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RESEARCH ARTICLE

ORGANIC SUBSTRATES FOR NEEM SEEDLINGS PRODUCTION

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ABSTRACT

The use of organic substrates of own formulation for seedlings production can be a viable alternative for producers by reducing costs and facilitating access. The aim of this study was to evaluate the early development of neem seedlings (*Azadirachta indica*) in different organic substrates. The experiment was performed in a completely randomized design, in a 4x5 factorial, with factors consisted of four organic sources (decomposed stem of the moriche palm [*paú de buriti*], earthworm humus, goat and poultry manure) in five proportions (0, 25, 50, 75 and 100% of source mixed with soil) with ten replications. At 30 days after the emergence, plant height; number of sheets; leaf area; dry mass of shoots; and length, volume and root dry matter were evaluated. The early development of the seedlings was better on the substrates with decomposed stem of the moriche palm [*paú de buriti*] and poultry manure, especially in the proportions of 50 and 75% of mixed source with soil, promoting better vegetative growth.

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INTRODUCTION

The neem [*Azadirachta indica* A. Juss. (Meliaceae)] is an exotic tree species originated in Asia and distributed in the Americas and other regions of tropical and subtropical climate (Azevedo et al., 2010). Its trunk produces red color resistant wood, its leaves are dark green, compound, imparipinnate and without stipules. The flowers are white and the fruit production begins with three to five years, and the fruits are yellow at maturity. The seeds are recalcitrant and tend to lose the germinating capacity in 90 days (Neves and Carpanezzi, 2008), making it difficult to store for long periods. It is a rustic plant,

of fast growing, which develops well in physically degraded soils with pH between 6 and 7; it settles well in regions with rainfall between 400-800 mm / year and can support long periods of drought (Freire et al., 2010), typical of Cerrado. The neem has great potential for economic exploration, providing financial returns due to its ability of multiple exploitation as wood production; acaricides and fungicides by the seeds and leaves (Duarte et al., 2012), and its use for afforestation and shading. The use of seedlings is common for the implementation of plant species, mainly arboreal. Its production in the nursery is advantageous, considering the time, the labor and costs involved in this process when compared to the direct sowing in the planting site. The use of good quality and vigorous seedlings is a key factor for the success of the transplanting and the establishment of plants in the field.

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The quality of the seedlings is affected by the conditions in which they are produced, especially by the substrate used. According to Wagner Jr. *et al.* (2007), the substrate is responsible for the fast and proper root growth, reflecting in the plant vigor. The substrate must support the changes, ensuring the development of a high quality plant in a short time and at low cost. It should present appropriate physical, chemical and biological characteristics to enable the full growth of root and shoot parts (Setubal and Afonso Neto, 2000). As it is used in the development stage in which the plant is very susceptible to microorganisms attack and is not very tolerant to drought, it must gather characteristics that promote moisture retention and nutrient availability to meet the plant's needs (Cunha *et al.*, 2006). The use of organic substrates of own formulation is a viable alternative for the production of seedlings, since the commercial substrates may be expensive or unavailable in certain regions. In addition, they do not always present the best responses, as reported by Menezes Junior *et al.* (2000), who observed better results with own substrates than with commercial ones in the production of lettuce seedlings.

There are many organic sources for substrates formulation and they vary between regions. The earthworm humus, decomposed stem of the moriche palm [*paú de buriti*], and the poultry and goat manure are easily found in large quantities in southwestern Piauí and can be a raw material of easy access and affordable for producers. Thus, the aim of this study was to evaluate the early development of neem seedlings (*Azadirachta indica*) in different organic substrates.

MATERIAL AND METHODS

The experiment was performed in a greenhouse at the Federal University of Piauí-UFPI, Campus Professora Cinobelina Elvas-CPCE, in Bom Jesus-PI, at 09°04'28''S and 44°21'31''W, with average altitude of 277m and average temperature of 26.5°C, although it is common to have temperatures around 40°C (Viana *et al.* 2002) from October to November, 2014. The experiment was performed in a completely randomized design, in a 4x5 factorial, with factors consisted of four organic sources: decomposed stem of the moriche palm [*paú de buriti*], earthworm humus, goat and poultry manure in five proportions: 0, 25, 50, 75 and 100% of source mixed with soil (Dystrophic Yellow Latosol) with ten replications. The soil was collected near the fruit cultivation sector of the Federal University of Piauí/Campus Professora Cinobelina Elvas, and the chemical analysis is presented in Table 1. The *paú de buriti* and the poultry manure were obtained in Estiva settlement in Redenção do Gurguéia-PI, the goat manure was obtained in the nursery of the CPCE and the earthworm humus was purchased in local shops.

