Research Article

Carnauba bagana substrate and application of humic substances on the production of yellow passion fruit seedlings[1]

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ABSTRACT

Owing to the high prices of agricultural inputs, it is necessary to seek alternatives that minimize costs during the seedling production stage, such as the use of agro-industrial residues to formulate substrates and the application of humic substances to increase the availability of nutrients. This study aimed to evaluate the production of yellow passion fruit seedlings in substrates based on carnauba bagana under increasing doses of humic substances. The design was completely randomized, in a 3 x 4 factorial scheme, referring to three substrates formulated with carnauba bagana (0, 50 and 100 %) plus soil and four doses of humic substances (0, 12.5, 25 and 50 g L^{-1}). The use of carnauba bagana in the substrate increased the leaf area, plant height, stem diameter, root length, root volume, shoot and root dry mass, and improved the seedlings quality. The application of humic substances to the substrate did not stimulate the seedlings development.

KEYWORDS: *Passiflora edulis*, *Copernicia prunifera*, alternative substrates.

RESUMO

Substrato de bagana de carnaúba e aplicação de substâncias húmicas na produção de mudas de maracujazeiro amarelo

Mediante os elevados preços de insumos agrícolas, é necessário buscar alternativas que minimizem os custos durante a etapa de produção de mudas, como a utilização de resíduos agroindustriais para a formulação de substratos e a aplicação de substâncias húmicas para o aumento da disponibilidade de nutrientes. Objetivou-se avaliar a produção de mudas de maracujazeiro amarelo em substratos à base da bagana de carnaúba sob doses crescentes de substâncias húmicas. O delineamento foi inteiramente casualizado, em esquema fatorial 3 x 4, referente a três substratos formulados à base de bagana de carnaúba (0, 50 e 100 %) acrescidos de solo e quatro doses de substâncias húmicas (0; 12,5; 25; e 50 g L^{-1}). O uso da bagana de carnaúba no substrato trouxe incrementos à área foliar, altura da planta, diâmetro do caule, comprimento radicular, volume radicular, massa seca da parte aérea e do sistema radicular, melhorando a qualidade das mudas. A aplicação de substâncias húmicas ao substrato não estimulou o desenvolvimento das mudas.

PALAVRAS-CHAVE: *Passiflora edulis*, *Copernicia prunifera*, substratos alternativos.

INTRODUCTION

Brazil is the world's largest producer of passion fruit (*Passiflora edulis* f. *flavicarpa*). Its production, in 2020, was estimated at 690,364 t, with an average yield of 14.87 t ha^{-1} (IBGE 2020), with the northeastern region being the leader in the national production. Almost all Brazilian passion fruit is produced in small- and medium-sized rural properties (IBGE 2017).

The success in the cultivation of this fruit depends on several factors, including seedling production. This step is considered fundamental for a good final performance of plants in the field (Siqueira et al. 2020). Thus, elaborate substrates must be used to provide conditions to the development of seedlings with physical, physicochemical, chemical and biological quality characteristics (Pascual et al. 2018, Muniz et al. 2020, Antunes et al. 2022), and that preferentially have a reduced cost and are easy to acquire (Schmitz et al. 2002).

The use of agro-industrial residues to formulate substrates is an alternative to minimize production costs, given the high prices of commercial substrates

[1] Received: Aug. 05, 2022. Accepted: Oct. 10, 2022. Published: Nov. 09, 2022. DOI: 10.1590/1983-40632022v5273631.

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and fertilizers (Muniz et al. 2020, Mendonça et al. 2021, Andrade et al. 2022). In addition, this can contribute to minimizing the environmental impact generated by the incorrect disposal of organic waste in the environment (Meneghelli et al. 2017). These materials add positive characteristics to the final substrate, contributing to the production of vigorous and high-quality seedlings (Reis et al. 2014).

Among plant-derived materials, bagana is obtained during the wax-extraction process from the carnauba tree (*Copernicia prunifera*), a species native to northeastern Brazil (Carvalho & Gomes 2017, Silva et al. 2019). This residue has shown positive results as a substrate for the production of seedlings of various fruit (Oliveira et al. 2020, Andrade et al. 2022) and flower (Silva et al. 2020) species.

