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Effect of humic substances and nitrogen fertilization on yellow passion fruit cultivation in the Brazilian semiarid region

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Fruit production of yellow passion fruit (*Passiflora edulis* Sims. f. *flavicarpa* Deg.) is affected by several factors, such as climate, soil and agricultural practices, including fertilization and irrigation, which are essential for high crop yield. Thus, an experiment was carried on to evaluate the effect of humic substances and different nitrogen doses supplied through fertigation on fruit production of yellow passion fruit in Brazilian semiarid. The experiment was carried on from December 2012 to December 2013 on Curaçá irrigation area, Juazeiro County, Brazil. The experimental design was randomized complete block in a split plot arrangement, considering humic substances (absence and presence) as main plots and N doses (180, 200, 260, 330 and 350 kg ha^{-1} yr^{-1} of N) as subplots with five replications and six plants in each parcel. The interaction of nitrogen doses and humic substances affected foliar nitrogen concentrations, stem diameter, production per plant and yield. No isolated effect of humic substances on any variable studied was significant. The higher yellow passion fruit yields are recorded at 290 and 350 kg ha^{-1} N doses without and with humic substances, respectively.

Key words: Passionflower, plant nutrition, organic acids.

INTRODUCTION

Brazil is the center of origin for yellow passion fruit (*Passiflora edulis Sims* f. *flavicarpa* Deg.), where 823,284 tons of passion fruit were produced in 2014, characterizing the country as the world's largest producer

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(FAOSTAT, 2015). Nearly 72.59% of Brazilian yellow passion fruit has been produced in Northeast region, and Bahia is the largest producer state (IBGE, 2015).

Yellow passion fruit production is affected by several factors, among which climate, soil and crop management practices, including fertilization and irrigation are predominant (Silva et al., 2015). The nutrients should be supplied at compatible levels according to the plant's requirements, preferentially using a fertigation technique, that's been currently proven effective (Borges et al., 2006), especially for yellow passion fruit crop which, according to Haag et al. (1973), demands large amounts of nutrient during development and budding phases; and nitrogen (N) is the most absorbed nutrient by this crop, observing an extraction of 205.5 kg ha^{-1} $year^{-1}$ and an N exportation through fruit harvest of 44.55 kg ha^{-1} for a fruit yield of 16.3 t ha^{-1}.

Fertigation allows applying, beyond soluble fertilizers, different inputs to plants such as herbicides, insecticides, bio-fertilizers and humic substances (HS). HS are formed by the transformation of biomolecules during the humification process of plants and animals residues in the environment (Silva and Mendonça, 2007).

Due to the beneficial effects of HS to soils and plants, it is possible to find in the scientific literature, some studies aiming to increase the efficiency of nutrients absorption, particularly for N, through the association of N fertilizing with HS for important commercial fruit crops such as grape (Ferrara and Brunetti, 2008), pineapple (Baldotto et al., 2009), custard apple (Cavalcante et al., 2012, 2014) and guava (Nunes et al., 2014), although for yellow passion fruit information are still scarce. A study on the effect of HS on fruit quality of yellow passion fruit was made by Silva et al. (2015). The study was justified by largely recognizing the influence of the HS on chemical, physical and biological soil properties and consequently in root growth, higher nutrient availability and chlorophyll biosynthesis (Ferrara and Brunetti, 2008).

Hence, the present study aimed to evaluate the effect of humic substances and different nitrogen doses supplied through fertigation on fruit production of yellow passion fruit in Brazilian semiarid.

METHODS AND MATERIALS

The experiment was carried out from December/2012 to December/2013 in Curaçá irrigated area, Juazeiro County, Bahia State, Brazil at the geographic coordinates of latitude 09° 07' S, and longitude 40° 04' W with altitude of 376 m. The climate of the region is classified, according to Köppen (1918), as hot and dry semiarid (Bswh).

During the execution of the experiment, the climatic data were collected by a meteorological station installed near the experimental farm (Figure 1), while physical and chemical characteristics of the soil from samples taken before executing the experiment are in Table 1. The soil is a Ultisols Ustult (American classification Soil Taxonomy).

