



ORIGINAL ARTICLE

## Alternatives substrates for production of seedlings camu-camu

### *Substratos alternativos para produção de mudas de camu-camu*

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**ABSTRACT:** Camu-camu is an Amazonian species with fruits that have high a concentration of ascorbic acid, a feature of interest to the food and pharmacological industries. However, for cultivation, studies are needed on seedling production and the use of alternative substrates. The objective of the present study was to develop camu-camu seedlings using different alternative substrates. For seedlings formation, seeds were removed from fruits collected from plants on the Urubu River in Boa Vista-RR, Brazil. A completely randomized experimental design was used, with ten substrates, five replications and five plants per replication. The treatments consisted of: [T1 - Soil; T2 - standard substrate: 3 parts sand soil + 1, T3 - commercial substrate: organoamazon<sup>®</sup>, T4 - standard substrate +75 % manure (E), T5 - standard substrate +50 % E; T6 - standard substrate + 25 % E, T7 - standard substrate +75 % rice hulls (C), T8 - standard substrate + 50% C; standard substrate T9 = 25% + C; T10 standard substrate +25% E + 25 % C]. The following characteristics were evaluated: plant height (cm), diameter (mm), shoot and root dry matter (g), root length (cm) and number of leaves. The substrate consisting of standard soil and sand at the ratio 3:1 gave favorable results for all the characteristics. Substrates with increasing proportions of rice hulls and manure did not improve the camu-camu tree seedling development.

**RESUMO:** O camu-camu é uma espécie amazônica com frutas que apresentam elevado teor de ácido ascórbico, característica que desperta interesse do setor industrial de alimentos e farmacológico. Para ser cultivado, há necessidade de estudos sobre formação de mudas e substratos a serem utilizados. Foi avaliado o desenvolvimento de mudas de camu-camu em diferentes substratos alternativos. Para a formação das mudas, as sementes foram retiradas de frutas coletadas de plantas localizadas às margens do Rio Urubu, Boa Vista-RR. O delineamento foi inteiramente casualizado, com dez substratos, cinco repetições e cinco plantas por unidade experimental. Os tratamentos consistiram de: T<sub>1</sub> - solo; T<sub>2</sub> - substrato padrão (SP), composto por 3 partes de solo + 1 de areia; T<sub>3</sub> (substrato comercial: organoamazon<sup>®</sup>); T<sub>4</sub> - SP + 75% de esterco bovino (E); T5 - SP + 50% E; T6 - SP + 25% E; T7 - SP + 75% de casca de arroz carbonizada (C); T8 - SP + 50% C; T9 - SP + 25% C; T10 - SP + 25% E + 25% C. Foram avaliados: o comprimento da parte aérea da planta (cm); o diâmetro do colo (mm); a massa seca de parte aérea e raízes (g); o comprimento de raízes (cm), e o número de folhas. De acordo com os resultados obtidos, concluiu-se que o substrato padrão composto por solo e areia, na proporção 3:1, foi o que proporcionou resultados favoráveis em todas as características avaliadas. Substratos com proporções crescentes de casca de arroz carbonizada e esterco bovino não proporcionaram bom desenvolvimento de mudas do camucamuzeiro.

## 1 Introduction

The camu-camu (*Myrciaria dubia* (Kunth.) McVaugh) is a typical Amazonian fruit tree that grows on river and lake banks throughout the Amazon basin. The species has attracted great interest in some countries because of its potential for ascorbic acid production, that varies from 845 to 6.100 mg in 100 g whole pulp (ESASHIKA; DE OLIVEIRA; MOREIRA, 2011; YUYAMA et al., 2011).

The growing market demand is causing more intense extraction of the species in its natural environment, bringing risk of genetic erosion and negative impacts on other organisms and the environment (PINEDO et al., 2001). It is essential to form camu-camu orchards on dry land to preserve the accessions. However, studies are needed on the formation of quality seedlings and the substrate to be used.

Camu-camu seedlings are basically formed by seeds in 6 to 8 months. To improve the production quality of the seedlings, the choice of substrate is essential. It should have good chemical and physical characteristics and preferentially raw material should be used that is accessible to the producers in the region, because commercial substrates currently found in the Amazon region are not economically viable and difficult to find in the local commerce.

