

Eficácia de substratos e substância húmica na produção de mudas de *Spondia purpurea*

L. por estaquia

Efficacy of substrate and humic substance on cuttings production of *Spondia purpurea*

L.

Efectividad de sustratos y sustancia húmica en la producción de plántulas de *Spondia purpurea* L.

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Resumo

Para a propagação de mudas por estaquia é importante a escolha de um substrato adequado. Adicionalmente, o uso de substâncias húmicas pode estimular o crescimento das mudas, como já conhecido para muitas espécies. Objetivou-se avaliar substratos a base de bagana de carnaúba e casca de arroz carbonizada associados a substância húmica - SH na produção de mudas de *Spondia purpurea* L por estaquia. Foram testadas três proporções (v:v) de substratos: 100% casca de arroz carbonizada; 50% bagana de carnaúba + 50% casca de arroz carbonizada; 100% bagana de carnaúba e quatro doses de substância húmica: 0 g L⁻¹; 12,5 g L⁻¹; 25 g L⁻¹; 50 g L⁻¹. Adotando delineamento inteiramente casualizado, em arranjo fatorial 3x4 com quatro repetições. Houve efeito significativo das doses de SH e da interação substrato e doses de SH sobre a área foliar e massa seca da parte aérea das mudas. O volume radicular foi afetado de forma isolada pelos fatores testados. As respostas de interação entre os substratos e as doses de substância húmica inferem que estas atuam de formas distintas sobre o desenvolvimento de mudas de seriguela de acordo com o tipo de substrato, logo, é fundamental a identificação e escolha da compilação que favoreça os melhores resultados. Há então a necessidade de se testar outros substratos associados a adição de SH, já sabido que condicionam incremento de variáveis biométricas das mudas de seriguela produzidas por estaquia.

Palavras-chave: Extrato húmico; Substrato alternativo; Propagação vegetativa.

Abstract

For the propagation of seedlings by cutting it is important to select an appropriate substrate. Additionally, the use of humic substances can stimulate the growth of the plants, as is already known for many species. We aimed to investigate the influence of substrates based on carnauba bagana and

carbonized rice husk associated with humic substance - HS in the production of *Spondia purpurea* L. seedlings by cuttings. We studied three proportions (v:v) of substrates, namely, 100% carbonized rice husk; 50% carnauba bagana + 50% carbonized rice husk; 100% carbonized rice husk and four doses of the humic substance, namely, 0 g L⁻¹; 12.5 g L⁻¹; 25 g L⁻¹; 50 g L⁻¹. The experiment was arranged in a completely randomized design, in a 3x4 factorial with four replicates. There was a significant effect of HS doses and substrate interaction and HS doses on the leaf area and dry mass of the aerial part of seedling seedlings. Root volume was affected in isolation by the factors tested. The interaction responses between the substrate and doses of humic substance infer that they act in different ways on development of seriguela seedlings according to the type of substrate, therefore, it is essential to identify and choose the compiled that favors the best results. There is then a need test other substrates associated with the addition of HS, already known to condition the increase of seriguela seedlings produced by cuttings.

Keywords: Humic extract; Alternative substrate; Vegetative propagation.

Resumen

Para la propagación de plántulas por corte, es importante elegir un sustrato adecuado. Además, el uso de sustancias húmicas puede estimular el crecimiento de las plántulas, como ya se sabe para muchas especies. El objetivo fue evaluar los sustratos basados en carnauba bagana y cáscara de arroz carbonizada asociada con la sustancia húmica - SH en la producción de esquejes de *Spondia purpurea* L. Se probaron tres proporciones (v: v) de sustratos: cáscara de arroz 100% carbonizada; 50% de carnauba bagana + 50% de cáscara de arroz carbonizada; 100% carnauba bagana y cuatro dosis de sustancia húmica: 0 g L⁻¹; 12,5 g de L⁻¹; 25 g de L⁻¹; 50 g L⁻¹. Adoptando un diseño completamente al azar, en un arreglo factorial 3x4 con cuatro repeticiones. Hubo un efecto significativo de las dosis de SH y la interacción del sustrato y las dosis de SH en el área de la hoja y la masa seca de las plántulas. El volumen de la raíz se vio afectado de forma aislada por los factores probados. Las respuestas de interacción entre los sustratos y las dosis de sustancias húmicas infieren que actúan de diferentes maneras en el desarrollo de las plántulas de acuerdo con el tipo de sustrato, por lo tanto, es esencial identificar y elegir la compilación que favorezca los mejores resultados. Existe entonces la necesidad de probar otros sustratos asociados con la adición de SH, que ya se sabe que condicionan el aumento de las variables biométricas de las plántulas de plántulas producidas por esquejes.