For the propagation of seedlings polyethylene bags were used as containers with dimensions of 22 x 5.5 cm in height and width, respectively. The substrate used in the production of seedlings was composed of soil : sieved sand : bovine manure at a 1:1:2 ratio. The planting holes were opened in the dimension 60 x 60 x 60 cm, received part of phosphate fertilizer in foundation and transplanting of seedlings was performed 60 days after seeding. The seedlings were transplanted in February 2013, at 3 x 3 m spacing distance, and conducted on vertical cordon, with a smooth wire no. 14, 1.8 m from the ground. Plants were drip-irrigated daily with three emitters per plant installed every 0.30 m for a flow of 1.6 L h^{-1} each one, following the potential evapotranspiration (ETo) and yellow passion fruit's Kc coefficient defined by Souza et al. (2009).

The nitrogen source used was urea (45% N), fertigated once a week, beginning at 30 days after transplanting (DAT), according to Borges and Coelho (2009) recommendation. Phosphorus and potassium fertilizations were performed using monoammonium phosphate MAP (50% of P_2O_5; 11% of N) and potassium chloride (60% K_2O), respectively. The phosphate fertilization, 120 kg ha^{-1} yr^{-1} of P_2O_5, was applied at 90 and 210 DAT, while potassium (140 kg ha^{-1} yr^{-1} of K_2O) was weekly parceled from 90 DAT until the end of the experiment. The nitrogen, potassium and phosphate fertilizers were supplied through fertigation system (Viqua® venturi injector of 1" at 10 bar operating pressure), according to soil analysis. Foliar fertilization with micronutrients was performed every 15 days from 90 DAT following plant demand.

The source of humic substances used in the experiment was the commercial product KS 100 (Omnia®) from leonardite, with composition of K_2O (15%), total organic carbon (45%), humic acids (70%) fulvic acids (8%), electrical conductivity (0.37 mS/cm) salt index (24), pH (10) and solubility (140 g L^{-1}). The fertigation with HS were performed once every 30 days, following the manufacturer recommendations (5 kg ha^{-1} of the product throughout the crop cycle) and the amount of K_2O discounted when the potassium fertigation was performed with the humic substances. All management practices for pruning, control of weeds, pests and diseases were performed following the instructions of Lima et al. (2002).

The experimental design was randomized complete block in a split plot arrangement, considering of humic substances (absence and presence) as main plots and N doses (180, 200, 260, 330 and 350 kg ha^{-1} yr^{-1} of N) as subplots with five replications and six plants in each parcel. The N doses were defined according to Borges and Coelho (2009) recommendations. Choice for N levels with differences ranging from 20 to 60 is not clear.

The following variables were evaluated: i) stem diameter (mm) at 270 days after transplanting (DAT), at 10 cm of height from the soil using a digital paquimeter (0.01 - 300 mm, Digimess®); ii) at the beginning of flowering, the leaf chlorophyll readings (chlorophyll *a*, *b* and total) were measured using a chlorophyll meter (Falker®, Brazil) in three leaves within the canopy of each replication (plant) between 0900 and 1000 H, following the methodology described by El-Hendawy et al. (2005). Readings were taken in the middle of the canopy, avoiding necrotic areas by the attack of pests and diseases; iii) the same leaves were collected immediately after performing leaf chlorophyll readings, and chemically analyzed. After washing and rinsing with distilled water, the leaves were dried at 70°C for 48 h. Total N concentrations were analyzed using the Kjeldahl method, following the methodology properly described by Malavolta et al. (1997); and iv) HS and N doses treatments were also evaluated by determining the cumulative fruit production (kg $plant^{-1}$) and fruit yield (ton ha^{-1}), when yellow passion fruits were harvested twice a week placed in plastic boxes and weighted using a Filizola® CF15 brand precision scale (0.5 grams of precision). After the registration of fruit production per plant, the fruit yield (ton ha^{-1}) was calculated in each treatment.