The substrates were placed in plastic tubes (12.5 cm long x 3 cm of diameter) in which the manual sowing of neem seeds (obtained from existing adult plants in CPE) was performed. After sowing, and for all the experimental period, irrigation was performed twice a day, in the morning and late afternoon. The emergence establishment occurred at eight days after sowing. The development of the plants was assessed 30 days after the emergence establishment by the variables: plant height (PH): measured with a digital caliper; number of leaves (NL): counting the total number of leaves/seedling leaf area

(LA): by measurement LI-3100 equipment Meter Area (LI-COR, Inc. (LI-COR, Inc. Lincoln, NE, EUA); root length (RL): measured with a digital caliper; root volume (RV): in a 10 mL test tube were added 70 ml of water and the roots were emerged, obtaining the final volume. Then, it was calculated the difference for the determination of variable; shoot dry mass (SDM) and root dry mass (RDM): by weighing up the parts in semi-analytical scale after oven drying. Data were submitted to analysis of variance and the means of significant parameters were compared by Tukey test at 5% of probability by the statistical program Assistat Versão 7.7 Beta (Silva and Azevedo, 2009). The quantitative factor was subjected to regression analysis, by the Sigma Plot 10.0.

RESULTS AND DISCUSSION

Overall, there was a significant effect of the substrates sources and proportions for all variables (Table 2). For the interaction, there was a significant effect, except for the leaf area. In general, the substrates formulated with *paú de buriti* and poultry manure showed the best results on the early development of the shoot parts of neem seedlings. The substrates in the proportions of 25, 50 and 75% of humus or goat manure promoted lower growth of seedlings. However, when they were used in the proportion of 100%, were equal to the *paú de buriti* and poultry manure (Table 3).

The plant height showed a quadratic behavior for the different sources, depending on the proportions used, except for the humus that followed an increasing linear trend (Figure 1A). *Paú de buriti* and poultry manure, at the proportion of 50%, showed an increase in the initial growth higher than 38%, compared to earthworm humus and goat manure. However, when they were used in the proportion of 100%, they showed similar results when they were not added to the substrate formulation, showing that the mixture of soil organic sources allows greater seedlings vigor. This fact shows high responsiveness to the proportions of organic matter during this phase, which is possibly related to the physical, chemical and biological conditions offered by organic substrates (Camargo *et al.*, 2011.). Silva and Santos (2014) also observed a greater height of neem seedlings that were produced in substrates with addition of up to 30% of organic matter. The humus and goat manure, even when pure, showed little effect on the plant height.

The number of leaves of the seedlings was similar for the sources in different proportions, except when earthworm humus was used in a proportion of 25%, which showed lower leaf emergence (Table 3). Regarding the proportions, all substrates showed a quadratic trend, with similar responses among *paú de buriti* and poultry manure, which were superior, and among the humus and goat manure (Figure 1B). Substrates produced with proportions of 25 and 50% of *paú de buriti* and poultry and goat manure showed the greatest number of leaves. For the substrate with humus, it was found in the proportion of 75%. There was fewer leaf emergence for all treatments, with sharp drop in the proportion of 100%, which was lower than when it was not used an organic source, except for the humus which still presented a greater value for the variable in this proportion. The substrates formulated with *paú de buriti* showed the greatest leaf area in all the proportions (Table 4).

Table 1. Chemical characterization of Dystrophic Yellow Latosol in 0-0.20m layer

pH	P	K	S	H+Al	Al	Ca	Mg	K	SB	T	m	V	OM
H ₂ O	-- mg dm ⁻³					cmol _c dm ⁻³					----- %		g/Kg
5.4	14.19	192.5	-	4.95	0.00	2.24	0.86	0.49	3.59	8.54	0.00	42.1	20.9

pH in water; P = phosphorus; S = sulfur; H + Al = hydrogen + aluminum; Al = aluminum; Ca = calcium; Mg = magnesium; K = potassium; SB = Sum of exchangeable bases; T = effective CEC; m = Aluminum Saturation Index; V = Base Saturation Index; and OM = Organic Matter.