In addition to alternative substrates, humic substances, the main constituent of organic matter, composed of humic and fulvic acids and humins, have been widely studied because of their ability to increase the absorption of nutrients and the tolerance of crops to environmental stresses, showing a potential use in seedling production (Canellas et al. 2015). The use of humic substances has already been reported in the production of ornamental (Fan et al. 2014), fruit (Cavalcante et al. 2013, Andrade et al. 2022) and horticultural (Hernandez et al. 2015) species, because of their positive effects on plant growth (Eyheraguibel et al. 2008).

Carnauba bagana and humic substances showed positive responses in the production of passion fruit seedlings, when evaluated in isolation (Cavalcante et al. 2013, Mendonça et al. 2021). However, their combined use in passion fruit seedling production has not been tested before. Therefore, this study aimed to evaluate the production of yellow passion fruit seedlings in substrates based on carnauba bagana under increasing doses of humic substances. The hypothesis was that seedling growth would be maximized at the combination of the highest carnauba bagana residue and humic substances concentrations.

MATERIAL AND METHODS

The experimental setup was established and conducted in a greenhouse with 70 % light interception, at the Universidade Federal do Maranhão, in Chapadinha, Maranhão state, Brazil (03º44'17"S, 43º20'29"W and 107 m a.s.l.), in December 2018. The region's climate is classified as humid tropical, with average annual rainfall of 1,613.2 mm and average annual temperature of 26.9 ºC (Passos et al. 2016).

A completely randomized design was adopted, in a 3 x 4 factorial scheme, referring to three substrates formulated with carnauba bagana [0, 50 and 100 % plus soil (v/v)] and four doses of humic substances [0 (control), 12.5, 25 and 50 g L^{-1}], with four replications and five seedlings per replication, totaling 240 seedlings.

The carnauba bagana was sourced from the carnauba agroindustry in the municipality of Vargem Grande, Maranhão state, mechanically crushed in a forage chopper and subsequently homogenized through a 5-mm-mesh sieve. The soil (Dystrophic Yellow Latosol) (Santos et al. 2013), equivalent to Oxisol (USDA 1996), used in the substrate formulation was collected from the topsoil (0-20 cm) layer. After the manual mixing of carnauba bagana with soil in the evaluated proportions, samples from each treatment were collected for chemical and physical characterization.

For the chemical characterization of the substrates (Table 1), the pH was determined in a solution of distilled water (5:1 v/v) (Brasil 2007), while the organic matter, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) contents were determined as described by Embrapa (1997). For K and P, a Mehlich 1 extract solution was used, as well as KCl 1.0 for Ca and Mg. The physical characterization (Table 2) was performed according to Schmitz et al. (2002).

Sowing was carried out in polyethylene bags (0.92 L capacity) filled with substrates corresponding to the treatments, using two passion fruit seeds (cv. Redondo Amarelo) per container. At 15 days after sowing (DAS), thinning was performed, leaving only the most vigorous plant in each container. Nutrient supplement applications were made with the aid of a syringe. With the exception of the control, which only received applications of distilled water, the humic substance applications were made using 1 mL of substrate at the concentrations of 12.5, 25 and 50 g L^{-1}, at five times: pre-sowing and at 14, 28, 42 and 56 DAS. The source of humic substances was Humitec WG®, composed of 68 % of total humic extract (52 % of humic and 16 % of fulvic acids). The product has a cation exchange capacity of 800 mmol kg^{-1}, maximum humidity of 13 %, pH

Table 1. Values for pH, organic matter (OM) and total contents of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) of substrates based on carnauba bagana (CB).

Substrates	pH	OM	N	P	K	Ca	Mg	S
	H_2O	g kg^{-1}	g kg^{-1}	mg kg^{-1}	cmol$_c$ kg^{-1}			
0 % CB	4.0	22.80	1.14	3.5	0.14	0.69	0.53	2.82
50 % CB	5.0	51.47	3.56	17.0	0.46	4.10	1.60	6.30
100 % CB	5.3	598.86	4.02	89.0	3.88	19.80	10.40	34.60

Table 2. Overall density, particle density and porosity of carnauba bagana (CB)-based substrates.

Substrates	Density (g cm^{-3})		Porosity (%)
	Global density	Particle density	
0 % CB	1.44	2.67	45.99
50 % CB	1.02	2.19	53.30
100 % CB	0.27	0.90	70.20

(100 g L^{-1}) of 11.5, salt content (10 g L^{-1}) of 48 %, electrical conductivity (100 g L^{-1}) of 7 mS cm^{-1} and solubility of 100 g L^{-1}.