Palabras clave: Extracto húmico; Sustrato alternativo; Propagación vegetativa.

1. Introduction

Spondia purpurea L. is a tropical fruit crop, that belongs to the *Spondia* genus, known as seriguela, cajá-vermelho, or some other common names, that vary with the cultivated

region (Lira Júnior et al., 2014; Quintão, 2016; Ramos, 2018). It is well known that the species has low seeds germination power. Therefore, the production of seedlings by cuttings has proved to be a more feasible alternative due to the propagation of a large number of seedlings associated with a short juvenile phase allowing thus greater plant uniformity in the orchard (Andrade, 2018a).

The identification of new materials along with the optimal composition of substrates for the production of fruit seedlings may be defined according to the species (Silva, 2012; Sousa et al., 2015). There is a great diversity of materials that can be used as substrates, which can enhance physical and chemical properties (Freitas et al., 2013), inducing water retention and aeration along with nutrient supply, according to plant's needs. In addition, to guarantee a good development of the plants, in a short period affordable cost may also be considered (Cunha et al., 2016).

The carbonized rice husk is a very common and easily obtained residue, which has a good potential to be used as substrates for seedlings of tree species development (Sousa et al., 2015). In addition, carnauba bagana is the residue of the extraction of wax from the leaves of *Copernicia prunifera*, usually found in greater abundance in the farming areas, considered by some producers as undesirable materials, being often burned in order to clean these areas (Gonçalves et al., 2019).

Among the materials that can be applied to substrates to stimulate the development of seedlings are humic substances, formed by fulvic and humic acids (Bernardes et al., 2011), originated in the humidification process, triggered by resynthesis of the carbon compounds generated in the decomposition of materials organic, forming “humus” (Baldotto & Baldotto 2013).

Humic substances can trigger a series of chemical and biochemical processes, related to the capacity of retaining nutrients in the substrate, the absorption and transport of cations, and physiological reactions in microorganisms and plants (Zandonadi et al., 2014)

This investigation aimed to study the influence of carnauba bagana and carbonized rice husk by the addition of humic substance on the production of *Spondia purpurea* L. seedlings by cuttings. We hypothesized that the cultivation of *Spondia purpurea* L. under carnauba bagana and carbonized rice husk by the addition of humic substance will enhance production of seedlings of siriguela, vegetatively propagated by cuttings. To study our hypothesis, siriguela plants were grown for three months under three substrates based on carnauba bagana + carbonized rice husks and four additional humic substance doses.

2. Material and Methods

The experiment was carried out from Nov 2019 to February 2020 in a greenhouse with 70% light interception, at Agricultural and Environmental Sciences Center (03°44'17" S and 43°20'29" O) of the Federal University of Maranhão, Chapadinha, Maranhão State, Brazil. The local climate is C2s2A'a', classified as humid tropical (Selbach & Leite, 2008), with annual rainfall ranging from 1.600 to 2.000 mm (Nogueira et al., 2012) and average annual temperature above 27 ° C (Passos et al., 2016). Climatic records were obtained from the Meteorological conventional station, in Chapadinha, Maranhão State, Brazil.

Adopting a quantitative exploration experimental research methodology. In this mode, it is possible to generate data sets or masses that can be analyzed using statistical mathematical techniques (Pereira et al., 2018).