Substrates are most important at the initial phases of plant development and can be of animal, vegetable or synthetic origin. According to Welter et al. (2011), the main characteristics of the raw materials to be used as substrate should take into consideration economic, chemical and physical factors.

The substrate used in the recipient while the seedlings are in the nursery should have good physical chemical and biological characteristics that enable rapid seedling growth, good dry matter content in the shoot and roots and easy acquisition, among other characteristics (YAMANISHI et al., 2004).

Organic materials available in quantity in the Northern region of Brazil, specifically Roraima, include rice husk ash, a product found abundantly because the state is a big rice producer. The product can be added to other substrates such as soil, sand and manure to form composts for fruit seedling production. In addition to rice husk ash, organoamazon® substrate is available in the region, a 100% natural and regional organic commercial product, consisting cattle, horse, chicken or sheep manure, rice husk ash, turf, sugarcane bagasse, grass cuttings, twigs and leaves. However, it is expensive and unfeasible for perennial fruit tree seedling production such as the camu-camu.

Considering the potential for economic and agronomic exploitation of the camu-camu and the difficulties in choosing a substrate that meets the nutritional requirements of plants in the nursery, in addition to the search for alternative materials accessible to regional producers, aimed to evaluate the development of seedlings camucamuzeiro using mixtures of substrates.

## 2 Materials and Methods

The experiment was carried out in the Fruit Cultivation Sector of the Embrapa Roraima, located in the municipality of Boa Vista-RR, referenced geographic coordinates 02°42'30"N

and 47°38'00"W, 90m altitude, in an area of cerrado (Savannah), where the wet season lasts 5 to 6 months a year, starting in April/May and finishing in September/October, with a total rainfall of approximately 1,200 mm. In the dry period (October to March) the rainfall is 300 mm, making an annual total of 1,500 mm, characterizing the Awi climate type.

The seeds to form the seedlings were obtained from fruits collected on the banks of the River Urubu, in the municipality of Boa Vista-RR. After collection, the fruits were taken to the Fruit Cultivation Laboratory at Embrapa Roraima, where the seeds were separated from the pulp by hand. They were then washed in running water until the pulp residue was completely removed, placed to dry with controlled temperature and relative humidity (25 °C and 60% RH) and later they were sown in a bed containing 1:1 (v/v) sand and sawdust substrate.

The bed was located in a greenhouse with an automatic spray irrigation system set at four hourly intervals, three times a day, for a period of 5 min each irrigation. The seedlings emerged fifteen days after sowing and when they reached a height of approximately 10 cm, standardizing height and stem diameter, they were transplanted to polyethylene bags (15 x 26 cm) containing different substrates according to each determined treatment. They were then placed on benches in a greenhouse with spray irrigation three times a day for five-minute periods.

A completely randomized experimental design was used, with 10 treatments, five replications and five plant per replication, totaling 250 plants. The treatments consisted of the following mixtures of substrates: T<sub>1</sub> - soil; T<sub>2</sub> - standard substrate (SP), consisting of three-parts soil + 1 sand; T<sub>3</sub> - commercial substrate : organoamazon®; T<sub>4</sub> - SP + 75% cattle manure (E); T<sub>5</sub> - SP + 50% E; T<sub>6</sub> - SP + 25% E; T<sub>7</sub> - SP + 75% rice husk ash (C); T<sub>8</sub> - SP + 50% C; T<sub>9</sub> - SP + 25% C; T<sub>10</sub> - SP + 25% E + 25%. Table 1 shows their chemical analysis.

The shoot length and the stem diameter of plants were assessed every 60 days for 270 days. The shoot length was measured using a ruler graduated in centimeters (cm) and this length was considered to be the height of the plant from soil level to the apex tip. The stem diameter was measured using a digital pachymeter in millimeters, measured at 1 cm above soil level.

At the end of the experiment (at 270 days), the following plant characteristics were assessed: root system length (RSL), shoot dry matter (CDM), root system dry matter (RSDM) and number of leaves.

The plants were removed from the polyethylene bags and the number of leaves counted. The roots were separated from the substrate by washing in running water until there were no soil residues and then the shoot was separated from the root system. The RSL was measured using a ruler graduated in centimeters. The plant fresh matter and the root system fresh matter were measured using analytical scales. The material was then placed in a forced air circulation chamber at ± 60 °C until constant weight to obtain the shoot and root dry matter.