At 60 DAS, a destructive analysis of the seedlings was performed, and the following variables were evaluated: leaf area (cm^2): the leaves were detached from the stem and scanned in a printer, and the leaf area was determined with the aid of the ImageJ® computer software (Lima et al. 2020); plant height (cm): determined from the ground level to the growth point of the plant with the aid of a tape measure; stem diameter (mm): determined at 1 cm close to the substrate using a digital caliper (Digimess, Shiko, China); root length (cm): the length of the longest root was measured with the aid of a tape measure; root volume (cm^3): after rinsing all the substrate debris from the root system, the root volume was calculated by measuring the displacement of the water column in a graduated cylinder; mass (g): the plant material was sectioned into shoot and root systems, placed in paper envelopes, dried in an oven with forced air circulation at 65 ºC until reaching constant mass, and then weighed to obtain the dry mass (g). To evaluate the quality of the seedlings, the Dickson quality index (DQI) equation was used (Dickson et al. 1960): DQI = TDM/[(PH/SD) + (SDM/RDM)], where: TDM is the total dry matter (g); PH the plant height (cm); SD the stem diameter (mm); SDM the shoot dry matter (g); and RDM the root dry matter (g).

The data were submitted to analysis of variance for diagnosis of significant effects ($p < 0.05$) and the treatments compared by the Tukey test using the InfoStat® software, version 2015.l. A principal component analysis from standardized data for mean zero and standard deviation one was also performed to explore the relationship between substrates and seedling quality parameters, using the Past 4.0 software.

RESULTS AND DISCUSSION

There was a significant effect ($p < 0.01$) of the carnauba bagana factor for all the studied variables (Table 3). In contrast, the doses of humic substances had no statistically significant effect ($p > 0.05$) on any of the variables. Interactions between carnauba bagana and humic substances were only observed for two variables: leaf area and root volume.

The increase in the carnauba bagana concentration in the substrate formulation promoted

Table 3. F-values for the variables leaf area (LA), plant height (PH), stem diameter (SD), root length (RL), root volume (RV), shoot dry mass (SDM), root dry mass (RDM) and Dickson quality index (DQI) of yellow passion fruit seedlings as a function of substrates and addition of humic substances.

Sources of variation[1]	F-value							
	LA	PH	SD	RL	RV	SDM	RDM	DQI
	cm^2	cm	mm	cm	cm^3	g	g	
CB	99.62**	29.71**	97.23**	22.87 **	25.38**	27.97**	12.56**	13.72***
HS	1.36^{ns}	2.44^{ns}	2.14^{ns}	1.99^{ns}	1.94^{ns}	1.36^{ns}	1.32^{ns}	1.58^{ns}
CB x HS	2.48*	1.87^{ns}	1.80^{ns}	1.37^{ns}	2.33*	1.27^{ns}	1.13^{ns}	1.39^{ns}

[1] CB: carnauba bagana; HS: humic substances. *** Significant at 0.1 %; ** significant at 1 %; * significant at 5 %; ^{ns} not significant.

an increase in leaf area (Figure 1). However, there was no difference between the proportions of 50 and 100 % of carnauba bagana in the absence of humic substances and at the humic substances doses of 12.5 and 25 g mL^{-1}. An increase in leaf area in response to the addition of carnauba bagana was reported in other studies (Mendonça et al. 2021, Andrade et al. 2022). This result may be attributed to an increase in the N concentration in the substrate (Table 1). According to Miyake et al. (2017), an ideal amount of N is extremely important for seedling development, because it is positively correlated with the chlorophyll content of leaves. Additionally, leaf area is related to dry matter accumulation (Silva Junior et al. 2020), which can affect the survival rate of seedlings in the field.

Nitrogen is the most limiting nutrient in the production of biomass, as it is a constituent of several plant cellular components, including chlorophyll, amino acids and nucleic acids. In this sense, it influences physiological processes such as protein synthesis and photosynthesis (Taiz et al. 2017). According to Mendonça et al. (2021), the most accumulated macronutrients in the shoots of passion fruit seedlings are N > Ca > K > Mg > P. In contrast, Antunes et al. (2022) reported the following order: K > N > Ca > P > Mg. However, the latter authors mentioned that the levels of nutrients accumulated in the shoots of passion fruit seedlings are influenced by the substrates used.

