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Impact of humic substances and nitrogen fertilising on the fruit quality and yield of custard apple

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ABSTRACT. The custard apple (*Annona squamosa* L.), also known as the sugar apple, is a fruit species native to Brazil that has been poorly studied, especially in relation to the effect of humic substances on its fruit quality and yield. An experiment was conducted from December 2010 to November 2011 to evaluate the fruit quality and yield of the custard apple as a function of nitrogen fertilising and the use of humic substances. The experimental design consisted of randomised blocks, with treatments distributed in a factorial arrangement (4 x 2), using four nitrogen doses (0, 100, 175 and 250 g of N plant^{-1}) and two humic substance applications (with and without humic substances), with four replications. The fruit yield and fruit characteristics, such as fruit mass, titratable acidity (TA), soluble solids (SS), pulp pH and SS/TA ratio, were recorded. The humic substances and the nitrogen levels significantly affected the soluble solids, titratable acidity and SS/TA ratio, while the pH pulp was only influenced by the humic substances. The humic substances promoted a quantitative increase in the fruit yield of 0.63 ton ha^{-1}. The fruit quality and yield of the custard apple depend on the nitrogen fertiliser and the interaction of the humic substances. Nitrogen fertilising of 100 g per plant, associated with humic substances, could be recommended for use in the production of custard apples.

Keywords: *Annona squamosa* L., fruit yield, humic acids.

Impacto de substâncias húmicas e adubação nitrogenada na qualidade de frutos e produtividade da ateira

RESUMO. A ateira (*Annona squamosa* L.), também conhecida como pinheira, é uma planta nativa do Brasil pouco estudada, especialmente em relação às substâncias húimicas e seus efeitos na qualidade de frutos e produtividade Nesse sentido, um experimento foi realizado de Dezembro de 2010 a Novembro de 2011 com o objetivo de avaliar a qualidade dos frutos, a produção e a produtividade da ateira em função da adubação nitrogenada e substancias húmicas. Adotou-se delineamento experimental em blocos casualizados com tratamentos distribuídos em esquema fatorial 4 x 2 referentes respectivamente, às doses de N (0, 100, 175 e 250 g de N planta^{-1}), e aplicação de substâncias húmicas (com e sem). Ureia foi usada como fonte de nitrogenio (45% de N) com quatro aplicações mensais de janeiro a abril de 2011. Foram avaliadas ao final do experimento a massa dos frutos, acidez titulável (AT), sólidos solúveis (SS), pH da polpa e ratio SS/AT. Ao final da colheita determinaram-se ainda a produção por planta (kg planta^{-1}) e a produtividade (t ha^{-1}). Produtividade e qualidade de frutos da ateira dependem da interação entre adubação nitrogenada e substâncias húmicas. A adubação nitrogenada com 100 g de N planta^{-1}, consorciada com substâncias húmicas pode ser recomendada para produção de ateira.

Palavras-chave: *Annona squamosa* L., produtividade, ácidos húmicos.

Introduction

The custard apple (*Annona squamosa* L.), also called the sugar apple, is a small, semi-deciduous tree that is 3-7 m in height and has a broad, open crown or irregularly spreading branches. It is commercially grown, mainly in Brazil, India and Taiwan (PAULL; DUARTE, 2011). This plant requires adequate soil moisture during the growing season, and to achieve higher fruit yields, the soil must be fertilised generously, especially with nitrogen, which is the nutrient most required by the custard apple (PLEGUEZUELO et al., 2011; CAVALCANTE et al., 2012a).

Nitrogen uptake by plant roots is directly affected by soil, plant and environmental factors, which include humic substances (FINZI et al., 2007). Humic substances act on the mechanisms

involved in cell respiration, photosynthesis, protein synthesis, water and nutrient uptake, enzyme activities (NARDI et al., 2000; TAHIR et al., 2011) and hormones (TREVISAN et al., 2010), thus stimulating plant growth. Humic substances, when applied directly onto the soil or substrate, have been used for plant production because these substances improve soil fertility and nutrient availability, according to Eyheraguibel et al. (2008).

Studies on the use of humic substances on fruit species are rarely reported in the literature; however, some investigations have been conducted with papaya (CAVALCANTE et al., 2011b), grape (FERRARA; BRUNETTI, 2008) and watermelon (SALMAN et al., 2005), with promising results. In addition, studies on fertilising yellow passion fruit with products containing humic substances developed under field conditions can be found in the scientific literature, including studies by Cavalcante et al. (2008) and Cavalcante et al. (2012b). However, the effect of humic substances on custard apple production, especially in combination with nitrogen fertilising, has been poorly quantified.

