

Custard apple production (*Annona squamosa* L.) in the Brazilian Northeast occurs throughout the year. Its management involves pruning, irrigation, and proper nutrition. The objective of the present work was to verify the influence of different doses of nitrogen and the use of humic substances on soil chemical attributes and tree custard apple nutrition. The experiment was conducted in a commercial orchard in the municipality of Anagé, Southwest region of the state of Bahia, in a randomized block design in a 4 x 2 factorial scheme, four nitrogen doses (0, 284, 568 and 852 g per plant, urea) with and without the application of humic substances (Ks100), with 4 replicates. Soil chemical characteristics, macro and micronutrient contents were evaluated in the leaves of the custard apple tree. Nitrogen rates reduced soil pH and the availability of calcium, magnesium and boron. The application of humic substances increased the content of potassium in leaves. Under the conditions studied, high nitrogen doses influence soil fertility and the application of humic substances does not improve the absorption of most of the nutrients, except potassium, and it did not influence the chemical attributes of the soil.

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Keywords: Annona squamosa, fertility, anonaceous, urea, fertilization.

# 15 1. INTRODUCTION

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Bahia is the largest producer of Custard apple (Annona squamosa L.) in Brazil, with an estimated area of 3575 ha in 2012, followed by Alagoas, Pernambuco, São Paulo and Ceará [1]. The socioeconomic importance of the crop has been increasing in recent years, especially in the semi-arid region, due to the hot climate, producing fruits with good quality throughout the year [2]. However, Custard apple development is reduced in the fall/winter period. According to [3], it is essential to increase the availability of nutrients in the soil through fertilization to meet the physiological requirements for obtaining good growth and development of the custard apple plant.

Researches with different doses of nutrients under field conditions, for the anonaceae, is an important strategy to optimize the production of this crop [4]. There is little information on the nutritional requirement of the custard apple plant [5]. According to [6], the plant extracts large amounts of nutrients from the soil. Since nitrogen is the most required nutrient, nitrogen fertilization increases the vegetative and reproductive stimulus of custard apple plant [3]. Working with doses of nitrogen and potassium in different production seasons, [7] observed linear increase in leaves nitrogen contents with the increase of nitrogen fertilization in the prunings performed in the fall/winter period. However, high doses of nitrogen can cause acidification in the soil and influence nutrient availability and plant nutrition [8, 9].

Another alternative is the use of humic substances, which provides an improvement in nutrient absorption [10]. According to [11], the use of humic substances improves the absorption of the applied fertilizer and increases the nutrient content in custard apple leaves. In addition, it favors plant photosynthesis [6]. However, the effectiveness of humic substances is influenced by the studied crop, soil conditions, dose, source, and form of application [12, 13, 14]. It may act directly on the growth of the plant or on improving the conditions for the development of the crop [10]. There is little information in the literature on custard apple nutrition, on the effect of humic substances and ideal nitrogen doses in autumn/winter pruning. According To [3], the custard apple producers modify the nitrogen/potassium ratio in the fertilization as a function of the time of the year, from 2 to 4 parts of nitrogen to 1 of potassium in autumn/winter pruning. Therefore, the objective of this work was to verify the influence of different doses of nitrogen and the application of humic substances on soil chemical attributes and custard apple plant nutrition.

# 41 2. MATERIAL AND METHODS

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# 43 2.1. Site of study

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The experiment was conducted from May 10th to October 24th, 2017, in a commercial custard apple orchard at Rancho Alegre farm, in the municipality of Anagé, Southwest region of the State of Bahia, located at 14°26' south latitude and 41°04' Longitude West of Greenwich, with 335 m of altitude, in which prevails the semi-arid climate, classified as Bwsh according to Köppen, with average precipitation of 656 mm year-1, average annual temperature of 22.3 °C, minimum of 19.0 °C and maximum of 29.0 °C.

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# 51 2.2. Experimental design

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The experimental design was a randomized complete block design with 4 x 2 factorial design, with 4 N doses (0, 284, 568 and 852 g of N per plant, in the form of urea), with and without the application of humic substances, with 4 replicates. The plots were composed of 4 plants, the first and the last plant were considered as borders.