The application of humic substances did not promote a significant increase in leaf area, if compared to the control. There was a decrease in leaf area in the substrate with 50 % of carnauba bagana when the humic substances dose increased from 25 to 50 g L^{-1}. The reduction in leaf area at the highest dose of humic substances possibly occurred because of a nutritional imbalance, considering the high complexity of the reactions that occur between the substrate and the applied humic substances. In the present study, humic substances composed predominantly of humic acids, which are considered to have a high molecular weight, were used. According to Rosa et al. (2009), high doses of high molecular weight humic substances can reduce the K uptake by plants, what would explain the reduction in leaf area at the dose of 50 g L^{-1} in the substrate with 50 % of carnauba bagana observed in the present study.

The increment of carnauba bagana in the substrate composition promoted significant increases in root volume for all doses of applied humic substances (Figure 2). There were significant effects of humic substances only for the substrate with 50 % of carnauba bagana, in which increases in humic substances up to a dose of 25 g L^{-1} resulted in increases in root volume. However, as observed for leaf area, it also decreased at the dose of 50 g L^{-1}. The root volume increased approximately 70 % for the dose of 25 g L^{-1} of humic substances, when compared to the control.

The increase in root volume with increasing bagana concentrations may be attributed to the higher porosity and lower density. In the study by Silva Júnior et al. (2014), using a substrate of carnauba residue plus rice husk, substrates with lower aeration space resulted in lower root volumes,

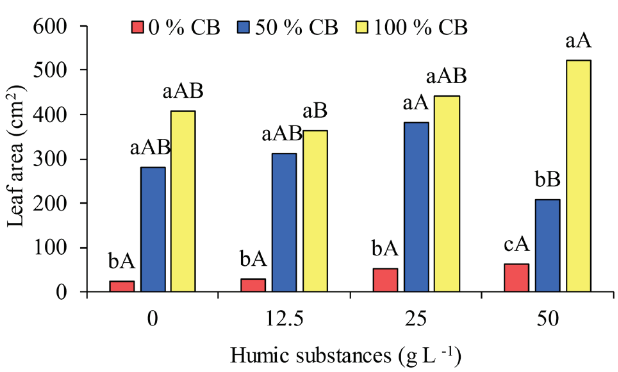

Figure 1. Leaf area of yellow passion fruit seedlings as a function of increasing doses of humic substances in substrates based on carnauba bagana (CB). Lowercase letters compare the different proportions of carnauba nuts within the same dose of humic substances, while capital letters compare the same proportion of carnauba nuts in different doses of humic substances.

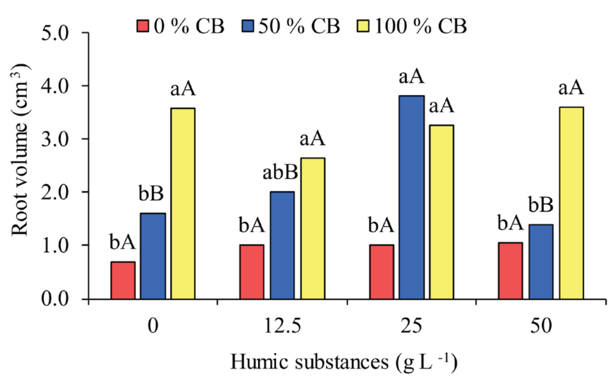

Figure 2. Root volume of yellow passion fruit seedlings as a function of increasing doses of humic substances in substrates based on carnauba bagana (CB). Lowercase letters compare the different proportions of carnauba nuts within the same dose of humic substances, while capital letters compare the same proportion of carnauba nuts in different doses of humic substances.

what was confirmed in the present study. The humic substances factor alone did not promote an increase in root volume (Table 1), what is consistent with the results of Cavalcante et al. (2013), who observed that the application of humic substances did not affect the root volume in yellow passion fruit. On the other hand, the dose of 25 g L^{-1} of humic substances together with the substrate with 50 % of carnauba bagana increased the root volume. According to Eyheraguibel et al. (2008), this result was possibly due to an increase in the number of lateral roots, resulting in an increase in total root length and root surface area. It should be noted that root volume is an important parameter, because it is directly related to the volume of soil explored by the roots.

