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## Biometric responses of sugarcane under high doses of vinasse<sup>1</sup>

### Respostas biométricas da cana-de-açúcar sob altas doses de vinhaça

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#### HIGHLIGHTS:

*The use of 600 and 1200 m<sup>3</sup> ha<sup>-1</sup> of vinasse in sugarcane cultivation contributes to greater biomass production.*

*The use of high doses of vinasse can impact the environment.*

*Application of 1200 m<sup>3</sup> ha<sup>-1</sup> of vinasse negatively affects the production of sugarcane in terms of alcohol and sugar.*

**ABSTRACT:** Rational use of vinasse as a fertilizer in sugarcane cultivation is a good option to increase crop development and yield due to its nutritional support, properly dispose of the by-product, and reduce costs of mineral nutrition. However, in the field, controlling the amount of vinasse can be difficult, interfering in sugarcane development. In this sense, this study aimed to evaluate high doses of vinasse related to biometric responses in the initial growth phase of sugarcane plants. The experimental design was completely randomized, and the treatments consisted of five vinasse doses (0, 150, 300, 600, and 1200 m<sup>3</sup> ha<sup>-1</sup>), with six replicates, except D0, three replicates, and one dose of mineral fertilizer with 70 kg ha<sup>-1</sup> of N, 120 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and 90 kg ha<sup>-1</sup> of K<sub>2</sub>O, with three replications. The biometric variables were evaluated in the following sequence: number of tillers per pot from 60 to 150 days after planting (DAP), plant height, number of green and dry leaves from 90 to 210 DAP, stem diameter, leaf area, and fresh and dry biomasses of roots and shoots at 210 DAP. Vinasse increased the biomass production through the number of tillers and the number of leaves compared to mineral fertilizer, promoting greater growth up to the dose of 600 m<sup>3</sup> ha<sup>-1</sup>. The vinasse dose of 1200 m<sup>3</sup> ha<sup>-1</sup> decreased the plant height.

**Key words:** *Saccharum officinarum*, agro-industrial waste, plant growth, mineral fertilization

**RESUMO:** O uso da vinhaça como fertilizante no cultivo da cana-de-açúcar é uma boa opção para melhorar o desenvolvimento da cultura devido seu aporte nutricional, destinar adequadamente o subproduto e diminuir os custos de plantio; no entanto, no campo, o controle da quantidade de vinhaça aplicada pode ser difícil, interferindo no desenvolvimento da cana-de-açúcar. Com isso, objetivou-se neste estudo avaliar altas doses de vinhaça em relação às respostas biométricas no crescimento inicial da cana planta. Utilizou-se o delineamento inteiramente casualizado, com tratamentos compostos por cinco doses de vinhaça (0, 150, 300, 600 e 1200 m<sup>3</sup> ha<sup>-1</sup>), com seis repetições, exceto D0 com três repetições, e uma dose de adubo mineral com 70 kg ha<sup>-1</sup> de N, 120 kg ha<sup>-1</sup> de P<sub>2</sub>O<sub>5</sub> e 90 kg ha<sup>-1</sup> de K<sub>2</sub>O, com três repetições. As variáveis biométricas foram avaliadas seguindo a sequência: número de perfilhos dos 60 aos 150 dias após plantio (DAP), altura de planta e número de folhas verdes e secas dos 90 aos 210 DAP, e diâmetro do colmo, área foliar e biomassa fresca e seca da raiz e parte aérea aos 210 DAP. A vinhaça aumentou a produção de biomassa através do número de perfilhos e do número de folhas em comparação ao adubo mineral, proporcionando maior crescimento até a dose 600 m<sup>3</sup> ha<sup>-1</sup>. A dose de vinhaça de 1200 m<sup>3</sup> ha<sup>-1</sup> diminuiu a altura de planta.

**Palavras-chave:** *Saccharum officinarum*, resíduo agroindustrial, crescimento de planta, adubo mineral

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## INTRODUCTION

Ethanol, whose raw material is sugarcane, is a biofuel widely consumed in Brazil. It generates a residue, popularly known as vinasse. Still, due to its composition, such as that presented by Pazuch et al. (2017), it can be considered as a by-product. It may replace part of the mineral fertilization for sugarcane plantations.