The data were analyzed using univariate statistical methods with the help of the Info-Gen statistical package (BALZARINI; DI RIENZO, 2012).

### 3 Results and Discussion

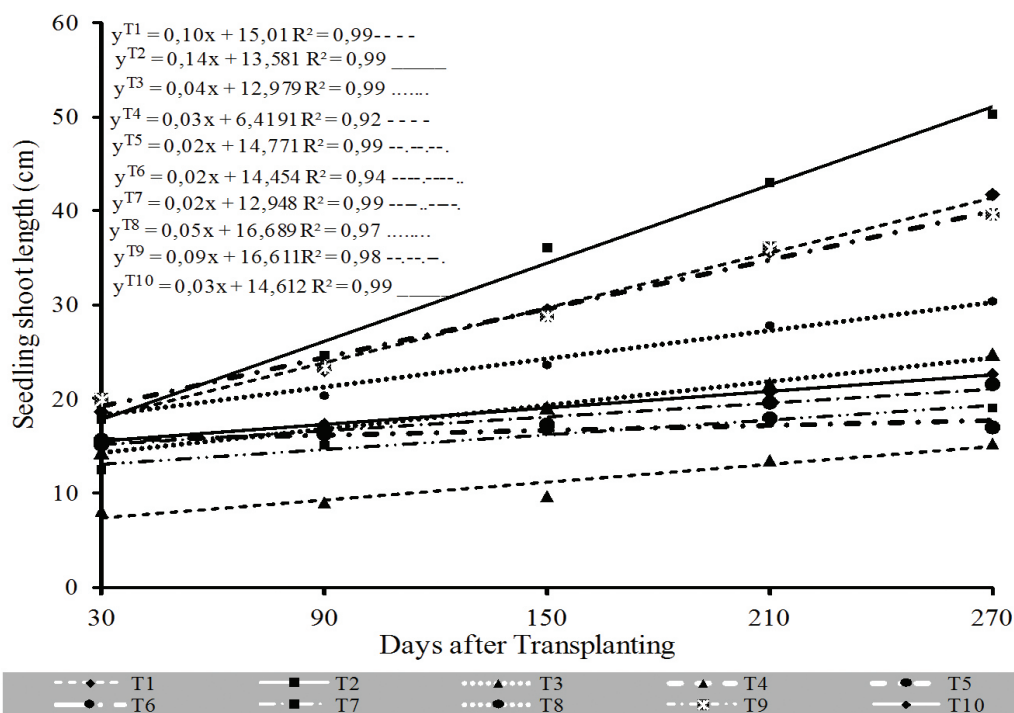
The results showed that there was significant difference regarding the characteristic of camu-camu seedling height for the different substrates used (Figure 1). T2, where the substrate consisted of standard substrate at the ratio of 3:1 of soil and sand, resulted in the greatest height for the camu-camu seedlings at 270 days after transplant, reaching 50.25 cm in height. When manure was added to the substrate (T4 = standard substrate + 75% cattle manure and T5 = standard substrate + 50% manure) the seedlings did not develop properly with heights of 15.27 cm and 16.98 cm, respectively. Further according to the substrate analysis, these treatments had high organic matter content (Table 1).

Organic matter-rich substrates usually result in greater development for most fruit trees, but this fact was not observed for the camu-camu in the present experiment. In 'Paluma' and 'Século XXI' guava trees the substrates prepared based on a mixture of soil (latosol) + sand plus + organic matter (corral manure) (3:1:1) and Plantmax® were the most indicated for seedling production compared to the substrates based on pure soil (latosol) and coconut fiber (ZIETEMANN; ROBERTO, 2007). When various substrates were used with pitanga trees no influence was observed from the substrates consisting of organic compost + soil + sand at the ratio of 1:1:3 and commercial substrate + sand + plus soil, at the ratio of 1:1:3, for the plant height characteristic (ABREU et al., 2005).