Statistical analyses included analysis of variance, mean separation of HS using Tukey's test, and simple regression to separation of N fertilizing doses. All the calculations were performed using the SAS Statistical Program (SAS, 2011), considering

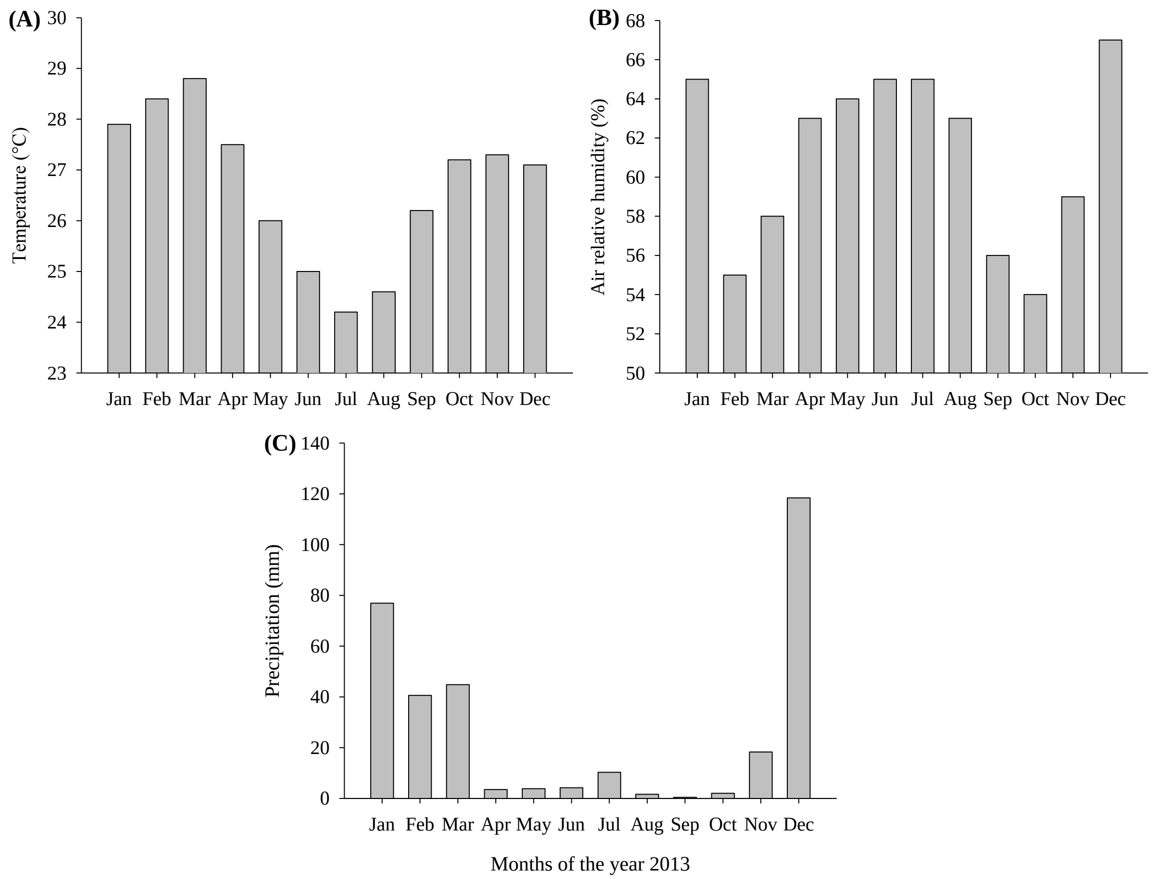

Figure 1. Monthly air temperature (A), air humidity (B) and precipitation (C) during the experiment.

Table 1. Chemical and physical characteristics of the soil (0-30 cm soil depth) where the experiment was carried out.

Chemical atributes		**Physical atributes**	
pH (water - 1:2,5)	5.6	Sand (g kg^{-1})	658
P (mg dm^{-3})	10.0	Silt(g kg^{-1})	277
K^+ ($cmol_c$ dm^{-3})	0.28	Clay (g kg^{-1})	65
Ca^{2+} ($cmol_c$ dm^{-3})	3.50	SD (kg dm^{-3})	1.52
Mg^{2+} ($cmol_c$ dm^{-3})	1.50	DP (kg dm^{-3})	2.51
Na^+ ($cmol_c$ dm^{-3})	0.29	Total porosity (%)	39.61
SB ($cmol_c$ dm^{-3})	5.57	Textural classification	Franco sandy
Al^{3+} ($cmol_c$ dm^{-3})	0.00		
H^+ + Al^{3+} ($cmol_c$ dm^{-3})	1.60	Carbon stocks in soil	
CEC ($cmol_c$ dm^{-3})	7.17	Fractions	Initial condition (t ha^{-1})
V (%)	78	TOC	6.54
O.M. (g dm^{-3})	12.1	CHF	2.41
C (g dm^{-3})	7.0	CHAF	1.11
E.C. (dS m^{-1})	2.0	CFAF	0.64