Since the first study published by Almeida et al. (1950), several studies have been conducted with vinasse to evaluate its effects on soil fertility and sugarcane yield (Silva et al., 2015; Nascimento et al., 2017; Cabral Filho et al., 2019). Given the results observed in these studies, soil fertility benefits that the practice of fertigation with vinasse promotes has been highlighted.

The use of vinasse in sugarcane cultivation has its benefits beyond the nutritional proposal. The agro-industrial waste has 90% of water (González et al., 2018), which helps in the water supply of sugarcane. Besides that, the use of waste in agriculture avoids some problems like environmental pollution.

Prado et al. (2017) affirmed that 450 m<sup>3</sup> ha<sup>-1</sup> of vinasse improved the raw material quality and increased the sugarcane yield. These doses can reduce the need for mineral fertilizer because the amount of nutrients present in its constitution correspond to the sugarcane nutritional needs. But the use of doses lower than 450 m<sup>3</sup> ha<sup>-1</sup>, as 120 m<sup>3</sup> ha<sup>-1</sup>, cannot bring the same benefits because of the reduced amount of nutrients offered, bringing responses as reduced stem production (Silva et al., 2018). Maradiaga-Rodriguez et al. (2018) found that the dose of 330 m<sup>3</sup> ha<sup>-1</sup> was the most indicated for better sugarcane growth.

When fertilized with vinasse, sugarcane may exhibit a distinct biometric behavior compared with that showed by the crop when fertilized only with mineral compounds. The residue does not have the same proportion of nutrients in a balanced way.

In the field, the control of the dose applied can be difficult, mostly when the vinasse is abundant, thus interfering in sugarcane development. The optimal dose is the most studied in vinasse experiments without information about the maximum dose that the sugarcane can support. This study aimed to evaluate the effect of high vinasse doses on biometric responses of sugarcane cultivar RB867515 in the initial growth.

## MATERIAL AND METHODS

The experiment was conducted between March and October 2018, at Departamento de Engenharia Agrícola of the Universidade Federal Rural de Pernambuco (UFRPE), campus of Recife, PE, Brazil (8° 01' 05" S and 34° 56' 48" W, and altitude of 6.49 m).

The experiment was conducted in a protected environment, in 100 dm<sup>3</sup> pots. The pots were filled with Ultisol collected at 0-20 cm and 20-40 cm soil layers in the Carpina Sugarcane Experimental Station (EECAC - UFRPE), in Carpina, located in the coastal tablelands of the Northern forest zone region of Pernambuco state, Brazil.

The chemical characterization of soil before applying the vinasse was: pH = 5.7; P = 6.5 mg dm<sup>-3</sup>; K = 0.075 cmol<sub>c</sub> dm<sup>-3</sup>; Ca = 1.50 cmol<sub>c</sub> dm<sup>-3</sup>; Mg = 0.75 cmol<sub>c</sub> dm<sup>-3</sup>; Na = 0.065 cmol<sub>c</sub> dm<sup>-3</sup>; sum of bases = 2.4 cmol<sub>c</sub> dm<sup>-3</sup>; cation exchange capacity = 5.7 cmol<sub>c</sub> dm<sup>-3</sup>; base saturation = 42%.

The pots had one hole at the bottom with a collector for the sampling of drainage water. The pot's base received a 10-cm-thick layer of crushed stone number zero, with a geotextile for drainage, separating it from the soil. The rest of the pot was filled with approximately 77 dm<sup>3</sup> of soil, half of this volume using soil from the 0-20 cm layer and the other half using soil from the 20-40 cm layer, resulting in the porosity of 46.15% and soil bulk density of 1.4 g cm<sup>-3</sup>.

The sugarcane cultivar RB867515 was cultivated from single-budded setts. Four buds were cultivated per pot, and, after germination, thinning was performed, leaving three plants per pot.

Six treatments were applied: five vinasse doses corresponding to 0 (D0), 150 (D150), 300 (D300), 600 (D600), and 1200 (D1200) m<sup>3</sup> ha<sup>-1</sup>, with replicates ranging from three to six, and mineral fertilizer (MF), with three replicates. The difference in the replications between treatments occurred because of the local experimental conditions and was allowed based on the experimental design.