Table 2. Análise de variância da altura de plantas (AP), número de folhas (NF), Área foliar (AF), massa seca da parte aérea (MSPA), comprimento de raiz (CR), volume de raiz (VR) e massa seca de raiz (MSR) de mudas de nim produzidas com substratos orgânicos formulados com diferentes fontes e proporções. Bom Jesus – PI, Brasil, 2014

Source of variation	Mean Squares						
	PH	NL	LA	SDM	RL	RV	RDM
Substrate sources (A)	18.51**	3.24*	1075.04**	0.18**	44.91**	0.016**	0.11**
Proportions (B)	34.39**	19.45**	1630.75**	0.03**	4.58**	0.007**	0.03**
(A) x (B)	8.52**	2.50*	160.29 ^{ns}	0.01**	10.29**	0.002*	0.01**
CV (%)	15.22	19.65	21.18	12.82	5.28	18.63	16.22

* and **: significant at 5 and at 1% respectively by F test ^{ns} no significant . CV: coefficient of variation

Table 3. Mean values of plant height, number of leave, leaf area and shoot dry mass of neem seedlings produced in own formulation of substrates, from four organic sources in five proportions. Bom Jesus - PI, Brazil, in 2014.

Substrate Sources	Source proportion (%)				
	0	25	50	75	100
	Plant Height (cm)				
Paú de buriti	8.28 a*	10.68 a	11.77 a	11.30 a	7.67 a
Humus	8.28 a	8.66 b	9.07 b	9.16 b	9.30 a
Goat manure	8.28 a	8.76 b	9.34 b	9.20 b	9.01 a
Poultry manure	8.28 a	11.24 a	11.44 a	10.44 ab	8.50 a
	Number of leave (unit)				
Paú de buriti	5.00 a	6.97 a	6.66 a	6.22 a	4.33 a
Humus	5.00 a	5.38 b	5.55 a	5.66 a	5.40 a
Goat manure	5.00 a	5.77 a	5.66 a	5.44 a	4.60 a
Poultry manure	5.00 a	6.77 a	6.66 a	5.77 a	4.33 a
	Shoot dry mass (g)				
Paú de buriti	0.22 a	0.26 b	0.28 b	0.26 bc	0.24 b
Humus	0.22 a	0.25 b	0.28 b	0.30 b	0.33 a
Goat manure	0.22 a	0.24 b	0.23 b	0.23 c	0.21 b
Poultry manure	0.22 a	0.34 a	0.37 a	0.39 a	0.37 a

* Means followed by the same letter in the column to the same variable and the same proportion, do not differ by Tukey test at 5% probability.

Table 4. Mean values of leaf area of neem seedlings produced in substrates own formulation, from four organic sources. Bom Jesus - PI, Brazil, 2014

Substrate source	Leaf Area (cm ²)
Paú de buriti	53.35 a
Humus	45.72 b
Goat manure	42.45 b
Poultry manure	45.57 b

* Means followed by the same letter in the column to the same variable and the same proportion, do not differ by Tukey test at 5% probability.

Table 5. Mean values of root length, root volume and root dry mass of neem seedlings produced in substrate of own formulation from four sources in five proportions. Bom Jesus - PI, Brazil, 2014

Substrate Source	Substrate Proportion (%)				
	0	25	50	75	100
	Root length (cm)				
Paú de buriti	11.72 a*	12.22 a	12.91 a	13.30 a	12.83 a
Humus	11.72 a	12.22 a	12.61 a	12.88 a	13.40 a
Goat manure	11.72 a	11.14 b	10.81 b	9.78 c	9.94 b
Poultry manure	11.72 a	12.57 a	12.25 a	11.17 b	8.93 c
	Root volume (cm ³)				
Paú de buriti	0.07 a	0.09 a	0.12 a	0.13 a	0.13 a
Humus	0.07 a	0.08 a	0.08 b	0.08 bc	0.06 b
Goat manure	0.07 a	0.08 a	0.08 b	0.06 c	0.05 b
Poultry manure	0.07 a	0.09 a	0.11 ab	0.12 ab	0.12 a
	Root dry mass (g)				
Paú de buriti	0.29 a	0.30 b	0.32 bc	0.28 bc	0.21 b
Humus	0.29 a	0.34 b	0.35 b	0.30 b	0.26 b
Goat manure	0.29 a	0.31 b	0.29 c	0.25 c	0.23 b
Poultry manure	0.290 a	0.42 a	0.44 a	0.45 a	0.42 a

* Means followed by the same letter in the column to the same variable and the same proportion, do not differ by Tukey test at 5% probability.