A completely randomized design (CRD) was carried out in a 3x4 factorial arrangement, with three substrates based on carnauba bagana (CB) and carbonized rice husks (CRH), namely, 100% CRH; 50% CB + 50% CRH and 100% CB and four doses of humic substance (HS), namely, 0 g L⁻¹; 12.5 g L⁻¹; 25 g L⁻¹ and 50 g L⁻¹, with 4 replicates, each replicate consisting of 4 units, totaling 16 seedlings per treatment.

For formulation of the substrates, the carnauba bagana was mechanically crushed with the aid of forage chipper. And the rice husk underwent carbonization with the aid of artisanal equipment consisting of a pierced tin (oven) and a metal pipe (chimney), the husk was spread around this carbonizer and turned until a dark color homogeneity was obtained.

As a container, 12 x 20 cm polyethylene bags were used, after being filled with the substrate corresponding to each treatment, a cutting was placed in each bag. Cuttings of a 1.5 cm diameter and 12 cm height, previously collected from a vigorous mother plant. Watering was carried out manually according to plant requirements in an attempt to keep the substrates at full soil field capacity.

The HS source used was the commercial product Humitec WG[®], classified as an organomineral fertilizer, composed of: 17% K₂O, 31% organic carbon, 68% total humic extract, 52% humic acids and 16% fulvic acids. Applications of 1 mL of HS were performed every 14 days.

The following parameters in the seedlings were evaluated: leaf area (cm²), determined with the computer program ImageJ[®]; length of the largest shoot (cm), with the aid of a millimeter ruler; diameter of the largest shoot (mm), obtained with a digital caliper (Digimess[®]); number of shoots, by counting the cuttings that had sprouting; percentage of

cutting survival (%), based on the number of live seedlings; root length (cm), measured with the aid of a ruler graduated in millimeters; root volume (cm³), performed by measuring the displacement of the water column in a graduated cylinder, according to the methodology described by Basso (1999); percentage of rooted cuttings, counting the number of rooted cuttings; and percentage of cuttings with callus, obtained by observing the calligraphic structure at the base of the cuttings and counting the number of cuttings with callus; shoot and root dry mass (g), obtained by drying in an oven with forced air circulation, at a temperature of 65°C, until reaching constant mass.

Using the statistical software Infostat[®] (Di Rienzo et al., 2015) the data were subjected to analysis of variance by the “F” test, when a significant effect was detected, the regression analysis was performed and the means of the substrates were compared by the Tukey test ($p < 0.05$).

3. Results and discussion

The analysis of variance revealed that humic substance doses and the interaction between substrates × humic substance significantly affected leaf area and shoot dry mass. Root volume was affected by substrates as well as humic substance doses (Table 1).

Table 1. Summary of analysis of variance for leaf area (LA), length of the largest shoot (LLS), diameter of the largest shoot (DLS), number of shoots (NS), percentage of cuttings survival (PCS), root length (RL), root volume (RV), percentage of rooted cuttings (PRC), percentage of cuttings with callus (%CC), shoot dry mass (SDM) and root dry mass (RDM) depending on different substrates and doses of humic substance.