Vinasse doses were applied 20 days before sowing in unique application. The amount of vinasse per pot corresponding to each dose was based on the pot area. Vinasse was collected at the Petribu Mill - located in the rural area of Lagoa de Itaenga, also in the coastal region of Pernambuco State. The vinasse was applied manually, with the quantity controlled through a beaker.

For the treatment with mineral fertilizer (MF), the fertilization consisted of 70 kg ha<sup>-1</sup> of nitrogen (N), 120 kg ha<sup>-1</sup> of phosphorus (P<sub>2</sub>O<sub>5</sub>), and 90 kg ha<sup>-1</sup> of potassium (K<sub>2</sub>O), according to the recommendations of Cavalcanti et al. (2008). Ammonium sulfate was used as the source of N, single superphosphate as the source of P<sub>2</sub>O<sub>5</sub> and potassium chloride as the source of K<sub>2</sub>O.

The composition of vinasse is presented in Table 1. The amounts of macronutrients plus sodium applied in treatments with vinasse doses, equivalent to mineral fertilization sources, are shown in Table 2, being extrapolated to kg ha<sup>-1</sup> proportional to the pot area.

Irrigations were carried out using water from the local supply system, manually, maintaining the soil moisture around field capacity for the development of the crop. During 210 days after planting (DAP), 160 L of supply water was applied in each pot.

**Table 1.** Chemical characterization of vinasse

Attributes	Values
pH	4.20
Electrical conductivity (dS m <sup>-1</sup> )	0.15
Carbon (mg L <sup>-1</sup> )	1032.50
Total nitrogen (mg L <sup>-1</sup> )	51.80
Total phosphate (mg L <sup>-1</sup> )	6.78
Calcium (mg L <sup>-1</sup> )	888.30
Magnesium (mg L <sup>-1</sup> )	395.30
Sodium (mg L <sup>-1</sup> )	729.10
Potassium (mg L <sup>-1</sup> )	1053.00

**Table 2.** Amounts of nitrogen (N), phosphorus (P<sub>2</sub>O<sub>5</sub>), potassium (K<sub>2</sub>O), calcium (Ca<sup>2+</sup>), magnesium (Mg<sup>2+</sup>), and sodium (Na<sup>+</sup>) applied according to each vinasse dose and mineral fertilizer (MF)

Dose (m <sup>3</sup> ha <sup>-1</sup> )	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>
	(kg ha <sup>-1</sup> )					
0	0	0	0	0	0	0
150	7.77	1.01	189.00	133.24	59.29	109.36
300	15.54	2.03	378.00	266.49	118.59	218.73
600	31.08	4.07	756.00	532.98	237.18	437.46
1200	62.16	8.14	1512.00	1065.96	474.36	874.92
MF	70	120	90	0	0	0

The number of tillers (NT) per pot was evaluated from 60 to 150 DAP. From 90 to 210 DAP, the following variables were evaluated: the total number of green leaves per pot (NGL), considering as valid leaves with at least 50% green area, with the count also including not fully developed leaves; the number of dry leaves per pot (NDL), considering leaves with less than 50% green area; and plant height (PH), considering in the measurement the distance from the base of the plant to the first visible ligule.

For the evaluated variables involving the time factor, such as number of tillers (NT), plant height (PH), number of dry leaves (NDL), and number of green leaves (NGL), analysis of variance was performed according to the treatments and time x treatment interaction.

At 210 DAP, leaf area per pot (LA, cm<sup>2</sup>) was determined through the equation proposed by Hermann & Câmara (1999), and stem diameter (SD), in mm, was measured at the base of each plant in the pot. At this time, the following variables were determined: stem fresh biomass (SFB), stem dry biomass (SDB), root fresh biomass (RFB), root dry biomass (RDB), tops + leaves fresh biomass (TLFB), utilizing all the region above the first visible ligule plus the green leaves, and tops + leaves dry biomass (TLDB). To determine dry biomass, the materials were placed in paper bags and dried in a forced-air circulation oven at 65 °C until reaching constant weight.