Hence, the present study aimed to evaluate the fruit quality and yield of the custard apple as a function of nitrogen fertilising and the use of humic substances in Brazil.

Material and methods

Plant material and growth conditions

Custard apple (Annona squamosa L.) plants that were five years old and propagated by seeds were used in this study.

The study was conducted from December 2010 to May 2011 (first trial) and from June 2011 to November 2011 (second trial) at "Campus Profa. Cinobelina Elvas," Federal University of Piaui, Piaui State, Brazil (Northeastern Brazil).

The physical and chemical characteristics of the soil developed for the experiment are shown in Table 1. The climatic data regarding air temperature and air humidity (thermo-hygrometer Instrutemp®, Brazil) collected during the experiments are shown in Figure 1.

The plants, spaced with 4 m between the rows and 3 m between the plants, were daily drip-irrigated with one self-regulating emitter at every 0.5 m, for a flow of 2.8 L h^{-1}. Pruning was performed following the instructions of Paull and Duarte (2011).

Table 1. Chemical and physical characteristics of the soil (0-20 cm soil depth) where the experiment was carried out.

Soil characterisitc	Value
pH (in water)	4.2
	——— $cmol_c\ dm^{-3}$ ———
Ca^{2+}	1.1
Mg^{2+}	0.4
$Ca^{2+} + Mg^{2+}$	1.5
Al^{3+}	0.5
$H^{+} + Al^{3+}$	3.4
CEC	5.0
P (mg dm^{-3})	5
K (mEq $100cm^{-3}$)	0.13
K (mg dm^{-3})	52
	——— % ———
Organic Matter	1.6
N	0.1
C organic	1.55
Al^{3+} saturation	25
Basis saturation	32
	——— % ———
Clay	39
Silt	10
Sand	51

P, K: Melich 1; H + Al: calcium acetate (extractor) 0.5M, pH 7; Al, Ca, Mg: KCl 1 M extractor; CEC: cationic exchangeable capacity.

The plot was limed with 1,500 kg ha^{-1} or 1.80 kg $plant^{-1}$ 60 days before the beginning of the experiment, and all of the plants were fertilised with 120 g of K_2O (potassium chloride, 60% K_2O) at 30, 60 and 90 days after pruning and with 120 g of P2O5 (triple superphosphate, 42% P_2O_5) at 30 days after pruning, according to the instructions by Anonymous (1993).

The nitrogen source used was urea (45% N) and was applied monthly (four fertilisings), according to the instructions of Anonymous (1993). The humic substances used in the experiment were extracted from leonardite, and the source adopted was Humitec®, whose complete composition included humic extract (16.5%), organic carbon (11.2%), humic acids (13.2%) and fulvic acids (3.3%). The humic substances were applied following the recommendations of the producer, i.e., 30 mL diluted in 3 L of water applied every 60 days after pruning, totalling two applications for each experiment.

Treatments and experimental design

The experimental design consisted of randomised blocks with treatments distributed in a factorial arrangement (4 x 2) of four nitrogen doses (0, 100, 175 and 250 g of N $plant^{-1}$) and two humic substances application (with and without humic substances), with four replications.

Variables Recorded and Statistical Analysis

During the fruit harvest time, i.e., April-May 2011 and October-November 2011, 10 fruits per parcel were manually harvested when their carpels were separated and the intercarpel tissue was yellowish green.