**Table 1.** Chemical analysis of the substrates.

Substrate	pH	P	MO	Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>+</sup>	Al <sup>3+</sup>	H+Al	SB	T	V	Zn	Fe	Mn	Cu
		mg dm <sup>-3</sup>	g kg <sup>-1</sup>	cmol <sub>c</sub> dm <sup>-3</sup>							%	mg dm <sup>-3</sup>			
T1	5.7	15.51	9.70	0.60	0.30	0.40	0.10	1.86	1.30	3.16	41.14	2.19	34.70	19.70	0.63
T2	6.7	13.41	26.10	10.40	0.50	0.28	0.00	0.95	11.18	12.13	40.95	2.23	34.71	19.71	0.65
T3	5.7	263.89	100.00	13.80	7.40	0.80	0.00	1.86	22.00	23.86	92.20	26.94	62.34	160.22	0.56
T4	5.8	314.87	61.50	10.20	5.00	0.29	0.00	1.66	14.95	17.15	90.30	24.44	13.50	90.92	0.64
T5	6.5	218.18	39.90	10.00	2.90	0.24	0.00	1.33	13.14	14.47	90.78	23.49	20.25	106.97	0.00
T6	6.2	151.18	38.40	9.90	1.60	0.24	0.00	1.19	11.74	12.93	90.76	24.34	27.86	123.11	1.03
T7	6.1	71.74	49.30	12.20	1.40	0.27	0.00	1.86	13.87	15.73	88.19	16.50	13.51	88.56	0.30
T8	6.6	93.01	42.90	11.00	0.90	0.31	0.00	1.08	12.21	13.28	91.93	20.76	17.99	127.27	0.56
T9	6.7	132.91	37.00	11.00	0.70	0.31	0.00	1.07	12.01	13.08	89.73	23.34	33.50	132.31	0.78
T10	6.4	170.23	41.40	9.90	1.80	0.31	0.00	1.19	12.01	13.20	89.44	23.10	15.13	100.54	0.69

MO – Organic matter; SB – sum of bases; T- CTC a pH 7,0; V – base saturation.



**Figure 1.** Effect of different substrates on camu-camu seedling length using different substrates T1 – soil; T2 – standard substrate (SP), consisting of three-parts soil + 1 sand; T3 – commercial substrate : organoamazon®; T4 – SP + 75% cattle manure (E); T5 – SP + 50% E; T6 – SP + 25% E; T7 – SP + 75% rice husk ash (C); T8 – SP + 50% C; T9 – SP + 25% C; T10 – SP + 25% E + 25% C. Boa Vista-RR, Brazil, 2013.

The results found in the present study confirmed the robustness of the camu-camu compared to other fruit trees that have been domesticated. The camu-camu develops in flooded soils poor in organic matter. However, studies are needed that lead to substrate choices so that quality seedlings can be produced in the nursery and form future commercial orchards. In this sense, Carneiro (1995) and Gomes et al. (2002) stated that shoot height combined with the stem diameter was one of the most important morphological parameters to estimate seedling growth after the final planting in the field.

Performance similar to the height was observed for seedling stem diameter. The greatest diameter (5.05 mm) was observed when the seedlings were grown in substrate consisting of 3:1 soil and sand (Figure 2). The seedlings with greater stem diameter are more resistant to adversities. Greater thickness is also important for plant propagation by grafting, used in the crop to clone plants with good physiological attributes.