**Table 3.** Mean values of leaf area (LA), stem fresh biomass (SFB), stem dry biomass (SDB), root fresh biomass (RFB), root dry biomass (RDB), top + leaves fresh biomass (TLFB), top + leaves dry biomass (TLDB), stem diameter (SD) and number of tillers (NT), according to mineral fertilizer (MF) and doses of vinasse applied to the soil (m<sup>3</sup> ha<sup>-1</sup>)

Contrasts	LA	SFB	SDB	RFB	RDB	TLFB	TLDB	SD	NT
	(cm <sup>2</sup> )	(g)						(mm)	-
MF	3475.1	1440.0	643.1	1116.0	474.7	398.7	141.3	21.0	10.2
vs	vs	vs	vs	vs	vs	Vs	vs	vs	vs
Vinasse (D150 to D1200)	3639.1	1420.5	622.8	1129.5	492.4	421.1	145.3	21.4	12.7
p-value	0.2	<0.0001	0.006	0.05	0.02	0.0001	<0.0001	0.001	0.0001
MF	3475.1	1440.0	643.1	1116.0	474.7	398.7	141.3	21.0	10.2
vs	vs	vs	vs	vs	vs	Vs	vs	vs	vs
D0	2853.2	1036.0	425.0	750.3	278.0	276.7	100.8	18.9	9.0
p-value	0.02	<0.0001	0.01	0.007	0.0006	<0.0001	<0.0001	0.0004	<0.0001
MF	3475.1	1440.0	643.1	1116.0	474.7	398.7	141.3	21.0	10.2
vs	vs	vs	vs	vs	vs	Vs	vs	vs	vs
D300	3375.8	1369.2	613.8	1077.8	415.2	379.7	133.8	20.9	11.9
p-value	0.3	0.0007	0.01	0.08	0.06	0.0008	0.0007	0.008	0.0002
MF	3475.1	1440.0	643.1	1116.0	474.7	398.7	141.3	21.0	10.2
vs	vs	vs	vs	vs	vs	Vs	vs	vs	vs
D1200	4114.0	1573.4	712.9	1302.4	646.6	544.2	177.5	22.3	14.1
p-value	0.3	0.0004	0.04	0.08	0.03	0.0006	0.0004	0.01	0.1
D0	2853.2	1036.0	425.0	750.3	278.0	276.7	100.8	18.9	9.0
vs	vs	vs	vs	vs	vs	Vs	vs	vs	vs
Vinasse (D150 to D1200)	3639.1	1420.5	622.8	1129.5	492.4	421.1	145.3	21.4	12.7
p-value	0.07	0.02	0.9	0.09	0.01	<0.0001	<0.0001	0.1	<0.0001

The data were subjected to analysis of variance using the PROC GLM procedure of SAS statistical package, 9.4 version (SAS Institute, 2010).

Based on the Mauchly test of sphericity, the results allowed evaluating the variables measured over time using the split-plot arrangement and orthogonal contrasts. Regression analysis was also performed.

The orthogonal contrasts were performed to compare mineral fertilization with treatments that received vinasse (MF vs. vinasse doses (D150 to D1200)); mineral fertilization with the non-application of vinasse (MF vs. D0); the individual response of mineral fertilization with the dose 300 m<sup>3</sup> ha<sup>-1</sup> (MF vs. D300); the individual response of mineral fertilization with the dose 1200 m<sup>3</sup> ha<sup>-1</sup> (MF vs. D1200); and non-application with the application of vinasse (D0 vs. vinasse doses (D150 to D1200)).

## RESULTS AND DISCUSSION

Through the evaluated contrasts, it can be observed that leaf area (LA) did not differ when comparing mineral fertilizer (MF) with vinasse doses (Table 3). Farinelli et al. (2017) evaluated the use of vinasse on corn plants and verified the absence of effect of the residue on leaf area. The leaf area is affected not only because of the number of leaves but also the green area photosynthetically active on the leaf that keeps it contributing to the plant development.

The low concentration of nitrogen in vinasse did not promote higher stem fresh biomass (SFB) and stem dry biomass (SDB) comparing to the mineral fertilizer (MF) until the D300 (Table 3) because nitrogen is the nutrient that most act in the production of biomass. The stem biomass was higher than MF, only with the application of 1200 m<sup>3</sup> ha<sup>-1</sup> of vinasse (D1200).

The MF vs. vinasse contrast (Table 3) showed that the applied vinasse doses (VD) did not promote values of root fresh biomass (RFB) production significantly different from

those obtained with the application of MF, probably related to the same cause of stem biomass, because of low concentration of nitrogen.

