

Original Article

Nutrient availability and growth of bell pepper plants under fertilization management with humic substances and cattle manure

Disponibilidade de nutrientes e crescimento de plantas de pimentão em manejo de adubação com substâncias húmicas e esterco bovino

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Abstract

Humic substances associated with cattle manure can enhance and reduce the costs of organic fertilization. In this sense, the objective was to evaluate the application of humic substances in association with cattle manure on soil fertility, growth and root nutrition in pepper plants. The experimental design used was randomized blocks, arranged in a factorial scheme with 4 doses of cattle manure (8, 18, 28 and 38 Mg ha⁻¹), 3 doses of humic substances (0, 8 and 12 kg ha⁻¹), with 4 replicates. The variables analyzed were plant height, stem diameter, leaf area, root growth, fresh and root dry weight, root macronutrient contents and soil fertility. Increases in plant height, leaf area and root dry matter (21.2, 58.21 and 8% respectively) were obtained by associating the application of humic substances with manure. Humic substances (12 kg ha⁻¹) in association with manure (8 Mg ha⁻¹) provided an increase in nitrogen content in the roots (36.05%) compared to the application of manure alone, and the application of humic substances (12 kg ha⁻¹) promoted an increase of 10.62% in potassium content in the roots. The availability of calcium in the soil regulated primary root growth. The association of humic substances with cattle manure promoted shoot growth, root dry weight and root nutritional increase in pepper plants.

Keywords: *Capsicum annuum*, humic acid, soil quality, phytomass root.

Resumo

Substâncias húmicas associadas ao esterco bovino podem potencializar e reduzir custos da adubação orgânica. Nesse sentido, objetivou-se avaliar a aplicação de substâncias húmicas em associação com o esterco bovino na fertilidade de solo, crescimento e nutrição radicular em plantas de pimentão. O delineamento experimental utilizado foi em blocos ao acaso, arranjados em esquema fatorial com 4 doses de esterco bovino (8, 18, 28 e 38 t ha⁻¹), 3 doses de substâncias húmicas (0, 8 e 12 kg ha⁻¹), com 4 repetições. As variáveis analisadas foram altura de planta, diâmetro de caule, área foliar, crescimento radicular, fitomassa fresca e seca de raiz, teores de macronutrientes da raiz e fertilidade do solo. Obtiveram-se incrementos em altura de planta, área foliar e fitomassa seca de raiz (21.2, 58.21 e 8% respectivamente) associando a aplicação de substâncias húmicas com esterco. As substâncias húmicas (12 kg ha⁻¹) em associação com o esterco (8 t ha⁻¹) proporcionaram incremento nos teores de nitrogênio na raiz (36.05%) em comparação com aplicação de apenas esterco, como também, a aplicação de substâncias húmicas (12 kg ha⁻¹) promoveu incremento de 10.62% no teor de potássio nas raízes. A disponibilidade de cálcio no solo regulou o crescimento primário da raiz. A associação de substâncias húmicas com esterco bovino promoveu crescimento da parte aérea, fitomassa seca de raiz e incremento nutricional radicular em plantas de pimentão.

Palavras-chave: *Capsicum annuum* L., ácido húmico, qualidade do solo, fitomassa radicular.

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1. Introduction

The species of the genus *Capsicum* have great economic and agricultural importance, especially the pepper (*Capsicum annuum* L.), the crop is widely produced and consumed worldwide, in 2022 the world production of peppers and fresh peppers was 36,972,494.42 tons (FAO, 2024). The great importance of the crop is mainly due to its culinary attributes and high nutritional value, in addition to its content of vitamin C, antioxidants and capsaicinoids (Wang et al., 2016).

Bell peppers have a high diversity in size, shape and color, the green pepper has a large amount of photosynthetic pigments such as chlorophyll *a* and *b*, while the yellow and red peppers have a predominance of carotenoid pigments such as violaxanthin, zeaxanthin, lutein and beta-carotene (Hallmann et al., 2019). Therefore, bell pepper is not only a vegetable with high market value, but also a contributor to human health, since it has a series of bioactive compounds, allowing it to increase the body's immunity against oxidative stress, as these reactive molecules are associated with several multifactorial diseases (Sathiyaseelan et al., 2021).