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# 57 **2.3. Plant and Area Characteristics**

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The custard apple plants of the experiment were 20 years old, originated from seed propagation, planted in 7 x 4 m spacing. They had a mean height of 3.65 m, crown diameter of 4.42 m and trunk diameter of 0.14 m, at 20 cm height from the soil. The irrigation used is of the micro sprinkler type, with flow rate of 30 liters hour-1, a daily irrigation shift of 3 hours, one sprinkler being used for each two plants. The soil analysis presented in Table 1 followed the methodology of [15], the soil texture is sandy clay loam.

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# Table 1. Soil chemical analysis of the area planted with custard apple, Anagé-BA, 2017

Depth	рН	P	K⁺	Ca⁺²	Mg⁺²	Al <sup>+3</sup>	H⁺	Na⁺	V	O.M.
	(H <sub>2</sub> O)	mg dm⁻³	cmol <sub>c</sub> dm <sup>-3</sup> %							
0-20cm	7.1	35	0.54	4.4	3.5	0	1.1	0.13	89	1.3
20-40 cm	7.0	14	0.53	4.2	3.2	0	1.2	0.11	87	0.8

66 \* Mehlich 1.

The climatic characteristics of the experimental period are presented in Figure 1, according to data from INPE meteorological station 32494.



Figure 1. Monthly precipitation, mean maximum and minimum temperatures from May to October, Anagé-BA, 2017

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In order to characterize the nutritional status of the custard apple plants, the fifth and sixth leaves of the middle third were collected in 20 plants randomly prior to the implantation of the experiment [16]. Data of the initial leaf nutritional characteristics of the custard apple plants are presented in Table 2, and analysis was performed according to the methodology described by [15].

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Ν	Р	K	Ca	Mg	S	В	Cu	Fe	Mn	Zn
			g kg <sup>-1</sup>					mg kg⁻¹		
32.7	2.27	14.6	11.49	4.16	2.13	63.97	17.87	160.04	65.65	10.85

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Pruning was done with scissors, in order to standardize the branches, leaving them with approximately 0.2 m, the manual
defoliation of these branches was also performed. After pruning, the plants were fertilized with 50 g of FTE BR 12 and 200
g of TOPMIX (52% of P2O5 and 11% of N), applied at 0.2 m depth in crown projection. 350 g of potassium chloride (58%
of K2O) was applied, in 7 doses every 15 days, starting 45 days after pruning.

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# 86 3.4. Treatments

The application of the humic substance was performed after pruning, applying 10 g per plant of commercial product Ks (00 (Omnia Brazil), distributed in the projection of the crown. The physico-chemical characteristics of this product, analyzed according to the methodology described by [17], are: N = 0.45%, P2O5 = (total) 0.08%, P2O5 (2% citric acid) = 0.02 %, K2O (H2O) = 2.00%, Ca = 0.26%, Mg = 0.30%, S = 0.12%, Fe = 0.26%, CO = 4.75%, pH = 9 , 65, moisture = 11.10%, density = 0.82 M / V, Si = 3.85%, AI = 1.85%, fulvic acid = 2.34% and humic acid = 3.65%. The different nitrogen doses were applied every 15 days from the pruning operation, using urea as the source of N (46% of N), applied under the projection of the crown.

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## 96 3.5. Variables studied and statistical analysis

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98 The nutritional status of the plants was evaluated collecting leaves following the methodology proposed by [16], at 156 99 days after pruning. The leaves were packed in paper bags and washed in water, rinsed with distilled water and dried in an 90 oven with forced air circulation at 65 °C until constant mass was obtained.

Soil samples were also collected in the crown projection in the 0-0.20 m layer, and the samples were dried in shade. After that, the samples were sent to the laboratory where the chemical analyzes of macro and micronutrients were carried out according to the methodology of [15].

After testing for normality and homogeneity the analysis of variance was performed using SISVAR Software [18], and quantitative data were submitted to regression analysis.

### 106 **3. RESULTS**

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#### 108 **3.1. Soil chemistry**

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The levels of remaining P, K, H + AI (Table 3), base saturation and soil organic matter at 156 days after pruning, when it was observed that they were not influenced by the applied treatments. According to the classes proposed by [19], the values of potassium, base saturation and soil organic matter content are very good, medium and low, respectively.