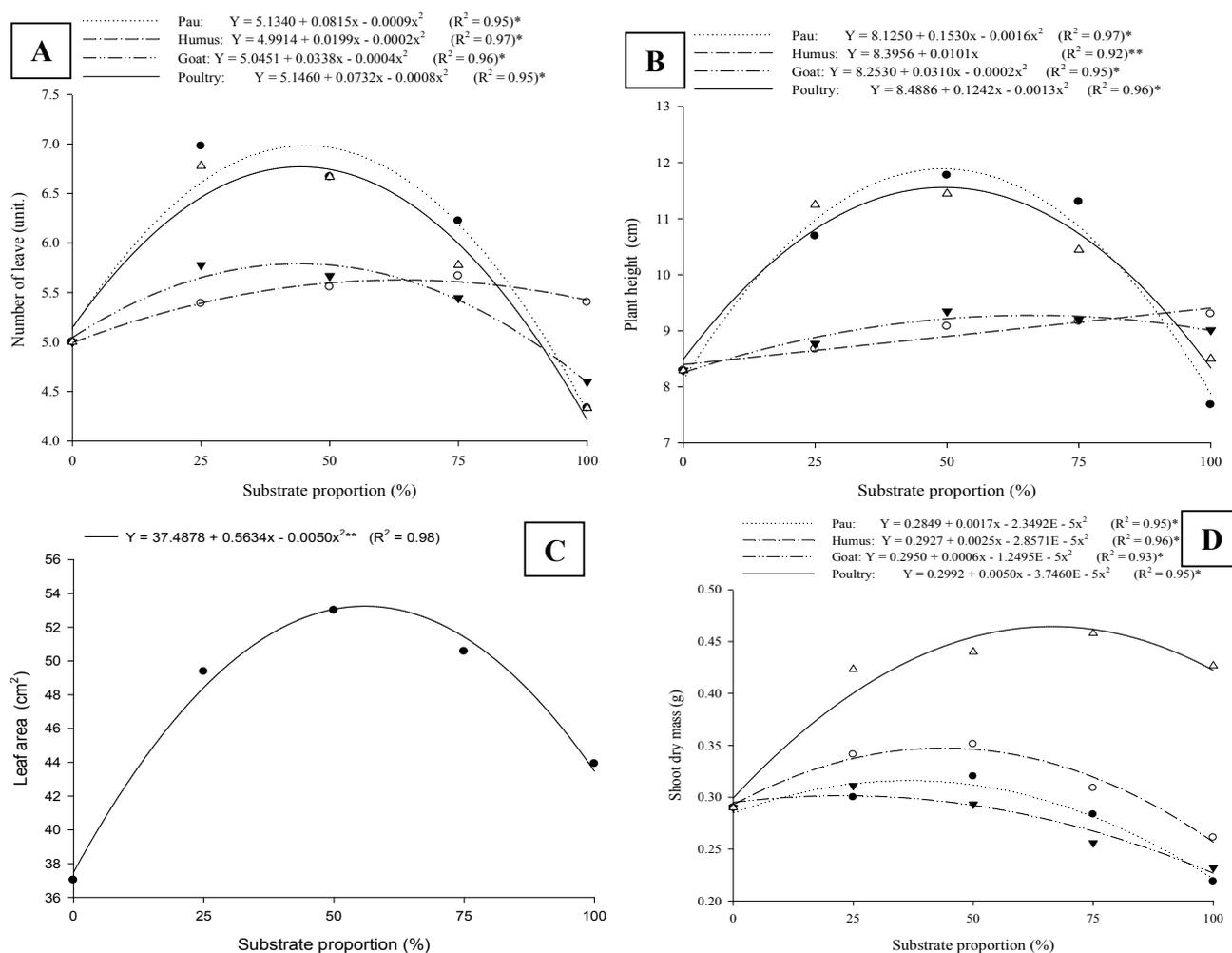


Figura 1. Response of plant height (A), number of leaves (B), leaf area (C) and shoot dry mass (D) of neem seedlings according to the organic substrate proportion. * and **: significant at 5% and 1%, respectively. Bom Jesus - PI, Brazil, 2014