Fertilization with cattle manure is a simple way to dispose of organic waste from livestock activities. Manure structures the soil, favoring the process of infiltration and water retention, formation of aggregates, improving aeration and reducing the erosion process of tropical soils (Faria et al., 2020). Cattle manure has been used in vegetable cultivation due to its great variability and quantity of nutrients, which can be made available for a prolonged period during the crop cycle, providing adequate growth and development, resulting in increased production (Yang and Zhang, 2022). Several recent studies have shown positive effects of using manure as a source of organic fertilizer in pepper crops (Morit et al., 2023).

Humic substances, due to their chemical characteristics, structural composition and interaction with soil microbiota, are excellent promoters of plant growth, and can act in tolerance to biotic and abiotic stresses, the formation of vascular tissues, apical dominance, especially in the induction and increase in root proliferation, resulting in increased absorption and assimilation of nutrients provided by manure (Korver et al., 2018). The effect on growth specifically is related to the action on metabolism, influencing ion transport, respiratory activity, chlorophyll content, nucleic acid synthesis and membrane enzymes H^+ -ATPases (Jindo et al., 2020). However, plant growth depends on the source, dose, content of bioactive molecules, molecular weight and method of application of the substance (Nardi et al., 2021). In this sense, studies to adapt application techniques to the environmental conditions of crop ecosystems are necessary to maximize the biostimulating effect of humic substances on plant metabolism.

The association of cattle manure with humic substances can be a viable management technique to reduce costs, increase production, and increase the agricultural profitability of the crop. In this context, the aim was to evaluate the application of humic substances in association

with cattle manure on soil fertility, growth and root nutrition in pepper plants.

2. Material and Methods

2.1. Experiment location

The research was carried out between August 2021 and January 2022 at the agricultural company Canteiro Cheiro Verde. The experimental place is located in the municipality of Nova Floresta, state of Paraíba, Northeastern Brazil (6° 27' 8" South latitude, 36° 12' 26" West longitude, 660 m altitude), with a climate classified by Köppen type As" (tropical climate with summer dry season, winter-autumn rainfall) (Alvares et al., 2013). The climate data are shown in Figure 1 (Agriempo, 2022).

2.2. Sources of organic fertilizer

The source of organic fertilizer used was cattle manure, consisting of the following chemical characteristics: Density = 874.5 kg m⁻³; EC = 5.02 dS m⁻¹; pH = 8.98; C to N ratio = 1.2; Moisture_{36°} = 9.09%; N = 15.8 g kg⁻¹; C = 19 g kg⁻¹; P = 5.2 g kg⁻¹; K = 15.3 g kg⁻¹; Ca = 12.7 g kg⁻¹; Mg = 14.4 g kg⁻¹; Na = 6.4 g kg⁻¹; S = 3.7 g kg⁻¹; Si = 14.8 g kg⁻¹; Cu = 23.9 mg kg⁻¹; Fe = 174.09 mg kg⁻¹; Mn = 774.2 mg kg⁻¹; Zn = 105.8 mg kg⁻¹; and B = 7.9 mg kg⁻¹.

A bioestimulant of the company Humik Growth Solutions™ was used extracted from leonardite, consisting of 70% humic acids, 15% fulvic acid, 14% potassium (K₂O), 1% calcium (Ca), 0.15% magnesium (Mg), 0.01% Copper (Cu), 0.002% zinc (Zn), 0.5% iron (Fe), 0.02% boron (B), and 1% nitrogen (N). It exhibited the following physicochemical characteristics: salinity index of 26%, water solubility of 300 g L⁻¹ at 20 °C, cation exchange capacity (CEC) of 200 cmolc kg⁻¹, and pH of 9.68 in a 1:10 (w v⁻¹) solution.