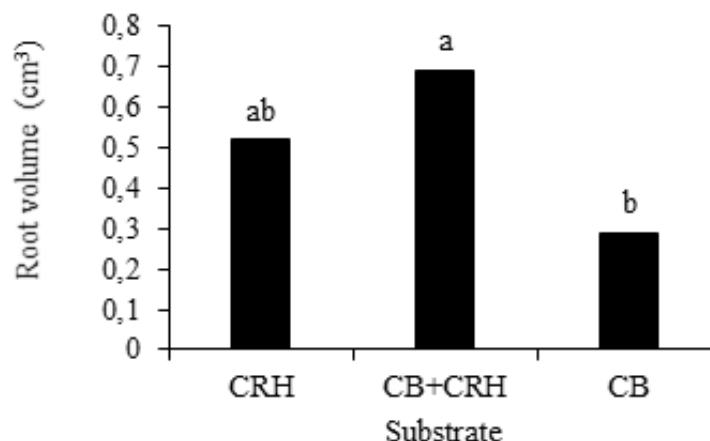
Variation source	D.F	Medium squares					
		LA (cm ²)	LLS (cm)	DLS (mm)	NS	PCS (%)	
Substrates	2	22343.96 ^{ns}	11.61 ^{ns}	0.67 ^{ns}	4.88 ^{ns}	13.02 ^{ns}	
humic substance	3	44228.93*	31.64 ^{ns}	0.54 ^{ns}	2.46 ^{ns}	243.06 ^{ns}	
S x HS	6	24673.32**	24.17 ^{ns}	0.46 ^{ns}	5.21 ^{ns}	412.33 ^{ns}	
Residue	34	8057.50	20.63	0.40	2.95	373.26	
C.V. (%)	----	138.92	89.95	24.96	64.58	42.15	
Variation source	D.F	RL (cm)	RV (cm ³)	PRC (%)	PCC (%)	SDM (g)	RDM (g)
Substrates	2	18.48 ^{ns}	0.47**	91.15 ^{ns}	507.81 ^{ns}	0.64 ^{ns}	0.01 ^{ns}
humic substance	3	55.99 ^{ns}	0.39**	295.14 ^{ns}	256.08 ^{ns}	0.79*	0.002 ^{ns}
S x HS	6	14.36 ^{ns}	0.10 ^{ns}	386.28 ^{ns}	646.70 ^{ns}	0.60*	0.01 ^{ns}
Residue	34	19.86	0.03	217.01	473.09	0.24	0.01
C.V. (%)	----	41.76	33.03	128.56	66.29	130.66	105.07

S= Substrates; HS= humic substance; D.F.=degree of freedom; C.V.= coefficient of variation; ** = significant at the 1% probability level, by the F test; * = significant at the 5% probability level, by the F test; ns = not significant. Source: Authors.

The influence of humic substances is evidenced by the detection of significant effect ($p < 0.05$) for the growth variables on the table above corroborates with several studies that highlight the significant results of the use of humic substance on the growth and development of seedlings (Bernardes et al., 2011; Baldotto et al., 2014; Borcioni et al., 2016; Gomes Júnior et al., 2019). It is noteworthy that the action of humic substances on growth variables and effects on the availability of nutrients depends on the type and concentration of the humic substance, the pH of the substrate and the plant species itself submitted to the applications (Muscolo et al., 2007; Nardi et al., 2009).

The CB + CRH substrate favored the highest increase on the leaf area (95.59 cm²) along with length of the largest shoot (5.69 cm), diameter of the largest shoot (2.66 mm), number of shoots (3, 34), root volume (0.69 cm³), percentage of cuttings with callus (37.50%), dry mass of shoot (0.53 g) and root (0.12 g). However, except root volume (Figure 1), none of the variables showed a statistical difference for the substrates.

Figure 1. Mean values for root volume as a function of different substrates.



Means followed by the same letter do not differ significantly by the Tukey test ($p < 0.05$). CRH: carbonized rice husk; CB: carnauba bagana. Source: Authors.

The average root volume obtained with the substrate CB + CRH was 137% higher than the average measured in seedlings grown with the substrate CB, and was statistically equal to the substrate PC with a difference of 32%. (Figure 1).

These results revealed that the highest average root volume of seedlings grown under CB + CRH substrate might be related to the good aeration provided by the component materials, as well as the supply of nutrients in favorable quantities for the development of the seedling root system.

The substrate can influence both the rooting percentage and the quality of the seedling root system (Franzon et al., 2010) Therefore, special attention should be paid to the selection of the substrate, as well as its additional nutrient source. The physical properties of CRH are the factors that qualify its use as a substrate, as they provide greater porosity and aeration. However, this material has low availability of nutrients, generally recommending its use associated with another source of organic matter (Silva et al., 2019).