However, for root dry biomass (RDB), there was a significant difference between MF and vinasse doses, in the contrasts MF vs. D0 and MF vs. D1200 (Table 3), with the largest values always obtained with the application of vinasse, because the higher amount of nutrients available on the D1200, thus the roots present greater development.

According to the means of the contrast comparing the application with the non-application of vinasse (D0 vs. vinasse), the difference between the means implies that, without the use of MF, the application of VD increases root growth by 77.1%.

The application of vinasse promoted higher top + leaves fresh biomass (TLFB) and top + leaves dry biomass (TLDB) than those obtained with the application of MF (Table 3). However, despite the low concentration of nitrogen, the vinasse also contains a high organic matter concentration responsible for part of the mineralized nitrogen available (Calcino et al., 2018).

Comparisons between MF and vinasse doses, including MF vs. D300 and MF vs. D1200, evidenced the effect of vinasse on stem diameter (Table 3). The vinasse was responsible for larger stem diameter than MF in the dose above D300, as occur in the D1200 when the SD was 2% higher than MF. However, Farinelli et al. (2017) observed that the application of concentrated vinasse mixed with other residues from the sugar-alcohol industry did not affect corn stem diameter.

For the effect of VD on SFB (Figure 1A), there was an increase in this variable according to the increase in vinasse doses, with the largest result at the dose 1200 m<sup>3</sup> ha<sup>-1</sup>. The stem biomass values obtained in the present study, with the application of vinasse doses, corroborate the results obtained by Prado et al. (2017).

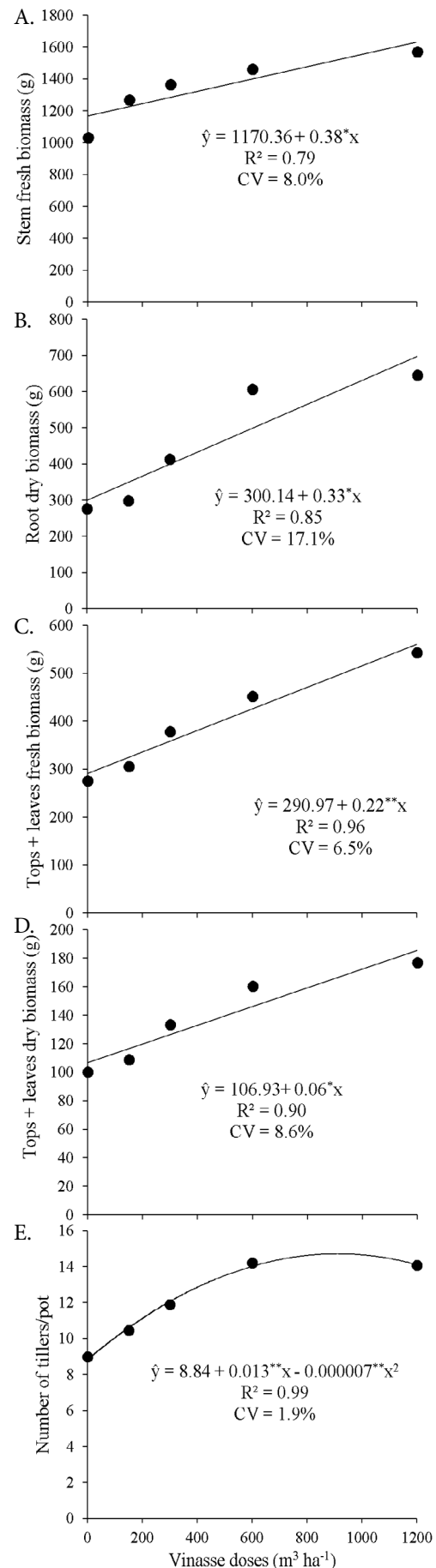
Barbosa et al. (2013) found that the vinasse dose of 338.6 m<sup>3</sup> ha<sup>-1</sup> increases the stem production of sugarcane. Also, Silva et al. (2014) evaluated that doses until 800 m<sup>3</sup> ha<sup>-1</sup> increase the stem yield.

For Silva et al. (2018), the increment in stem dry biomass (SDB) depends on a higher amount of nutrients in vinasse because of the greater amount of nutrients present in the residue.