The best responses from the tested sources were obtained with the proportion of 50% (Figure 1C), therefore, it is observed that the substrates formulated with the mixture of soil with organic source were better than the pure ones. The addition of organic matter promoted greater seedling development, by serving as a carbon supply and improving the characteristics of the substrates, increasing the number of leaves and leaf area, allowing a higher production of photoassimilates and consequently greater plant height (Faustino *et al.*, 2005). Regarding the shoot dry mass, the poultry manure stood out with the highest values in all proportions, except in the control (0%) and with 100% of humus. At 75%, the goat manure and *paú de buriti* showed the worst results for shoot dry mass accumulation of seedlings 30 days after emergence (Table 3). All the substrates, except the ones with poultry manure, showed similar or lower results than the pure soil, when it was used proportions from 75% of source (Figure 1D). Malavolta (1989) verified that the poultry manure has a high concentration of nitrogen (N), which is directly connected to the photosynthetic process and the production of photoassimilates that are used for the growth of plant tissues. And the availability of N from organic waste ensures an initial supply that is demanded by plants (Quadros *et al.*, 2000), which possibly contributed to the best results of this source. The reduction of the growth parameters with the increasing proportions, particularly when the organic sources were used

pure, may be due to the effect that these compounds have on pH characteristics and electrical conductivity (Albano *et al.*, 2014) or, in some cases, due to the excess of N that is provided. The substrate source and proportions used affect the development of roots and individually and associated (Table 2). Considering the results of the variables regarding the substrate source within each proportion used (Table 5), it is observed that the substrates with *paú de buriti* and earthworm humus were the ones which promoted greater root growth in all proportions, except at 25 and 50%, when the poultry manure provided similar results to these. In the proportion of 75%, there was a lower root growth in goat manure, and 100% with the poultry manure. Due to the increase of the proportions of organic sources (Figure 2A), the root growth was linearly stimulated by the earthworm humus, with an increase of 14% when used pure, and impaired by goat manure. The *paú de buriti* and poultry manure presented quadratic behavior for the variable, and the highest values were observed in the proportion of 75 and 25% of the sources, respectively. According to Diniz *et al.* (2006), substrates with a high organic matter content ensures a high number of pore spaces, and a low apparent density, which promotes root development. Abreu *et al.* (2010) point out that treatments with earthworm humus shows great quantities of organic matter, because the worms present action on humification processes, contributing to the fragmentation of vegetables waste and incorporation, as well