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	P(rem)	K <sup>+</sup>	H+AI	V	O.M.
	mg dm <sup>-3</sup>	cmol <sub>c</sub> dm <sup>-3</sup>	mmol <sub>c</sub> dm <sup>-3</sup>	%	g dm <sup>-3</sup>
With HS	54.45 <sup>ns</sup>	0.64 <sup>ns</sup>	41.18 <sup>ns</sup>	46.63 <sup>ns</sup>	16.47 <sup>ns</sup>
Without HS	57.38	0.53	29.43	54.73	13.42
Mean	55.92	0.58	35.31	52.20	14.95
C.V. (%)	8.97	37.19	63.70	33.74	33.29

Table 3. Mean values of remaining phosphorus, potassium, hydrogen + aluminum, base saturation and organic matter

after to the application of humic substances (HS), general mean and coefficient of variation (C.V.) in soil cultivated with

117 <sup>ns</sup> non-significant by F test (P=0.05)

custard apple, Anagé-BA, 2017

119 Micronutrients (Table 4) were not influenced by the application of humic substances and there was no significant 120 regression for the nitrogen doses. According to the classes proposed by [19], the levels found in the present experiment 121 for copper, manganese and zinc are high and iron is low.

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### Table 4. Average micronutrient content as a function of the application of humic substances (HS), general mean and coefficient of variation (C.V.) of soil cultivated with custard apple, Anagé-BA, 2017

	Cu	Fe	Mn	Zn	
	-	mg	) dm <sup>-3</sup>	111	
With HS	2.85 <sup>ns</sup>	14.83 <sup>ns</sup>	14.98 <sup>ns</sup>	10.99 <sup>ns</sup>	
Without HS	2.68	12.83	12.24	11.44	
Mean	2.77	13.84	13.60	11.21	
C.V. (%)	40.70	38.76	43.54	57.78	

# 125 <sup>ns</sup> non-significant by F test (*P=0.05*)

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The increase of the nitrogen doses caused reduction of 1.21 in soil pH, in the calcium content of 15.18 mmol<sub>c</sub> dm<sup>-3</sup> and 9.71 mmol<sub>c</sub> dm<sup>-3</sup> in the magnesium content (Figure 2 (A, C and D)). corroborating with the results found by [8] working with doses of urea in dwarf coconut. This variation in pH caused the availability of aluminum to increase 4.48 mmolc dm<sup>-3</sup> as a function of N rates (Figure 1B). This same behavior was observed by [9] working with doses and sources of nitrogen in soil cultivated with mombaça grass.

The phosphorus content increased by  $3.21 \text{ mmol}_{c} \text{ dm}^{-3}$  as a function of the growth of the nitrogen doses (Figure 2E). Different behavior from that observed by [9], where the levels of phosphorus reduced with increasing doses of nitrogen. Boron levels reduced by 0.06 with the nitrogen rates.



Figure 2. PH values (CaCl<sub>2</sub>, A) and aluminum (B), magnesium (C), calcium (D), phosphorus (E) and boron (F) values of soil cultivated with custard apple as a function of nitrogen doses, Anagé-BA, 2017. \* Significant (*P*= 0.05).

#### 140 3.2. Nutrition of the plants

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The application of humic substances increased the potassium contents and did not influence the contents of the other 142 nutrients in the custard apple leaves (Table 5). This behavior differs from that observed by [11], working with custard 143 144 apple, which observed an increase in nitrogen contents with addition of humic substances. Working with pineapple seedlings, [20] observed increases in potassium content in their leaves with the use of humic substances. The levels of N 145 are in the ideal range according to [3], since P, K and Mg are above and S and B are below. Studying nitrogen rates and 146 application of humic substance [11] obtained lower levels of N, P, K, Mg and S. Working with organic fertilizer [21] found 147 similar levels of N, Mg and S, lower than P, K and higher Ca. The boron and manganese contents are below the average 148 levels shown by [22]. Iron and copper contents are close to those found by the same authors. The contents of B, Cu and 149 Zn are close to those found by [5] and below those found by [21]. The same authors found smaller Fe and Mn contents. 150

	Ν	Р	К	Mg	S	В	Cu	Fe	Mn
		g kg <sup>-1</sup> mg kg <sup>-1</sup>							
With HS	30.6 <sup>ns</sup>	1.98 <sup>ns</sup>	14.65	3.53 <sup>ns</sup>	2.21 <sup>ns</sup>	38.48 <sup>ns</sup>	6.26 <sup>ns</sup>	145.68 <sup>ns</sup>	62.65 <sup>ns</sup>
Without HS	31.29	2.04	16.71	3.62	2.24	43.35	6.48	146.13	65.03
Mean	30.97	2.01	15.18	3.58	2.23	36.18	6.37	145.91	63.84
C.V. (%)	4.48	10.58	9.25	12.74	9.28	42.55	7.28	9.87	18.72