Among the VD evaluated, the largest value (696.14 g) of RDB was observed at 1200 m<sup>3</sup> ha<sup>-1</sup> (Figure 1B). Pina et al. (2015), evaluating the influence of different organic residues, including vinasse, associated with chemical fertilizer (NPK) on sugarcane rooting and yield, found that the vinasse dose of 300 m<sup>3</sup> ha<sup>-1</sup> promoted greater rooting than that obtained under mineral fertilization.

Figures 1C and D show that the respective increases of fresh biomass and dry biomass of tops + leaves according to the vinasse doses applied, where the highest values for both variables were obtained with the dose of 1200 m<sup>3</sup> ha<sup>-1</sup>, mostly because the amount of organic matter, K<sub>2</sub>O, Ca<sup>2+</sup>, and Mg<sup>2+</sup> presents, which are macronutrients required for the vegetative growth.

Corroborating the results obtained, Ueno et al. (2014) also observed that vinasse contributes to increasing the



\*, \*\* - Significant at  $p \leq 0.05$  and  $p \leq 0.01$  by F test, respectively

**Figure 1.** Stem fresh biomass (A), root dry biomass (B), top + leaves fresh biomass (C), top + leaves dry biomass (D), and number of tillers (E) according to the vinasse doses applied to the soil

production of shoot dry biomass. According to Mariano et al. (2016), organomineral fertilizers contribute more to biomass accumulation than mineral sources of nutrients.

Although D1200 promoted higher biomass yield than MF and among the other doses applied, one should rethink the effects of an exacerbated biomass production on crop yield and the possible adverse effects resulting from this practice, mainly when project this effect on the sugarcane yield and the quality of the crop derived products, such as sugar and alcohol.

For the evaluated variables involving the time factor, only the number of tillers was not significant for the time x treatment interaction. Vinasse promoted higher sugarcane tillering compared to MF from D300 (Table 3). Compared to its non-application, the vinasse application resulted in a 41.1% increase in tillering. Figure 1E shows that the highest means were observed at 928.6 m<sup>3</sup> ha<sup>-1</sup>.

The vinasse contains nutrients in an organic form, allowing faster absorption and utilization by the sugarcane than when supplied in the form by mineral fertilization. Its supply in a high concentration allows the plant to start its cycle with more available nutrients and, consequently, a more accelerated vegetative development.

Amounts of potassium supplied below the equivalent to D150 do not promote higher sugarcane tillering (Flores et al., 2012) because this stage is more dependent on nitrogen and phosphorus (Jadoski et al., 2010), nutrients that are found at low concentration in vinasse, especially at the dose D150, thus justifying the need for using doses greater than D300 to enable a greater development than that obtained with MF.

For PH, the effect of treatments is observed only from 120 days after planting; MF did not differ from D300 and D1200, and there was a difference only in the comparison between the application or not of VD (Table 4).

At 180 DAP, it becomes clear that D600 and D1200 hampered the plant height (PH) in sugarcane compared to MF. The absence or low concentration of nutrients in the soil enabled a lengthening of the crop.

Apparently, the plant height was more sensitive to the vinasse high nutrients concentration, as K, Ca, and Mg, which began to have significance at D600 and D1200, thus affecting its growth.

For being the predominant mineral in vinasse, potassium alone does not affect the plant height because, according to Flores et al. (2012), the crop responded positively to the increased supply of mineral sources of K<sub>2</sub>O. Crusciol et al. (2020) verified that organomineral sources of K contributed to the development of sugarcane plant height.