The substrate containing 100 % of carnauba bagana contributed to the greatest plant height, followed by 50 % of carnauba bagana, showing, respectively, 131 and 73 % higher values than the control (0 % of carnauba bagana) (Table 4). An increase in plant height in response to the addition of carnauba bagana was also reported by Mendonça et al. (2021), although they did not observe any difference between the substrates with 50 and 100 % of carnauba bagana. The increase in seedling growth may be attributed to the improvement in the chemical characteristics of the substrates (Table 1), as the addition of carnauba bagana increased the pH, organic matter and macronutrient levels, resulting in a better seedling nutrition, as reported by Mendonça et al. 2021 and Andrade et al. 2022. According to Costa et al. (2021), the addition of organic material as a conditioner can improve the chemical, physical and hydric characteristics of the substrate.

For stem diameter, the substrate containing 100 % of carnauba bagana also stood out in relation to the other substrate proportions, followed by the substrate with 50 % of carnauba bagana, with averages of 3.16 and 2.83 mm, respectively (Table 4). Substrates containing higher proportions of carnauba bagana possibly resulted in a greater accumulation of photoassimilates by the seedlings, what is of paramount importance in metabolic processes (Albano et al. 2014). Another factor that may have contributed to this result may be related to the nutritional balance of K provided by this treatment. According to Valeri & Corradini (2005), this nutrient promotes stem thickening. Seedlings that present a better balance between stem diameter and seedling height have a greater resistance to adverse conditions in the field (Antunes et al. 2022).

Table 4. Means of the variables plant height (PH), stem diameter (SD), root length (RL), shoot dry mass (SDM), root dry mass (RDM) and Dickson quality index (DQI) of yellow passion fruit seedlings as a function of substrates.

Substrates	PH	SD	RL	SDM	RDM	DQI
	cm	mm	cm	g	g	
0 % CB[1]	12.15 c	1.56 c	18.39 b	0.17 b	0.05 b	0.02 b
50 % CB	21.00 b	2.83 b	25.30 a	1.29 a	0.28 a	0.13 a
100 % CB	28.09 a	3.16 a	28.69 a	1.68 a	0.31 a	0.14 a

[1] CB: carnauba bagana. Equal lowercase letters in the column do not differ by the Tukey test at 5 % of significance.

For root length, it was observed that the substrates with 50 and 100 % of carnauba bagana were 37.6 and 56.0 % higher than the control, respectively (Table 4). These results show that higher levels of organic matter in the substrate (Table 1) resulted in a greater root development, in addition to the granulometric conditions of these substrates, which contributed to better aeration conditions (Table 2). Total porosity is also an important factor to consider, as it is indicative of the physical quality of the substrate, and should present a good relationship between macro- and microporosity (Schmitz et al. 2002, Antunes et al. 2022). According to Pascual et al. (2018), the recommended total porosity should be in the range of 50-80 %. In this sense, substrates with 50 and 100 % of carnauba bagana are within the recommended range (Table 2).

There was an increase in shoot dry mass with the addition of carnauba bagana (Table 4). The substrates with 50 and 100 % of carnauba bagana were statistically similar, and both were superior to the control ($p < 0.01$), with values of 1.29 and 1.68 g, when compared with 0.17 g, respectively. The gradual increase in shoot dry mass averages is probably due to increases in the N and K contents present in the carnauba bagana, what corroborates the results of Albano et al. (2017), who observed a significant increase in this variable after the application of semi-decomposed carnauba residue substrate in 'Formosa' papaya seedlings.

For root dry mass, the best responses were obtained by substrates with 50 and 100 % of carnauba bagana, with averages of 0.28 and 0.31 g, respectively (Table 4). The increases in root dry mass were in the order of 460 % for the substrate with 50 % of carnauba bagana and 520 % for the substrate with 100 % of carnauba bagana, if compared to the control.

The increase in nutrient availability, as previously mentioned, in addition to better physical characteristics, such as the reduction in density provided by the addition of carnauba bagana to the substrate, may have favored the largest increases in root dry mass.