Table 5. Average leaf nutrient content due to the use of humic substances (HS), general averages and coefficient of variation (C.V.) the custard apple, Anagé-BA, 2017

154 <sup>\*</sup>, <sup>ns</sup> significant and non-significant by F test (*P=0.05*)

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Leaf calcium levels (Figure 3A) showed tendency to increase with increasing nitrogen doses, increasing 1.98 g kg<sup>-1</sup> and zinc contents (Figure 3B) had reduction of 1.08 mg kg<sup>-1</sup> depending on the nitrogen doses. The levels of zinc and calcium

are below ideal according to [3, 22]. Calcium levels are close to that observed by [5, 11].

13,5 A В 10,4841\*+0,0026\*N R<sup>2</sup>=0,97 ŷ=15.3317\*-0.0035\*N R<sup>2</sup>=0.6813 13,0 12.5 15 Calcium (g kg 12,0 Zinc (mg kg 1 14 11,5 11.0 13 10,5 10,0 12 852 284 568 200 400 600 800 Nitrogen doses (g plant<sup>1</sup>) Nitrogen doses (g plant<sup>1</sup>)

- Figure 3. Calcium (A) and zinc (B) content in custard apple leaf as a function of nitrogen doses, Anagé-BA, 2017. \* Significant (*P=0.05*).
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- 163 4. DISCUSSION
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- 165 4.1. Soil Chemistry
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Soil acidification was probably caused by nitrification reaction from ammonium to nitrate. The urea initially has an alkaline reaction in the soil, later the nitrification performed by microorganisms of the genus Nitrosomonas and Nitrobacter cause acidification in the soil [23, 24]. Therefore, it can be affirmed that the application of nitrogen fertilizers acidifies the soil [25].

The reduction in Ca and Mg contents occurred due to changes in availability caused by pH. According to [26], this can occur due to the leaching loss of cations ( $Ca^{2+}$  and  $Mg^{2+}$ ), being replaced in the soil cation exchange complex by aluminum.

The phosphorus content increased with the nitrogen doses probably due to the favoring of the decomposition of the pruning remains. The degradation of straw is favored by the application of nitrogen [27], because the microorganisms involved require nitrogen for decomposition.

The reduction in soil boron availability with increased nitrogen rates is probably associated with pH variation. By studying the variation in boron adsorption as a function of pH, [28] found that low and very high values favor adsorption, reducing availability. In this work, the pH at the highest nitrogen dose reached 4.5, being within the favorable range of adsorption. In addition, [29] reports that in acidic pH, organic matter is the main source of boron, with the increase associated with the same behavior described for phosphorus.

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## 182 **4.2. Nutrition of the plants**

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The increase in potassium leaf content with the use of humic substances is associated with increased absorption. The application of humic substances increases the electrochemical gradient between the roots and the soil [30] and acts on the expression of the genes encoding H + ATPases proteins and their activation [13]. The same authors report that the action of the humic substances are directly related to pH, this shows that the lack of results in the other nutrients may be associated with this.

The low calcium contents occurred due to the high levels of potassium and magnesium in the soil, which compete for the same absorption sites [29], which is justified by the high levels of foliar potassium shown above. The increase in foliar calcium content with nitrogen doses is associated with reduced competition with magnesium contents, which reduced the effect of pH.

Applications of nitrogen doses led to an imbalance in the nutrition of the plants, where boron, zinc, manganese, and sulfur contents are limiting their development. Sulfur and manganese are directly linked to the nitrogen assimilation reduction process [29].

Therefore, other studies should be carried out, varying the sources of nitrogen, in order to avoid the effect of acidification of the soil, and to verify if the use of high nitrogen doses in the crop / crop cycle conducted in the autumn / winter is justified. Improving also fertilization with micronutrients and with sulfur.

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### 200 **5. CONCLUSION**

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Under the conditions in which the present work was conducted, we concluded that high nitrogen levels cause acidification in the soil, reducing the availability of calcium, magnesium and boron and increasing the levels of aluminum and phosphorus. The application of humic substance in soil cultivated with custard apple does not affect the absorption of most of nutrients with the exception of potassium, causing an increase, and it does not influence the chemical attributes of the soil.

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