After soil preparation, the manure was applied as a basal dressing and broadcast on the experimental plots. The humic substances were applied to the soil as topdressing, using a solution at a ratio of 1:10 (w v⁻¹), divided into four intervals: eight days after transplanting (DAT), during vegetative growth (31 DAT), at the onset of fruiting (58 DAT), and harvest (87 DAT).

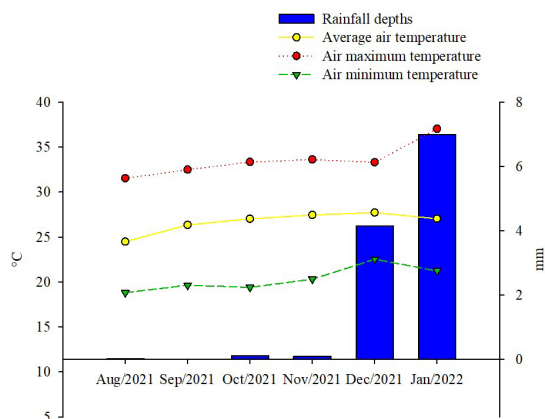


Figure 1. Average air temperature and precipitation depth data during the experimental period.

2.3. Experimental design

The experimental area was divided into four sections measuring 20.8 m (length), 0.8 m (width), and 0.2 m (height). Plants were grown in double rows, with a spacing of 0.4 m between rows and plants and 0.6 m between beds, resulting in an evaluation area of 0.64 m² per plot. The experimental unit consisted of 48 plants. A randomized block experimental design with four replicates was used, in a 4 × 3 factorial arrangement consisting of four cattle manure doses (8, 18, 28, and 38 Mg ha⁻¹) and three humic substance doses (0, 8, 12 kg ha⁻¹). The cattle manure doses were determined through field research in the production region (non-published data), adjusting the doses based on the quantity applied by local growers; the humic substance doses were based on the manufacture's recommendations.

2.4. Conducting the experimente

The soil was scarified and tilled using a scarifier attached to a mini-tractor, and the beds were formed and standardized using an automatic bed shaper. The beds were then covered with plastic mulch. The green bell pepper hybrid used was Kolima (Top Seed®), which has a 105-day growth cycle and produces square-shaped fruits (block type) with thick walls and commercial length and weight of 10 cm and 240 g, respectively. The seedlings were initially sown in 200-cell trays measuring 0.53 m (length), 0.27 m (width), and 0.42 m (height). The substrate used for seeding contained 60 kg of coconut fiber, 20 L of vermicompost, 5 L of ashes, and 100 mL of effective microorganisms consisted of unsalted cooked rice and sugarcane molasses colonized by microorganisms from native forests (Andrade et al., 2020). The seedlings were transplanted to the growing site 35 days after sowing (DAS), when they reached a height of 0.15 m and exhibited five definitive leaves.

Irrigation was performed during periods with no rainfall, using a drip irrigation system with drip tape. The irrigation schedule involved a maximum of 30 min per day with a flow rate of 1.5 L h⁻¹. This resulted in an estimated average daily water application depth of 7.5 mm, split into two applications of approximately 3.75 mm each. These irrigations were performed in the early morning and late afternoon. The soil moisture was continuously monitored using analog tensiometers installed of 0.20 and 0.40 m depths in the planting beds. Irrigation was carried out to ensure a soil moisture above 70% of field capacity.

Fruiting initiation was observed at 22 DAT; excessive shoots were pruned at 42 DAT to improve air circulation within the foliage, increase light incidence, and prevent excessive vegetative growth. Additionally, immature fruits were thinned. Manual weeding was performed between the planting beds to control weed growth. Plant staking was unnecessary, and no significant pest or disease incidences were observed throughout the crop cycle.