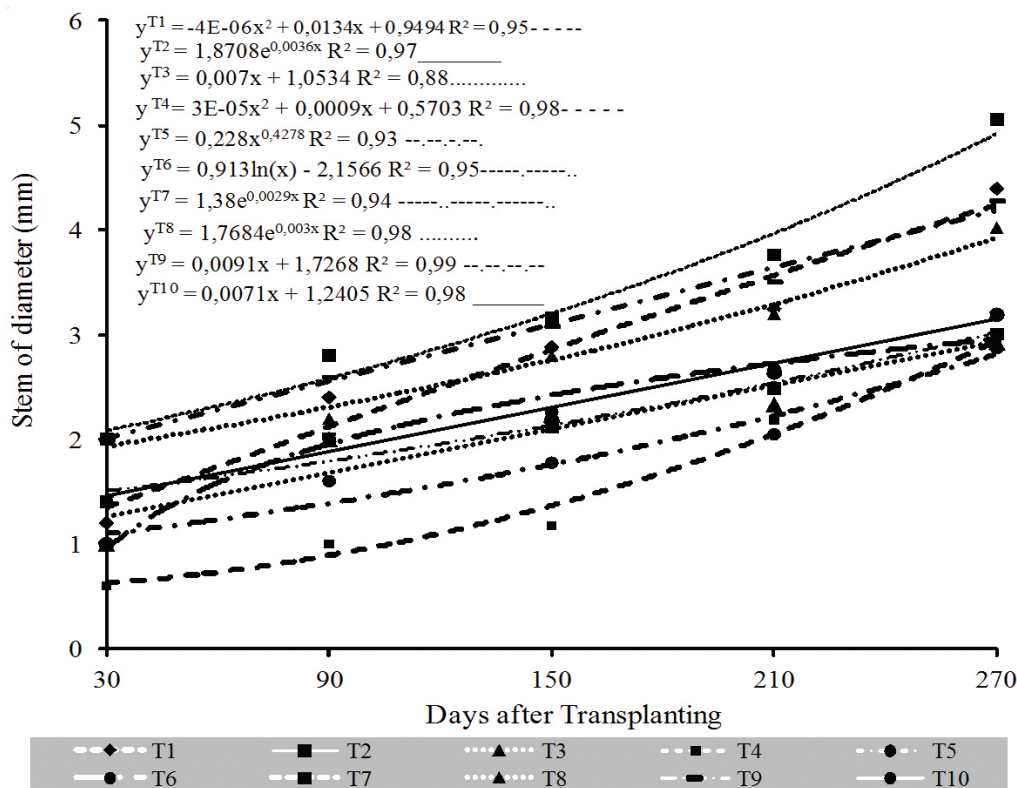
For treatments T3, T4 and T5, substrates consisting of organoamazon<sup>®</sup>; standard substrate + 75% cattle manure, standard substrate + 50% manure, respectively, reached diameters less than 3 mm, considered low for seedlings at 270 days of age.

The analysis of the substrates showed that they presented adequate nutrient contents and were rich in organic matter (Table 1) that may have influenced not only this characteristic but also the others, because the camu-camu tree is adapted naturally to flooded soils poor in organic matter

(PENN JUNIOR, 2006; MATHEWS; YUYAMA, 2010). Similar results were reported by Lima et al. (2006) who studied alternative substrates containing rice husk ash, powdered dry coconut bark and carnauba palm leaf straw which did not contribute satisfactorily to obtain quality Caribbean cherry seedlings. Mixtures in the substrate to produce araticum were tested and it was reported that the treatments with ash and lime did not differ statistically in height plant or stem diameter. Thus good substrates are those that result in good growth for seedling height and stem diameter (FERREIRA et al., 2009).

There was significant difference for the shoot dry matter variable. Seedlings grown in the substrate consisting of soil and sand at 3:1 presented greatest shoot dry matter 3.35 g (Figure 3). On the other hand, the seedlings cultivated in commercial substrate (T3), and treatments T4 and T5 consisting of soil and manure and T6, T7 and T10 consisting of rice husk ash, standard soil and manure, respectively, presented a smaller quantity of dry matter. However, when the proportion of rice husk ash was lower, 50 to 25%, and mixed with soil, there was an increase in the root dry matter T1, T8 and T9 with values of 2.15; 1.48 and 2.26 g, respectively (Figure 3).

Other *species* present similar development performances regarding rice husk ash application. Saidelles et al. (2009) observed that the species *Eucalyptus saligna* performed similarly to *Apuleia leiocarpa*, where the best treatments had the lowest proportions of rice husk ash mixed with soil to form the substrate. This characteristic should be taken into



**Figure 2.** Effect of different substrates on plant diameter in camu-camu seedlings using different substrates. T1 - soil; T2 - standard substrate (SP), consisting of three-parts soil + 1 sand; T3 - commercial substrate : organoamazon<sup>®</sup>; T4 - SP + 75% cattle manure (E); T5 - SP + 50% E; T6 - SP + 25% E; T7 - SP + 75% rice husk ash (C); T8 - SP + 50% C; T9 - SP + 25% C; T10 - SP + 25% E + 25% C. Boa Vista-RR, Brazil, 2013.



consideration to obtain plants suitable to withstand transplant, because Caldeira et al. (2008) stated that the shoot and root ratio in the seedling should be 2:1 and the root and shoot ratio 1:2. It is important to analyze this ratio when the seedlings go to the field, because the seedling shoot should not be much greater than the root, because of possible problems regarding water absorption for the shoot and thus the composition of the substrate used is important.