The reduction in density and increase in porosity may contribute to a greater water retention by the substrate, affecting its availability for seedlings (Costa et al. 2021) and increasing root development, what may have occurred in the present study. According to Pascual et al. (2018), the density for organic substrates should be in the range of 0.30-0.75 g cm^{-3}. The substrates of the present study containing 0 and 50 % of carnauba bagana presented density values higher than those recommended for organic substrates. However, it is important to note that one of the tested substrates had 100 % mineral composition (0 % of carnauba bagana), and the other a mixture of mineral and organic material (50 % of carnauba bagana). The substrate with 100 % of carnauba bagana showed a lower density value (0.27 g cm^{-3}) in relation to the others, but it is noteworthy that the result was close to the recommended minimum.

An increase in the Dickson quality index (DQI) was observed with the addition of carnauba bagana (Table 4). The substrates containing 50 and 100 % of carnauba bagana were superior to the control, but did not differ from each other. These results corroborate those by Mendonça et al. (2021), who observed increases in the DQI of passion fruit seedlings with the addition of carnauba bagana. This parameter is considered a good indicator of seedling quality, as it considers the height/diameter and shoot dry mass/root dry mass ratios, and their relationship with the total dry mass of the seedling. According to Medeiros et al. (2016), the DQI of passion fruit seedlings may vary depending on the genotype and treatment.

It was possible to observe the relationship between the studied parameters and substrates in the principal component analysis (PCA) (Figure 3). The principal component 1 explained 68.12 % of the data variability, whereas the principal component 2 explained 20.01 %, adding up to 88.13 % of the total variability. The principal component 1 showed a positive correlation of the parameters evaluated for substrates with 50 and 100 % of carnauba bagana, and a negative correlation with the substrate with 0 % of carnauba bagana.

The PCA also revealed that the substrates containing the proportions of 50 and 100 % of

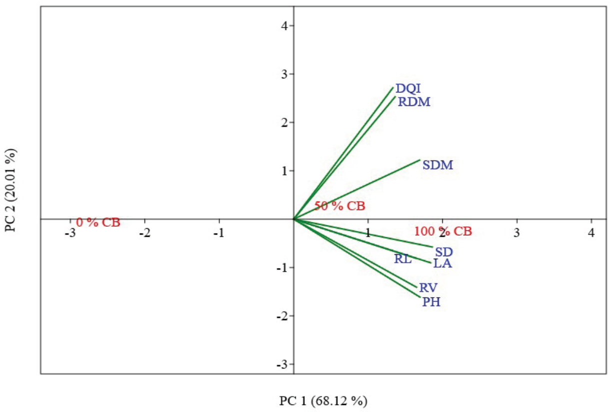

Figure 3. Principal component analysis (PCA) related to the parameters evaluated in yellow passion fruit seedlings produced on substrates based on carnauba bagana (CB). DQI: Dickson quality index; RDM: root dry mass; SDM: shoot dry mass; SD: stem diameter; LA: leaf area; RL: root length; RV: root volume; PH: plant height.

carnauba bagana were responsive by morphological variables, dry mass accumulation and DQI. These results are consistent with the univariate analysis, which showed a clear trend of improvement in the parameters evaluated in response to the addition of carnauba bagana (Table 4). This relationship between the higher proportions of carnauba bagana in the substrates and the increments in seedling development is due to the improvement in the chemical (Table 1) and physical (Table 2) properties of the substrate, which influenced the availability of water and nutrients, as aforementioned.

In general, the humic substances had no significant effects when evaluated in isolation (Table 3). Some studies have reported the beneficial effects of humic substances on various agricultural crops (Eyheraguibel et al. 2008, Hernandez et al. 2015, Andrade et al. 2022), but their effects are probably dependent on the plant species, application rate and management used (Trevisan et al. 2010). In addition, the humic substances source may also be influential, since humic substances isolated from leonardide (the source used in the present study) have lower positive effects, when compared to other sources such as humic substances isolated from peat, compost or vermicomposts (Canellas et al. 2015). This may account for the absence of significant effects of humic substances in the present study; however, it does require a more detailed investigation for confirmation.

CONCLUSIONS

1. The use of carnauba bagana in the substrate formulation is favorable for the formation of yellow passion fruit seedlings. Thus, taking into account the quality assessment of the seedlings produced, the substrates containing 50 and 100 % of carnauba bagana increased many growth parameters, when compared to the control, and could be used in the production of seedlings. However, in order to reduce the costs of the raw material, it is recommended to use 50 % of carnauba bagana in the substrate.
2. The application of humic substances to the substrate does not stimulate the development of yellow passion fruit seedlings.

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