension of 1.08% *S. platensis* increases the growth and biomass production of the seedlings; and when used in the concentration of 0.88% improves the quality of the 'Formosa' papaya seedlings.

Suspensions of *S. platensis* applied on leaves do not affect the growth and quality of seedlings of papaya.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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