P, K^+ e Na^+ Mehlich-1 extractor (HCl^+ H_2SO_4); $Al3^+$, Ca^{2+}, Mg^{2+} extractor KCl 1M; OM= organic matter; SB= base saturation; V= base saturation; CEC= cation exchange capacity; SD= soil density and DP= density of particles; E.C.= electric conductivity; TOC= total organic carbon; CHF; CHAF e CFAF= carbon in the humin fraction, humic and fulvic acids, respectively.

Table 2. Stem diameter (SD), leaf nitrogen concentration (N), leaf chlorophyll index (*a*, *b* and total), fruit production (FP) and fruit yield (FY) of yellow passion fruit as a function of nitrogen fertilizing and humic substances grown in Brazilian semiarid.

Source of variation	SD	N	Chl *a*	Chl *b*	Total Chl	FP	FY
	mm	g kg^{-1}	CI			kg plant^{-1}	t ha^{-1}
HS (Value "F")	0.36^{ns}	0.01^{ns}	4.91^{ns}	0.38^{ns}	2.09^{ns}	0.98^{ns}	1.06^{ns}
Without HS	27.36^{a}	53.22^{a}	33.54^{a}	10.04^{a}	43.59^{a}	12.28^{a}	13.60^{a}
With HS	28.22^{a}	53.46^{a}	34.84^{a}	10.47^{a}	45.32^{a}	13.64^{a}	15.20^{a}
LSD	3.31	8.41	1.35	1.58	2.74	3.16	3.48
N doses(Value "F")	2.17^{ns}	1.00^{ns}	0.40^{ns}	1.88^{ns}	0.85^{ns}	4.11**	3.23*
180	26.99	50.08	33.75	9.31	43.05	12.53	12.56
200	26.80	61.25	34.47	10.04	44.52	10.71	13.37
260	28.55	50.54	34.81	11.62	46.43	13.31	14.78
330	28.94	52.91	33.62	10.11	43.74	13.98	15.54
350	27.69	51.95	34.33	10.22	44.55	14.27	15.85
HS X N (Value "F")	2.88*	1.19**	0.28^{ns}	0.95^{ns}	0.25^{ns}	3.28*	3.95*
CV%	7.24	26.93	7.27	18.85	9.72	17.19	17.21

HS = Humic substances; LSD = least significant difference; CI = chlorophyll index; ns = no significant; ** = significant at the 0.01 level of probability; * = significant at the 0.05 level of probability; means followed by the same letter in the columns do not differ statistically by Tukey test at 0.05 and 0.01 probability.

significant at $P \leq 0.01$.

RESULTS AND DISCUSSION

The interaction between the nitrogen (N) doses and humic substances (HS) affected the stem diameter ($p < 0.05$), leaf nitrogen concentration ($p < 0.01$), fruit production and yield ($p < 0.05$), a result that shows interdependence between the studied factors for these variables, although any effect on chlorophyll index has been registered. It is also observed that humic substances and N single doses did not significantly affect any of the studied variables (Table 2).

For treatments with HS, the stem diameter (SD) presented a better significant data adjustment to the quadratic regression model as a functions of N levels, characterized by the SD increase followed by decrease with the increasing of N doses applied through fertigation (Figure 2A and B); while for treatments without HS, SD increased with N doses enhancement, increasing 4.1 mm from 180 kg ha^{-1} to the maximum estimated dose of 281.33 kg ha^{-1} N (Figure 2A). This result is in agreement with Rebequi et al. (2011) who registered an increase of 4.9 mm on SD of yellow passion fruit from the lowest to the highest N dose evaluated, but it disagrees with Santos et al. (2011) that did not find significant difference for SD of yellow passion fruit, as a function of nitrogen fertilization doses.