The root system length differed among the substrates used and values of 31.86, 33.81 and 37.84 cm were observed in treatments T1, T2 and T8, respectively (Figure 4a). None of the treatments with manure in addition to commercial substrate in their composition responded well for this characteristic. Scalon et al. (2011) assessed germination and growth of pau-ferro (*Caesalpinia ferrea* Mart. ex Tul.) in different substrates and verified that the substrate with soil and sand resulted in greater root length. The authors attributed this fact to fewer

nutrients and less water available in the substrate that forced the roots to greater axial growth in search of nutrients.

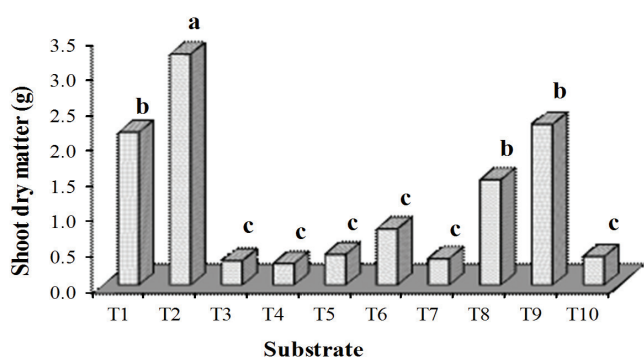
However, in treatments 8 and 9, with the addition of rice husk ash at proportions of 25 and 50%, a positive response was obtained regarding root length and dry matter. According to Melo, Bortolozzo and Vargas (2008), rice husk ash is very porous that may balance the mixture of other materials. Thus the chemical analyses of the substrate recorded high pH values: 6.6 and 6.7 (Table 1) that may have influenced the good development in root length and dry matter.

Root dry matter of 2.32 was found with the standard soil and sand-based substrate (T2) (Figure 4b). Performance was similar to the root length for the other characteristics.

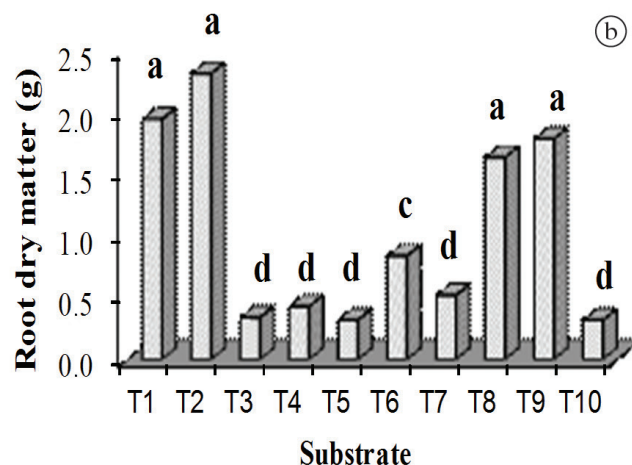
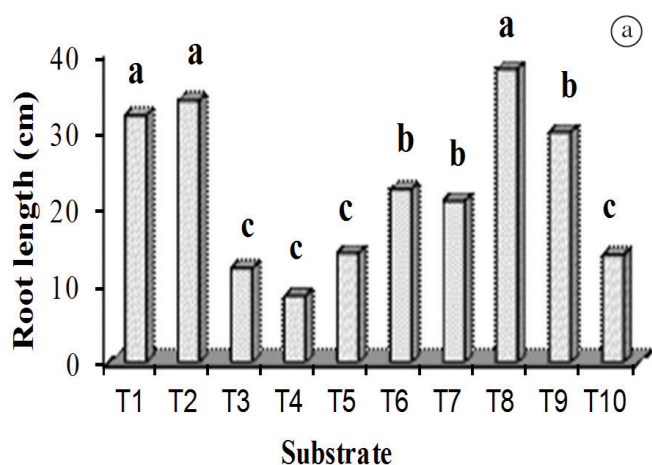
Root dry matter has been recognized by different authors as one of the most important and best parameters for estimating seedling survival and initial growth in the field (SAIDELLES et al., 2009). As a general rule, when formulating substrates for seedling production using seeds, approximately 20 to 40% of a more porous material (vermiculite, rice husk ash, charcoal) is recommended in a mixture of 60 to 80% of a less porous material (subsoil, organic compost, humus). However, depending on the species and management used in the seedling production (substrate, irrigation, recipients, etc.), this proportion may be different, and tests should be carried out at the site before using any composition (WENDLING; GATTO, 2002).