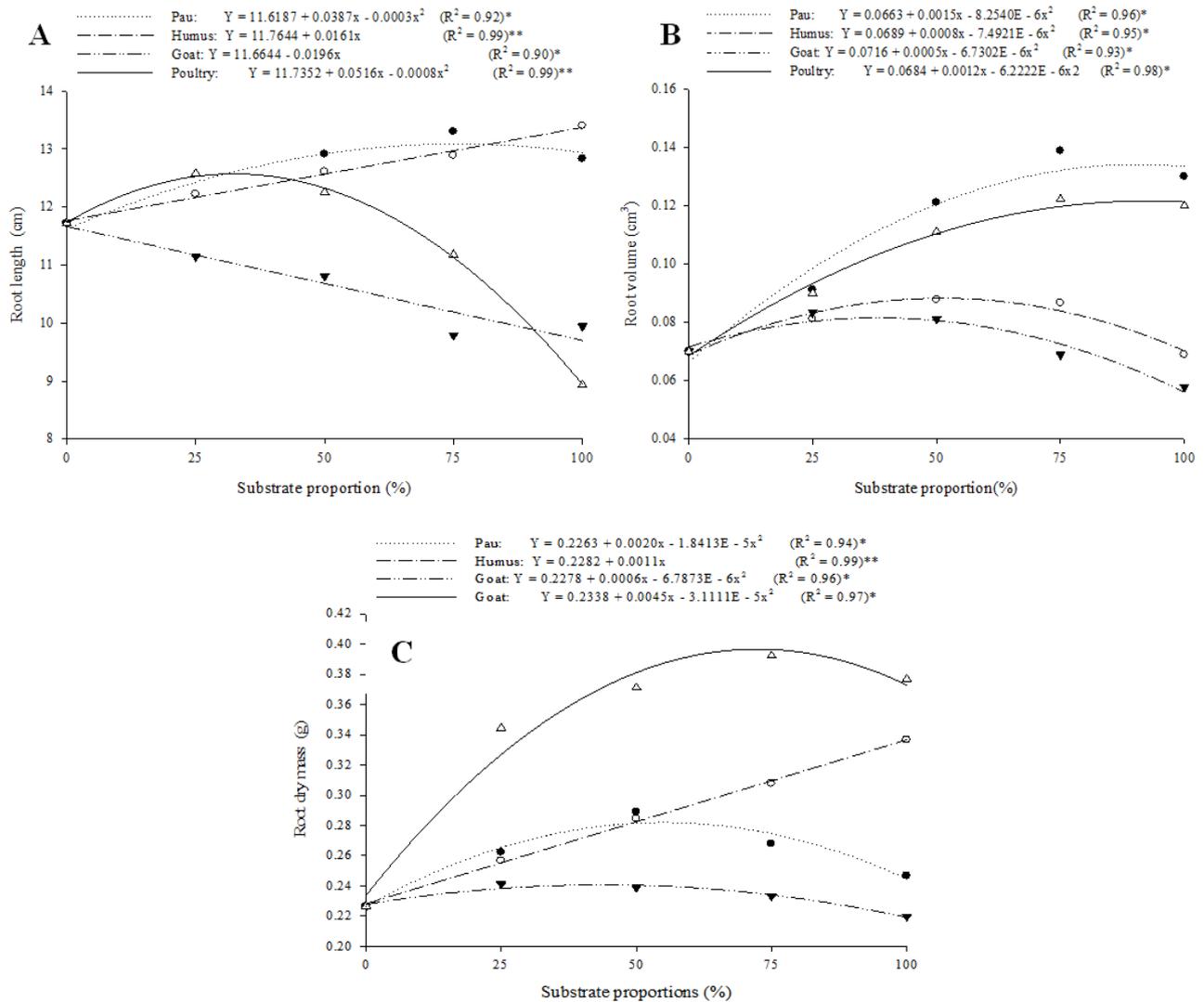


Figure 2. Response of root length (A), root volume (B) and root dry mass (C) of neem seedlings according to the organic substrate proportions. * and **: significant at 5% and 1%, respectively. Bom Jesus - PI, Brazil, 2014

as total, exchangeable and assimilable elements (Ca, K, Mg and P), making them more available (Wolinsk-Miklós, 1997). The humus also increase the microporosity of the soil, increasing aeration and water retention (Abreu *et al.*, 2010). From 50% of source, the root volume differed, where the *paú de buriti* followed by the poultry manure provided the highest values for the variable (Table 5). The superiority of these sources was also observed in the proportions of 75 and 100%, while the humus and goat manure presented lower volumes of seedling roots. Comparing the substrates, according to the increasing proportions of sources, all of them showed a quadratic response (Figure 2B). The best results were obtained in *paú de buriti*, followed by poultry manure, humus and goat manure. For the first two, the proportion of 75% stood out as the best; for the other two, the best proportion was of 50%, being observed a reduction in the roots volume in the highest proportions of the source, even lower than those observed in the proportion of 0%, showing that it is necessary to heed to the proportion of the organic source for the formulation of the substrate, since higher amounts may be detrimental to the development of the roots of the neem seedlings. The root dry mass of, within each proportion, indicates a greater accumulation in seedlings produced from poultry manure in all proportions (Table 5). The other sources were equal in the proportion of 25 to 100%.

And at 50 to 75%, the goat manure was inferior to the root dry mass accumulation. In Figure 2C it is observed that only the humus showed a linear correlation to the root dry mass, due to increasing proportions of the source. These results are similar to those observed by Goés *et al.* (2011), who observed that the production of tamarind seedlings was favored with the increasing of earthworm humus proportion in the substrate. The chicken manure promoted greater root dry mass, especially for the proportion of 75%, with an increase of 72% when compared to the proportion of 0%; and the goat manure showed the worst results. For *paú de buriti*, the best response was obtained at 50% of proportion proportion of the formulated substrate. In general the substrates made from *paú de buriti* and poultry manure were the ones who promoted better early development of neem seedlings with greater root and shoot growth.

Conclusion

The use of substrates made from organic sources as *Paú Buriti*, earthworm humus, chicken and goat manure are viable alternatives for the production of neem seedlings. The early development of the seedlings was better on the substrates with decomposed stem of the moriche palm [*paú de buriti*] and

poultry manure, especially in the proportions of 50 and 75% of mixed source with soil.

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