Treatments with HS presents the maximum estimated dose of 260 kg ha^{-1} N reaching a peak a 28.9 mm, which corresponds to the reduction of 30.16% of N fertilizer applied as compared to the plants grown without HS (Figure 2B), that could occurred because HS increases nutrient absorption, among them, N (Primo et al. 2011). Thus, the plants treated with HS had a better N use, reaching the maximum stem diameter with a lower dose of N. Nunes et al. (2014) evaluated the growth and leaf nutritional status of guava grown with HS and soil mulching, and registered no significant effect on stem diameter. On the other hand, Baldotto et al. (2009) observed significant increases on growth and development of pineapple seedlings propagated by tissue culture as a function of humic acids isolated from vermicomposting during the acclimatization period. These facts may have occurred due to the benefits provided to the plants by the humic acids addition, which are formed by heterogeneous molecular aggregates and stabilized by hydrogen connections and hydrophobic interactions, favoring root system development (Zandonadi et al., 2007) and nutrients accumulation (Chen et al., 2004).

As shown in Figure 2C, plants grown without HS at 180 kg ha^{-1} of N presented nearly 56.5 g kg^{-1} of N, with exponential decrease of N concentration with N doses increasing, a result in disagreement with Venancio et al. (2013) who verified linear increase of N leaf concentrations with higher N doses applied, with higher N concentration of 40.49 g kg^{-1} of N corresponding to the higher N dose applied (210 kg ha^{-1} of N). According to adequate range of supply defined by Prado and Natale (2006), (43-55 g kg^{-1}), the plants were N adequately supplied, and there was no visual toxic symptoms of N excess, although the recorded values are strongly higher than those reported by Silva Júnior et al. (2013) in a study on the bio-fertilizers use as a HS source for yellow passion fruit plants.