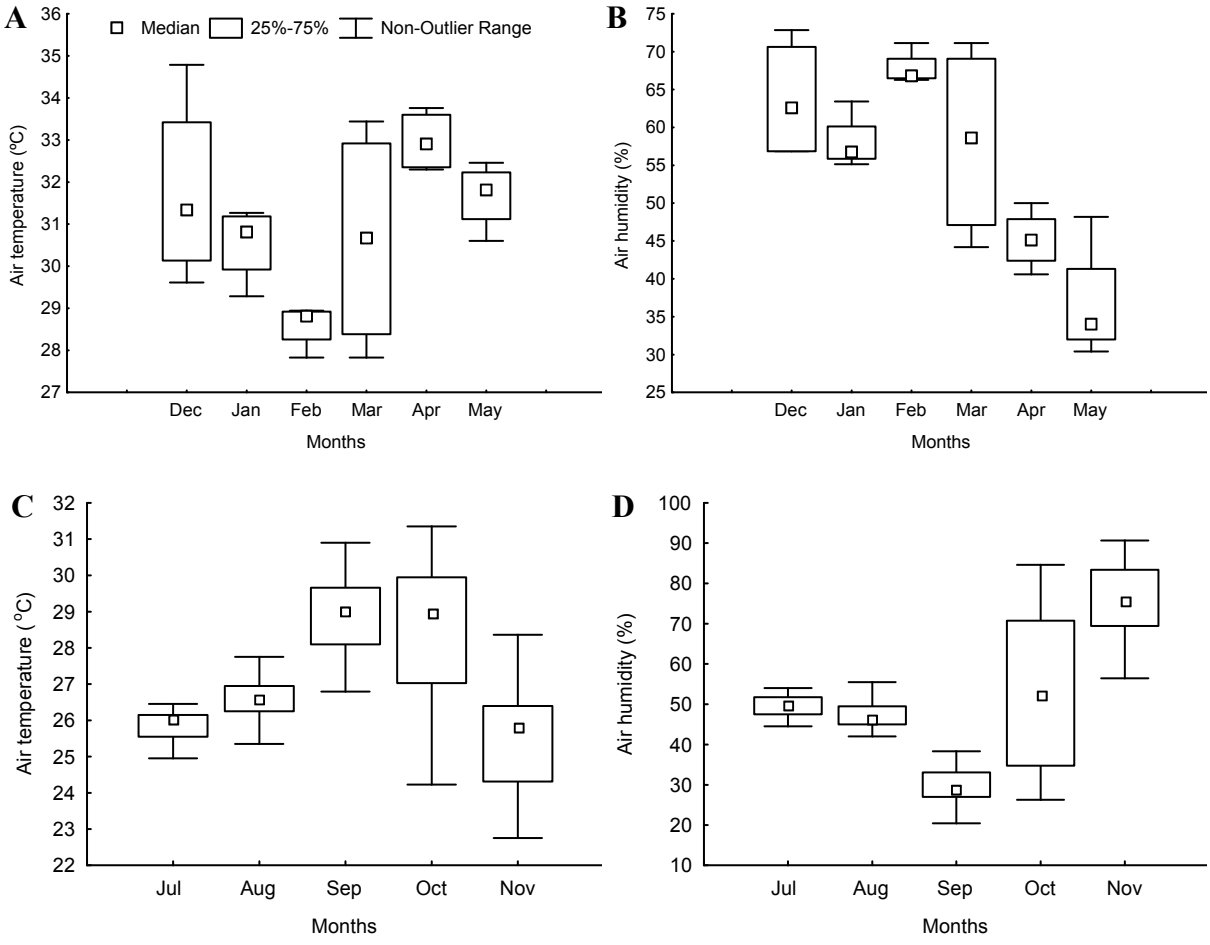

Figure 1. Average temperature and air humidity during the execution of the experiments. (A and B – first trial; C and D – second trial

The fruits were placed in paper bags and taken to the Food Laboratory at the Federal University of Piaui, Bom Jesus, Brazil. This parameter for fruit selection was recommended by Kavati and Piza Junior (1997) for commercial farms

The fruit analyses of the custard apple fruits included the usual parameters: i) the fruit mass was measured using a Sartorious® (Göttingen, Germany) brand precision balance (0.01 g precision) and expressed in g; ii) for the titratable acidity (TA), 20 g of macerated fruit pulp was taken from yellow passion fruits and brought to a final volume of 100 mL by adding distilled water. A 20-mL sample was taken from the mixture, and three to four drops of phthalein were used as an indicator. This suspension was titrated with 0.1 N sodium hydroxide (NaOH). The results were expressed as a percentage; iii) the soluble solids (SS), expressed as °Brix, were measured using an Abbe® refractometer (Bausch and Lomb, Rochester, NY, USA); iv) the pulp pH was measured using a Marconi® pH meter; v) after chemical analyses, the relation between the soluble solids and the titratable acidity (SS/TA ratio) was calculated; and vi) the fruit yield were measured as ton ha^{-1}.

Statistical analyses included analysis of variance (ANOVA), a mean separation of humic substances using the Tukey test and regression analysis of the nitrogen doses using the combined data of two consecutive trials. All calculations were performed using the Sigmaplot software, and the terms were considered significant at $p \leq 0.01$.