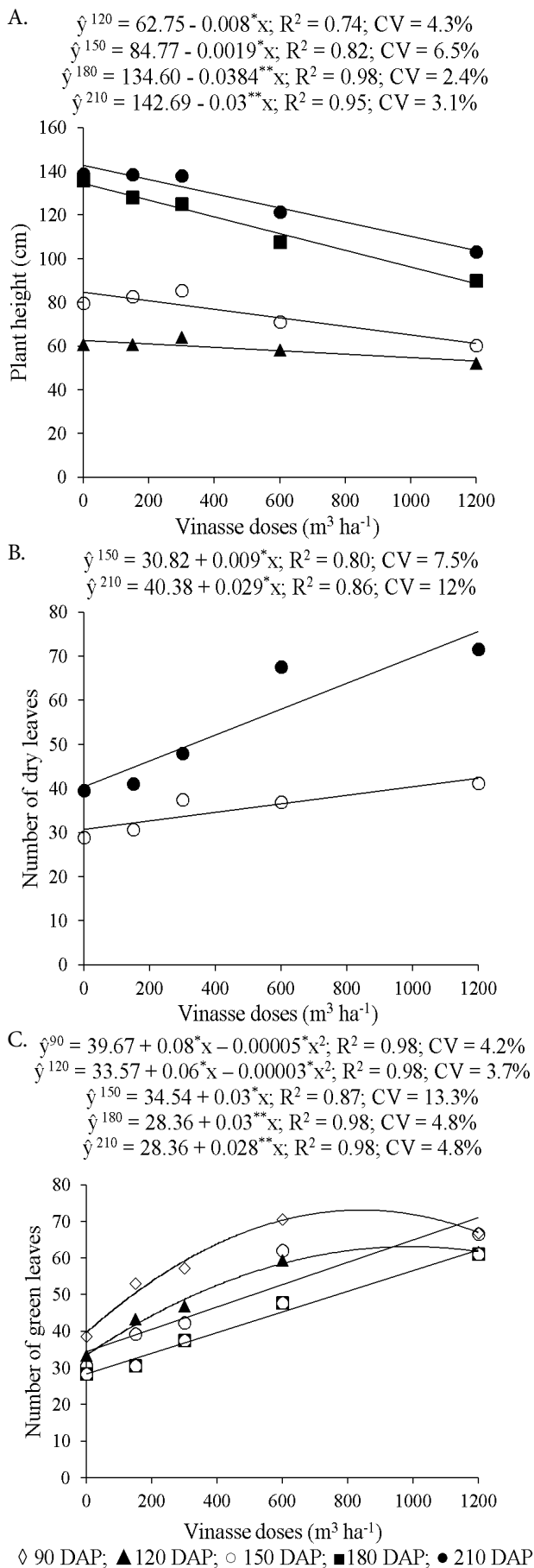
Concerning NDL, mineral fertilization differed from vinasse doses from 180 DAP (Table 4). Vinasse, in general, promoted greater vitality for the leaves, generating 6.76% lower NDL than MF. Only at D1200, NDL was higher than the value obtained with MF.

The NGL was sensible to the vinasse doses, increasing the leaves emissions significantly until D1200. At 180 DAP, the NGL produced by plants that received vinasse was 55.17% higher compared to the value obtained with MF, probably due to the slow release of nutrients that happens with the mineralization of organic compounds, allowing that until the mentioned period, the presence of available nutrients was high for such doses.

**Table 4.** Mean values of plant height, number of dry leaves, and number of green leaves at 90, 120, 150, 180, and 210 days after planting according to mineral fertilizer (MF) and doses of vinasse applied to the soil (m<sup>3</sup> ha<sup>-1</sup>)