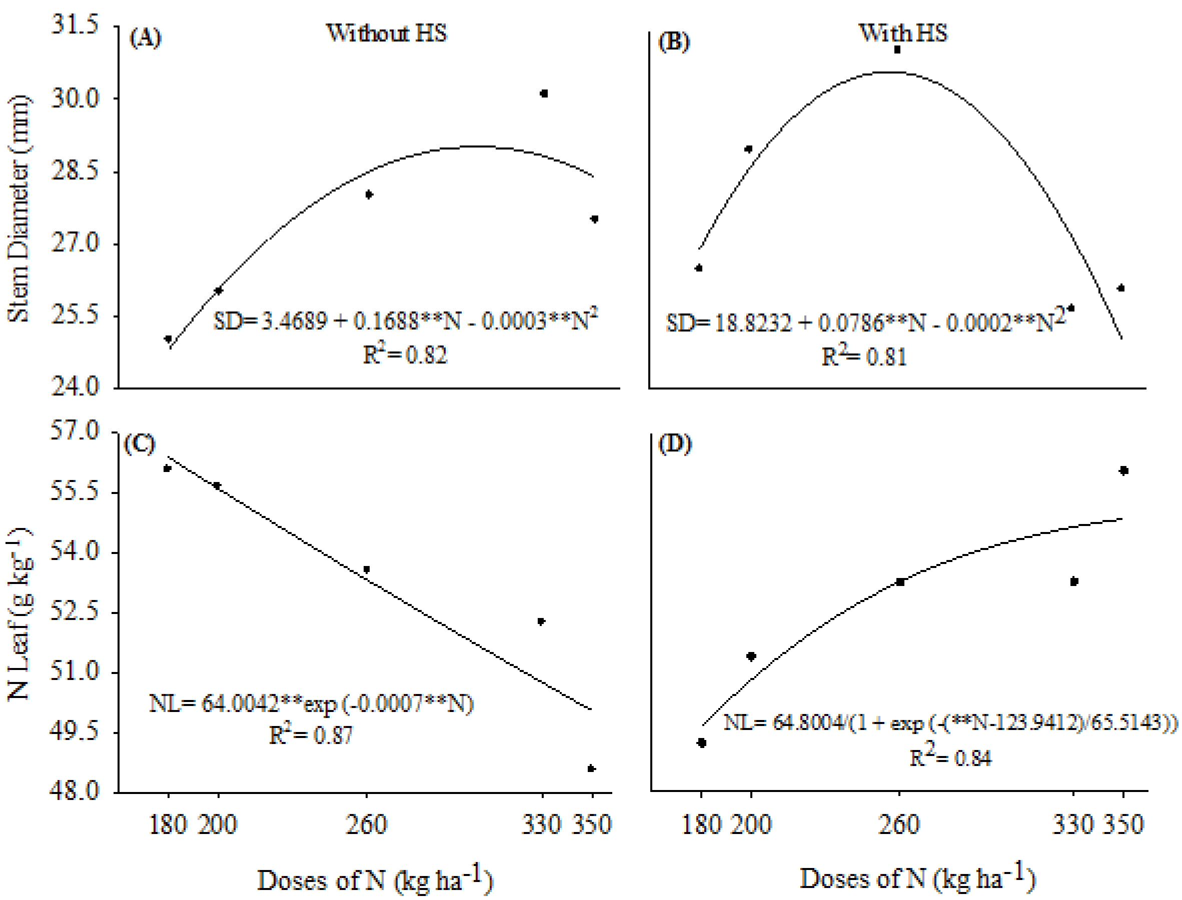

Figure 2. Stem diameter (A and B) and leaf nitrogen concentration(C and D) of yellow passion fruit as a function of N doses and humic substances.

The N leaf concentrations of yellow passion fruit presented exponential growth with N increase using HS (Figure 2D), with the highest dose (64 g kg^{-1} of N) recorded at the maximum leaf N level (350 kg ha^{-1} of N). This N leaf concentration increase can be explained by the positive effect provided by HS to nitrogen root absorption as ammonium nitrate (Keeling et al., 2003). In addition, according to Figure 2D, it is possible to infer that there was a "luxury consumption" in plants that received HS because those plants presented an adequate N leaf concentration for a satisfactory yield when fertilized with 260 kg ha^{-1} N (Prado and Natale, 2006).

The leaf chlorophyll indexes *a*, *b* and total of yellow passion fruit were not affected by N doses, independently of HS use (Table 2) that is congruent with the results of Cavalcante et al. (2014) for custard apple crop. On the other hand, increases on leaf chlorophyll as a function of foliar spray of humic acids (5 and 20 mg L^{-1}) of grape were reported by Ferrara and Brunetti (2008). Primo et al. (2011) and Baldotto et al. (2009) found that humic acids provided higher photosynthetic pigments levels and significant increase of chlorophyll *a* and *b* ratio, as compared to the control.

Yellow passion fruit plants grown without HS produced more than 14.4 kg plant^{-1} when fertilized with 290 kg ha^{-1} year^{-1} N (Figure 3A), a result lower than that quoted by Cavalcante et al. (2012b), who obtained a fruit production of 17.81 kg plant^{-1} with NPK soil fertilizing. However, these results exceed the values recorded by Cavalcante et al. (2005) who obtained a fruit production of 7.1 kg plant^{-1} and 8.4 kg plant^{-1} presented by Cavalcante et al. (2007) in soil fertilized with liquid bovine bio-fertilizer. The positive response to N doses can be attributed to the low soil organic matter content, even for a semiarid region (Table 1), as Bayer and Mielniczuk (2008) stated that organic matter is the main soil N source.

The plants fertigated with HS increased fruit production from 12.0 to 15.2 kg plant^{-1} (26.70%) from the lowest to the highest N dose applied (Figure 3B), which is congruent to the results of Cunha et al. (2015) who observed significant interactions between N doses and HS on custard apple (*Annona squamosa* L.) production and yield, also in a semiarid climate. This increment can be explained by the effect of HS on soil chemical and biological properties (Pimenta et al., 2009) with direct effect on nutrient uptake (Primo et al., 2011) and