Regarding number of leaves, the plants from substrates T2 and T8 presented 53.92 and 56.64 leaves per plant, while the seedlings in the other treatments presented a smaller number of leaves (Figure 5).

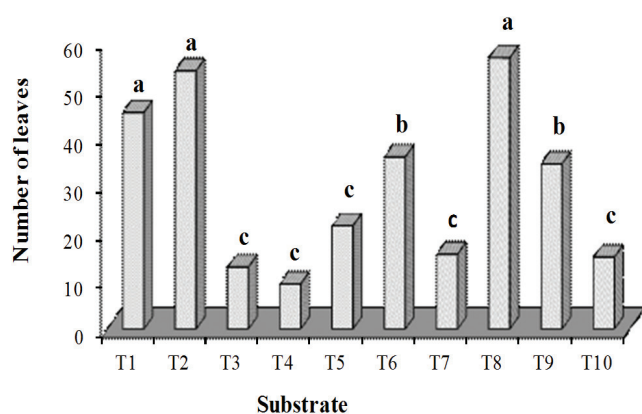
Increase in number of leaves in seedling production is a beneficial factor, because they are the principal location where photosynthesis occurs and they are also reserve centers. They further contribute to new tissue formation, such as roots, and in this way are more important than the stems (PEREIRA et al., 1991; HARTMANN et al., 2002). According to Costa et al. (2009), for passion flower fruit seedlings, the



**Figure 3.** Shoots dry matter of camu-camu seedlings in function of different substrates T1 - soil; T2 - standard substrate (SP), consisting of three-parts soil + 1 sand; T3 - commercial substrate : organoamazon<sup>®</sup>; T4 - SP + 75% cattle manure (E); T5 - SP + 50% E; T6 - SP + 25% E; T7 - SP + 75% rice husk ash (C); T8 - SP + 50% C; T9 - SP + 25% C; T10 - SP + 25% E + 25%. Boa Vista-RR, Brazil, 2013.



**Figure 4.** Root length (a) and root dry matter (b) of camu-camu seedlings in function of different substrates. T1 - soil; T2 - standard substrate (SP), consisting of three-parts soil + 1 sand; T3 - commercial substrate : organoamazon<sup>®</sup>; T4 - SP + 75% cattle manure (E); T5 - SP + 50% E; T6 - SP + 25% E; T7 - SP + 75% rice husk ash (C); T8 - SP + 50% C; T9 - SP + 25% C; T10 - SP + 25% E + 25%. Boa Vista-RR, Brazil, 2013.



**Figure 5.** Number of leaves on camu-camu seedlings in function of different substrates T1 - soil; T2 - standard substrate (SP), consisting of three-parts soil + 1 sand; T3 - commercial substrate : organoamazon<sup>®</sup>; T4 - SP + 75% cattle manure (E); T5 - SP + 50% E; T6 - SP + 25% E; T7 - SP + 75% rice husk ash (C); T8 - SP + 50% C; T9 - SP + 25% C; T10 - SP + 25% E + 25%. Boa Vista-RR, Brazil, 2013.

substrate “soil = organic compost + vermiculite, at the 1:1:1 v/v volumetric ratio is the best alternative for yellow passion fruit seedling production, and has resulted in a greater number of leaves. Substrates were tested for *Eugenia uvalha* formed by vermiculite, plantmax<sup>®</sup>, sand, plantmax<sup>®</sup> + turf, rendmax<sup>®</sup> (1:1 v/v) and riverbank soil and it was concluded that the vermiculite and sand substrate resulted in the best biometrical indices in the assessments of the *Eugenia uvalha* tree seedlings, including a greater number of leaves.

This characteristic is important, but not decisive, in camu-camu trees for characterizing a quality seedling, because there may be leaf fall during the assessments and even due to factors external to the plant.

## 4 Conclusions

The substrate consisting of soil and sand at the ratio of 3:1 is recommended for the formation of camu-camu tree seedlings.

Increasing proportions of rice husk ash and cattle manure in the composition of the substrate did not result in good development of camu-camu tree seedlings.

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