Results and discussion

As observed in Table 2, the humic nitrogen levels significantly affected the soluble solids (SS), titratable acidity (TA) and SS/TA ratio, while the pH pulp was only influenced by the humic substances. In addition, significant interactions between the N levels and humic substances were registered for all the fruit variables recorded in the study.

Independent of the humic substance use (Figure 2A and B), the pulp pH of the custard apple fruits

increased with increasing nitrogen levels until it reached 100 g per plant, and this increase was immediately followed by decay. Cavalcante et al. (2012c) reported that fruit pH is a characteristic used to evaluate the acid components of fruits and their shelf life, reporting that less acid fruits are more often recommended for fresh consumption, while acid fruits are more appropriate for the food industry. It is important to note that the use of humic substances reduced the pulp pH by nearly 1.94%. This is a relevant fact for this fruit species because most custard apple fruits are consumed as fresh fruit.

Table 2. Fruit characteristics [mass, pulp pH, soluble solids (SS), tritatable acidity (TA), "SS/TA ratio" and yield] of custard apple fruits as a function of humic substances and nitrogen. fertilizing.

	pH	SS	TA	SS/TA ratio	Fruit mass	Fruit yield
		ºBrix	%		g	ton ha^{-1}
N levels ("F" value)	2.12^{ns}	5.93**	4.94**	3.23**	1.250^{ns}	1.69^{ns}
HS ("F" value)	6.04**	1.82^{ns}	4.17^{ns}	8.61**	0.487^{ns}	0.69^{ns}
Without HS	5.35 a	34.87 a	4.73 a	7.60 a	173.487 a	6.98 a
With HS	5.25 b	33.87 a	5.10 a	6.79 b	167.412 a	7.61 a
Interaction (N x HSs)	14.01**	2.29**	8.78**	5.66**	0.647^{ns}	0.35**
V.C.	2.25	6.10	10.20	10.87	14.44	28.98

VC = variation coefficient; **significant at $p \leq 0.01$ probability error; ns: non significant; HS = humic substances. Data followed by different letters in columns are significantly different according to Tukey test ($p \leq 0.01$).

The effects of the nitrogen fertilising levels on the soluble solids of the custard apple fruits were significantly impacted by the use of humic substances. In the plants that received the humic substances, the soluble solids of the fruits increased by approximately 2.8% after using 0 to 100 g of N per plant and decreased by 6.25% after using 100 to 250 g of N per plant (Figure 2C). Conversely, the plants that were not treated with humic substances presented an exponential decay with the increasing nitrogen levels (Figure 2D). The reduction of fruit soluble solids as a function of an increasing nitrogen level was previously reported by Silva et al. (2002), and this reduction could be explained by the positive and direct effect of humic substances on the root absorption of ammonium nitrate (KEELING et al., 2003).

Independent of the treatment, the averages presented in Figures 2C and D are much higher than the 25.5-27.5 ºBrix range reported by Pereira et al. (2003), the 19.7-21.1 ºBrix range registered by Silva et al. (2007) and the 15.3-22.8 ºBrix range found by Cavalcante et al. (2011a) in a study of fruit quality as a parameter for the selection of custard apple genotypes in Brazil.

As observed in Figure 2E, the increase in nitrogen levels promoted a significant decrease in the fruit titratable acidity of 19.61% from the minimum (0 g of N per plant) to the maximum (250 g of N per plant) nitrogen levels. When comparing the titratable acidity results of the present study with those registered in the scientific literature, it is possible to infer that the 0.59% average recorded here is higher than the 0.22-0.25% range reported by Dias et al. (2003), who examined the effect of pruning on fruit quality, and higher than the 0.15% recorded by Bolívar-Fernández et al. (2009), who compared different custard apple genotypes. Accordingly, Cavalcante et al. (2012c) argued that high titratable acidity is important for fruit processing because this high acidity reduces the necessity of adding artificial acid components, although this is not a limiting factor in genotype selection when other fruit quality parameters are satisfactory. Low titratable acidity is relevant to consumption as a fresh fruit.

Without the use of humic substances, the SS/TA ratio followed the same tendency of the soluble solids (Figure 2F), i.e., a higher average value registered after using less than 100 g of N per plant, while the use of humic substances produced a sequential increase of this variable with an increase in nitrogen levels (Figure 2G), showing that the soluble solids were more influential on the SS/TA ratio and titritable acidity. Additionally, all of the averages are lower than those reported by Silva et al. (2007) and Cavalcante et al. (2011a).