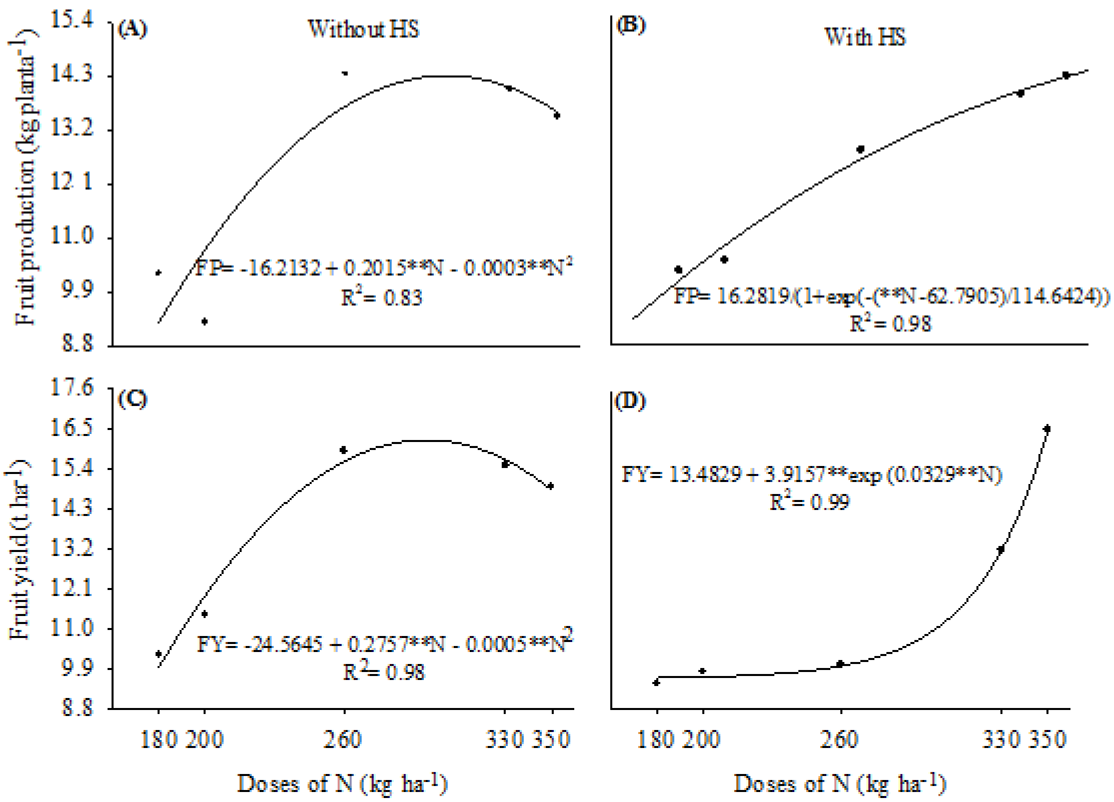

Figure 3. Fruit production (A and B) and fruit yield (C and D) of yellow passion fruit of yellow passion fruit as a function of N doses and humic substances.

consequently to the plants. According to Eyheraguibel et al. (2008), the HS effects promote significantly higher water consumption, and consequently better plant growth and production. Additionally, much of the humic acid's biostimulants effects have been credited to the HS activity, which is similar to the plant hormones of auxin class, that is, they can promote plant growth in relatively small concentrations (Baldotto et al., 2009).

The average fruit yield of the fertigated yellow passion fruit presented different data distributions to the HS effects (Figures 3C and D). In plants without HS, the maximum estimated fruit yield was 16.5 t ha^{-1} for 290 kg ha^{-1} N dose, and, therefore, below the 17.5 t ha^{-1} of the treatments with the N dose of 350 kg ha^{-1}, in plants with HS. These results are above the Brazilian average fruit yield of 13.42 t ha^{-1} $year^{-1}$ (Agrianual, 2014), but, however, they are lower than those recorded by Venancio et al. (2013) who evaluated the production, fruit quality and leaf nitrogen content in yellow passion fruit under nitrogen fertilization, and obtained an average yield of 18.5 t ha^{-1} yr^{-1} under the conditions of Aquidauana - MS.

Conclusions

The stem diameter, leaf N concentrations and fruit production of yellow passion fruit are affected interdependently by nitrogen doses and humic substances. The leaf indexes of chlorophyll *a*, *b* and total are not affected by the nitrogen fertigationor by humic substances. The higher yellow passion fruit yields are recorded at 290 and 350 kg ha^{-1}N doses without and with humic substances, respectively.

Conflict of Interests

The authors have not declared any conflict of interests.

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