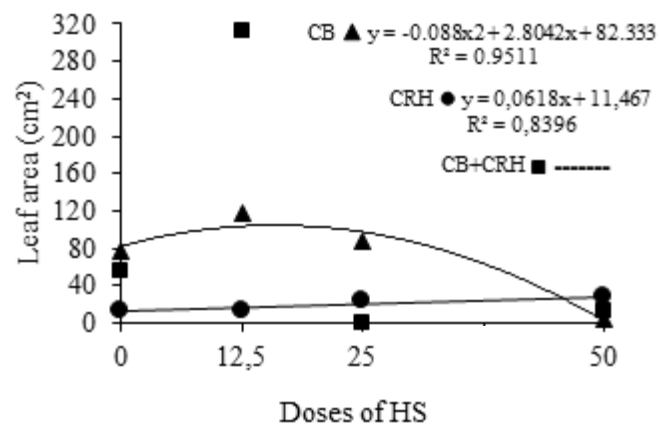
A study carried out to evaluate the use of CRH in the production of *Ficus enormis* seedlings via cutting, pointed out that this material mixed with other organic materials resulted in an improvement in the quality of the seedlings, in comparison to the substrate composed only by CRH (Fragoso et al., 2016). Brito et al. (2017) reported a significant increase in the root volume of three varieties of lettuce with the use of carnauba bagana with carbonized rice husk as a substrate, which further favored other evaluated parameters on the investigation, recommending the use of this substrates as an alternative to the commercial.

A quadratic regression model between substrates and humic substance was observed

on the leaf area. The statistical analysis indicates that the proposed quadratic model for those parameters was sufficiently accurate for predicting the response within the range of assayed conditions.

The maximum estimated leaf area was 104.67 cm^2 under 15.9 g L^{-1} of HS (Figure 2). Nitrogen has a fundamental role in vegetative development and acts in the biosynthesis of proteins and chlorophylls (Andrade et al., 2003). Thus, it is likely that the action of the humic substance in combination with the availability of nitrogen by the substrate might be caused such a result. Besides that, humic substances exert effects such as increases in the accumulation of leaf nutrients and synthesis of chlorophylls, stimulating the development of the aerial part (Baldotto et al., 2009).

Figure 2. Average values for leaf area as a function of different substrates and doses of humic substance (HS).



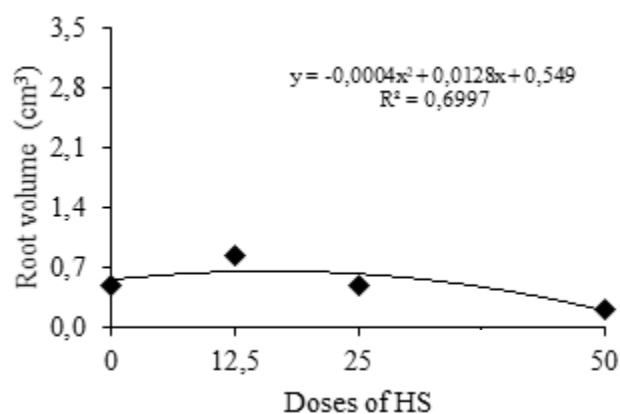
CRH: carbonized rice husk; CB: carnauba bagana. Source: Authors.

The averages of the leaf area variable of seedlings grown on the CB + CRH substrate and submitted to HS doses application did not adjusted any regression model. When the substrate used was CRH, the leaf area of the seriguela seedlings showed lower averages, adjusting a linear model, surpassing the average of the CB substrate seedlings with humic substance doses close to 50 g L^{-1} (Figure 2). This substrate has nitrogen as well as other nutrients, in insignificant amount, and is therefore considered inert.

In line with our results, Andrade (2018 b) reported an increase in the leaf area of papaya seedlings due to the addition of humic substance to the substrate, in which a 100% carnauba bagana in interaction with the dose 12.5 g L^{-1} humic substance, showed significant gain in leaf area by 479.73 cm^2 .

The root volume of seriguela seedlings varied according to the humic substance doses and adjusted a quadratic regression model, by the maximum estimated dose of 16 g L⁻¹ providing an RV average of 0.65 cm³ (Figure 3). Our findings, thus, indicated that humic substances can increased the amount of root hair and lateral roots in the plant, in agreement with (Canellas et al., 2006). Corroborating Aguiar et al. (2009) reported that humic substences induce a higher incidence of fine and lateral roots in corn seedlings.