Regarded individually, the soluble solids and titratable acidity characteristics can represent a false indication of fruit flavour, while the soluble solids/titratable acidity ratio is considered a practical form, mainly for consumption as fresh fruit (CAVALCANTE et al., 2007).

The average mass of the individual fruits was not affected by either the nitrogen levels or the humic substances (Table 2). The fruits produced in this study are heavier, on average, than those recorded by Cavalcante et al. (2011a) but lighter than the 274 g obtained by Costa et al. (2002) and the 220 g recorded by Girwani et al. (2011) for the custard apple hybrid-4 ('1/6 British Guinea' × 'Atemoya') in India. In addition, the higher average value of the present study is comparable to the maximum one registered by Kumar et al. (2011) in India.

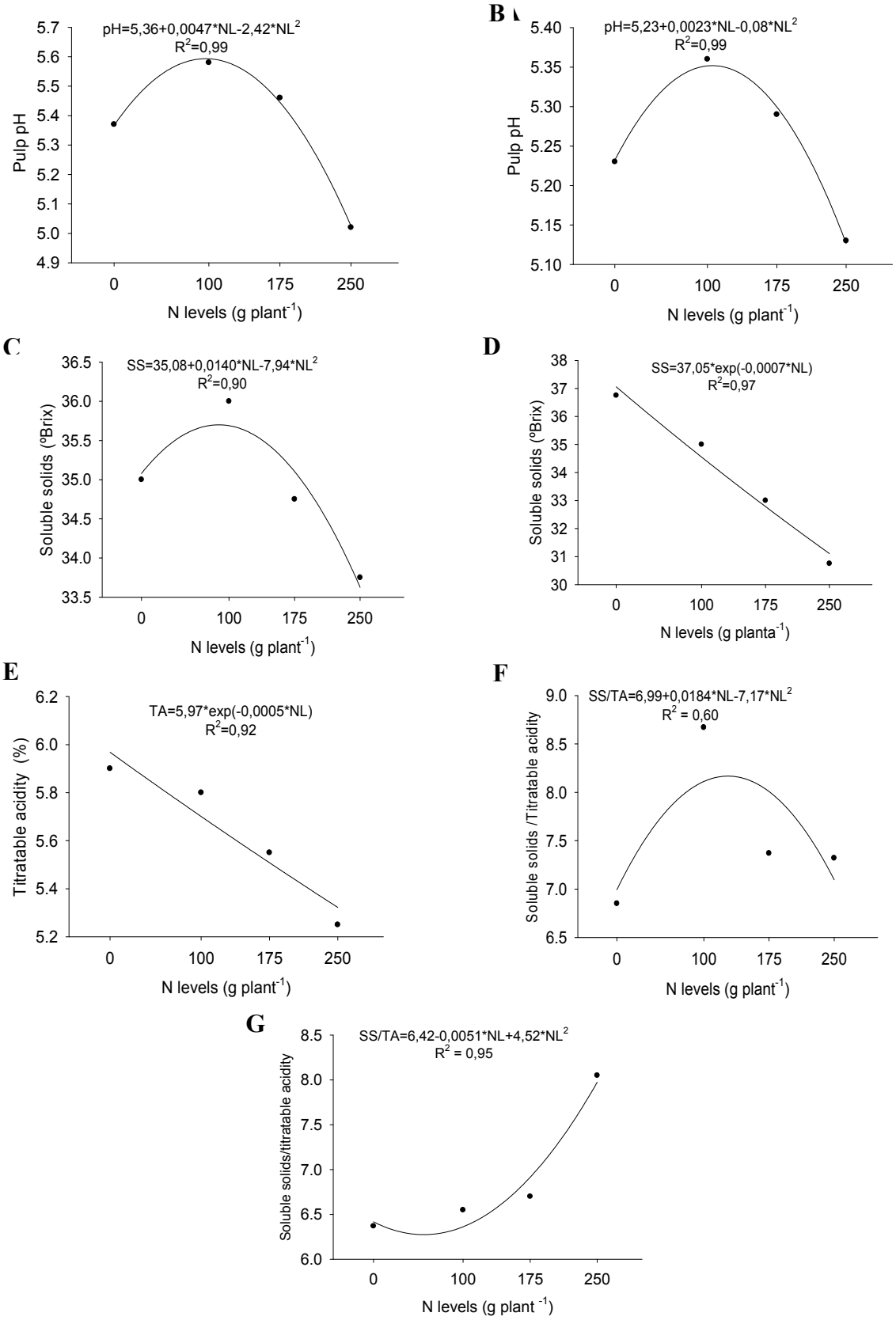

Figure 2. Pulp pH (A e B), soluble solids (C e D), titratable acidity (E) and SS/TA "Ratio" SS/AT (F e G) of custard apple fruits as a function nitrogen levels and humic substances. [A, C and F: without humic substances; B, D and G: with humic substances].

The fruit yields of the custard apple were similar for the plants with and without humic substances, as observed in a quadratic adjustment with a minimum fit of 0.80 with humic substances and 0.85 without humic substances and at a peak at 100 g of N per plant (Figures 3A and B). Accordingly, from 0 to 100 g of N per plant, an increase in fruit yield of 10.00 and 5.89%, respectively, was registered for the plants without and with humic substances. This increase could be explained by the good soil fertility conditions before the experiment (Table 1) and may be associated with the beneficial effects of humic substances on the soil and plant, as previously described by Tahir et al. (2011). In addition, among all nutrients, nitrogen presents fundamental