Contrasts	Time of evaluation (days after planting)				
	90	120	150	180	210
	Plant height				
MF	44.3	61.8	81.4	120.8	130.5
vs	vs	vs	vs	vs	vs
Vinasse (D150 to D1200)	47.6	58.7	74.9	112.7	125.3
p-value	0.4	0.8	0.9	0.03	0.3
MF	44.3	61.8	81.4	120.8	130.5
vs	vs	vs	vs	vs	vs
D0	45.1	60.6	79.8	135.8	138.8
p-value	0.3	0.02	0.001	<0.0001	0.0004
MF	44.3	61.8	81.4	120.8	130.5
vs	vs	vs	vs	vs	vs
D300	48.6	64.1	85.6	125.0	138.1
p-value	0.1	0.3	0.3	0.1	0.9
MF	44.3	61.8	81.4	120.8	130.5
vs	vs	vs	vs	vs	vs
D1200	47.5	52.2	60.4	90.1	103.1
p-value	0.7	0.7	0.8	0.1	0.4
D0	45.1	60.6	79.8	135.8	138.8
vs	vs	vs	vs	vs	vs
Vinasse (D150 to D1200)	47.6	58.7	74.9	112.7	125.3
p-value	0.6	0.001	<0.0001	<0.0001	<0.0001
	Number of dry leaves				
MF	21.0	32.0	35.6	50.6	60.0
vs	vs	vs	vs	vs	vs
Vinasse (D150 to D1200)	18.9	36.3	36.6	47.4	57.1
p-value	0.09	0.1	0.1	0.03	0.04
MF	21.0	32.0	35.6	50.6	60.0
vs	vs	vs	vs	vs	vs
D0	23.6	28.3	29.0	33.3	39.6
p-value	0.01	0.02	0.008	0.003	0.0004
MF	21.0	32.0	35.6	50.6	60.0
vs	vs	vs	vs	vs	vs
D300	21.0	35.5	37.5	43.5	48.0
p-value	0.2	0.1	0.05	0.1	0.29
MF	21.0	32.0	35.6	50.6	60.0
vs	vs	vs	vs	vs	vs
D1200	17.0	39.3	41.3	55.1	71.6
p-value	0.3	0.4	0.1	0.03	0.03
D0	23.6	28.3	29.0	33.3	39.6
vs	vs	vs	vs	vs	vs
Vinasse (D150 to D1200)	18.9	36.3	36.6	47.4	57.1
p-value	0.08	0.1	0.04	0.05	0.002
	Number of green leaves				
MF	53.0	47.0	41.3	29.0	32.3
vs	vs	vs	vs	vs	vs
Vinasse (D150 to D1200)	62.0	52.7	52.6	45.0	44.2
p-value	0.0006	0.01	0.001	0.02	0.06
MF	53.0	47.0	41.3	29.0	32.3
vs	vs	vs	vs	vs	vs
D0	38.6	33.3	30.6	25.6	28.3
p-value	<0.0001	0.0002	<0.0001	<0.0001	<0.0001
MF	53.0	47.0	41.3	29.0	32.3
vs	vs	vs	vs	vs	vs
D300	57.3	46.8	42.3	35.8	37.5
p-value	0.003	0.04	0.02	0.07	0.08
MF	53.0	47.0	41.3	29.0	32.3
vs	vs	vs	vs	vs	vs
D1200	67.0	61.6	66.6	59.1	61.1
p-value	0.04	0.08	0.07	0.5	0.5
D0	38.6	33.3	30.6	25.6	28.3
vs	vs	vs	vs	vs	vs
Vinasse (D150 to D1200)	62.0	52.7	52.6	45.0	44.2
p-value	0.03	0.007	<0.0001	<0.0001	<0.0001

The D0 vs. vinasse contrast shows that vinasse increases the production of leaves in sugarcane. The maximum difference was recorded at 180 DAP, with 75.30% more leaves produced than the non-application of vinasse.



\*, \*\* - Significant at  $p \leq 0.05$  and  $p \leq 0.01$  by F test, respectively

**Figure 2.** Plant height (A), number of dry leaves (B) and number of green leaves (C) of sugarcane plants according to the vinasse doses and days after planting

Analyzing the effect of vinasse singly, Figure 2A shows that the two lowest PH values were obtained with D600 and D1200. The higher means of PH were promoted at a low concentration of vinasse (D0 and D150). The means observed with MF are consistent with the results of Maradiaga-Rodriguez et al. (2018).

The linear behavior between the doses of vinasse (Figure 2B) shows that the increase in the by-product applied amount resulted in higher NDL, especially at D600 and D1200, which led to similar means.

The regression analysis in NGL (Figure 2C) shows the maximum point occurred between 800 and 1000  $m^3 ha^{-1}$  (between 90 and 120 DAP), i.e., the nutrients present in vinasse enabled sugarcane to produce a greater number of leaves from D600. Souza et al. (2018) observed that a dose of 200  $m^3 ha^{-1}$  was sufficient to increase leaf production.

The supply of excess nutrients at doses D600 and D1200 may have enabled the increase in plant biomass production. Still, the development of the stem, roots, and plant height can respond positively to mineral fertilization instead of vinasse. However, according to the purpose of sugarcane production, this does not indicate a positive point because the excessive development of sugarcane biomass can negatively affect its yield at the end of the cycle, which does not occur when the doses D300 and D150 are applied.

## CONCLUSIONS

1. Vinasse doses of 600 and 1200  $m^3 ha^{-1}$  increased the number of tillers and leaf areas compared to mineral fertilizer and decreased plant height and stem and roots biomass during the initial stage of crop growth.
2. With the application of 150 and 300  $m^3 ha^{-1}$  of vinasse, it is necessary to supplement dose with mineral fertilizer.

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