Figure 3. Average values for root volume as a function of different doses of humic substance (HS).



Source: Authors.

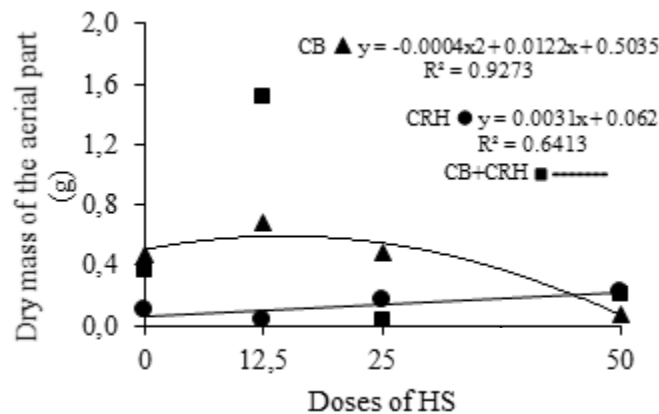
Consequently, the increase in root volume (Figure 3) promotes an increase in the area of exploration of the root system, leading to a greater access to water and nutrients present in the substrate, reflecting in greater development of the aerial part (Canellas & Olivares, 2014). Studies indicate root development as the main effect of using HS. For instance, it is reported that the use of different doses of HS promoted the growth of lettuce plants, with an important action on the root system. Taking the emission of roots, of smaller diameter in large amounts, when added to the substrate in the highest tested concentrations (Borcioni et al., 2016).

On the other hand, it is important to note that some studies warn that plant species tend to respond to the action of these substances up to a certain level, and that high concentrations can result in reduced growth and development of plants (Wangen et al., 2013; Baldotto et al., 2014). This explains the reduction in root volume with the use of humic substance in concentrations greater than 15,25 g L⁻¹.

For the shoot dry mass, similar results to the leaf area was observed, in the CB substrate as a function of the HS doses. The means adjusted to the quadratic model with the maximum estimated of 0,59 g obtained with the dose of 15.25 g L⁻¹. This relevant increase in

the aerial part of the seedlings is related to a larger leaf area provided by the addition of humic substances in association with the CB substrate. The averages with the use of the CB + CRH substrate did not adjusted a model and with the CRH substrate there was also an increasing linear effect, however, presenting lower averages (Figure 4).

Figure 4. Average values for dry mass of the aerial part as a function of different substrates and doses of humic substance (HS).



CRH: carbonized rice husk; CB: carnauba bagana. Source: Authors.

Analogous to that obtained in our study (Figure 4), Wangen et al. (2013) reported increase in the production of fresh mass and dry mass from the aerial part of Malaysian cabbage (*Brassica chinensis* L.) with the application of humic substances.

Additionally, Gomes Júnior et al. (2019), when evaluating doses of humic substance in the production of mangosteen seedlings, reported an increase in the content of photosynthetic pigments of chlorophyll a and b, that lead to an important role in increasing the availability of nutrients, acting mainly on the photosynthetic apparatus of the seedlings.

And, since the ability to produce photoassimilates and absorb nutrients in adequate quantities conditions the increase in the plant's biomass, consequently, they influence the production of more vigorous and resistant seedlings, with good performance when transplanted to the field (Silva et al., 2019).

In this way, meeting the demand for propagation materials for the formation of more productive orchards. Adopt the use of substances as humic substances in a technical and assertive manner, in the appropriate dosages for each species.

4. Conclusions

The use of humic substance in association with tested substrates favors the growth of seedlings.

The interaction responses between the substrate and doses of humic substance infer that they act in different ways on development of *Spondia purpurea* seedlings according to the type of substrate, therefore, it is essential to identify and choose the compiled that favors the best results.

There is then a need test other substrates associated with the addition of humic substance, already known to condition the increase of seriguela seedlings produced by cuttings.

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