importance because it forms part of amino acids, proteins and nucleic acids and because it directly or indirectly acts on many biochemical plant processes (SHI et al., 2012). However, its association with humic substances and its physiological consequences for custard apple plants has been poorly studied.

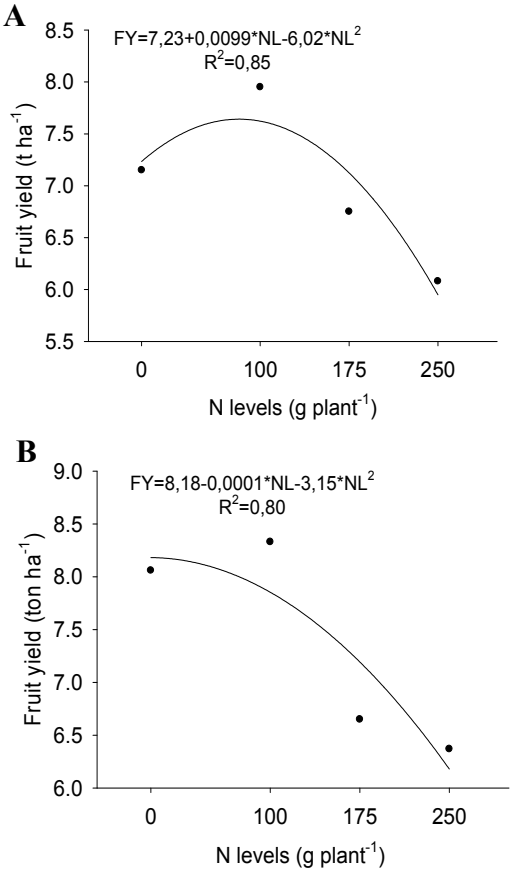

Figure 3. Fruit yield of custard apple fruits as a function nitrogen levels and humic substances. [A: without humic substances; B: with humic substances.

Despite the lack of significant statistical effects (Table 2), the plants that received humic substances presented a quantitative difference in fruit yield of 0.63 ton ha^{-1} in relation to those that did not receive this product. That yield is considered a high income for fruit growers in Brazil.

Independent of the humic substance use, the average yield values quoted in the present study are higher than the average values quoted in the scientific literature, such as 3.2 ton ha^{-1} (KAVATI; PIZA JR, 1997) and 6.10 ton ha^{-1} (COSTA et al., 2002) in Brazil and 5.0 ton ha^{-1} (PATEL et al., 2010) in India.

In general, the average values registered in the study are higher than those verified by Kumar et al. (2011), who recommend a substantially higher amount of fertiliser. The previous authors indicated that for high custard apple yields, it is necessary to use 400 g of N per plant, a level not evaluated in the present study.

Conclusion

Thus, the results of this study indicate that i) the fruit quality and yield of the custard apple depend on the interaction of nitrogen fertilising and humic substances; and ii) under the soil, climate and plant conditions of this study, 100 g of N per plant of cover fertilising, associated with humic substances, could be recommended for the production of custard apples.

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