2.5. Variables analyzed

Plant height (PH) was measured from the soil base to the last leaf incision of the plant, the measurement was made using a ruler and the values were expressed in centimeters (cm). The stem diameter (SD) was measured

using a digital caliper and the values expressed in millimeters (mm). The leaf area (LA) was calculated after the product of the length and width of three leaves in the median part of the plant crown and corrected with the correlation coefficient of 0.60 proposed by Tivelli et al. (1997), expressed in square centimeters (cm²). Growth evaluations occurred at 56 (DAT).

The roots of the pepper plants were collected at the end of the production cycle (135 DAT), detached from the plant from the collar, and washed with distilled water. Subsequently, the root length (RL) was measured using a graduated ruler (mm). The root fresh weight (RFW) (g plant⁻¹) was determined on a digital scale and then the samples were dried in a forced air circulation study at 65°C to determine the root dry weight (RDW) (g plant⁻¹).

The nutritional contents of root macronutrients were quantified at the end of the production cycle (135 DAT). The plant material was stored in *Kraft* paper bags and subsequently placed in an oven with controlled air circulation at 65 °C for drying until constant mass was obtained. The plant material was then crushed in a *Willey-type* mill and chemically analyzed at Laboratório de Matéria Orgânica do Solo (LABMOS) of the Departamento de Solos e Engenharia Rural of the Universidade Federal da Paraíba (DSER – UFPB).

The nutritional contents of nitrogen (N), phosphorus (P) and potassium (K) were quantified according to the methodology described by Thomas et al. (1967). N was determined by the *Kjeldahl* method, after wet digestion with sulfuric peroxide. The P contents were determined colorimetrically by the molybdenum blue method. The K contents were determined by flame photometry model 910 Analyser®.

The soil in the experimental area was classified as Latossolo Amarelo Eutrófico típico (EMBRAPA, 2018), being collected at a depth of 20 cm between the cultivation lines and close to the plants nutritional absorption bulb in the pre-installation and pre-fruited periods at 58 days after transplanting (DAT). After that, the soil was sieved through a sieve with a 2 mm mesh opening, dried in the shade for 48 hours (Air-Dried Fine Soil) and analyzed according to Teixeira et al., (2017).

The pH was determined using the H₂O water method, measuring the effective electrochemical concentration of H⁺ ions in the soil solution. Phosphorus (P) contents were determined colorimetrically by the molybdenum blue method. Sodium (Na) and potassium (K) by flame photometry, both by extraction with Mehlich-1. Exchangeable calcium (Ca) and magnesium (Mg) were extracted by KCl solution (1 mol L⁻¹) and determined by complexometry with EDTA. The potential acidity (H⁺Al) of the soil was extracted using a calcium acetate solution buffered at pH 7.0, and determined volumetrically using a NaOH solution and the exchangeable acidity (Al³⁺) was quantified using the volumetric method by titration with sodium hydroxide, after extracting aluminum from the soil using KCl (1 mol L⁻¹). Organic matter (OM) was quantified by the wet digestion method with sulfuric acid and potassium dichromate, determined via titrimetry with ammoniacal ferrous sulfate.

2.6. Statistical analysis

The obtained data were tested for normality (Shapiro-Wilk) and subjected to analysis of variance ($p \leq 0.05$). Polynomial regression models were fitted to analyze the effects of cattle manure doses, and Tukey's test was used to compare means for the humic substance doses ($p \leq 0.05$). Principal component and cluster analyses were performed to analyze correlations between variables (growth, root nutrition and soil chemicals). These analyses were conducted using the R 4.3.3 statistical software (R Core Team, 2024), whereas multivariate analyses were conducted using the FactoMineR 2.4 package (Le et al., 2008). Figures were generated using SigmaPlot® 12.5 software.

3. Results

3.1. Aerial and root growth

Plant height (PH), leaf area (LA), root fresh weight (RFW) and root dry weight (RDW) were positively influenced by the interaction of cattle manure with humic substances. However, root length (RL) and stem diameter (SD) was influenced only by cattle manure ($p \leq 0.05$).

The application of 8 kg ha⁻¹ of humic substances (HS) associated with 8 Mg ha⁻¹ of cattle manure (CM) resulted in the highest plant height (37.69 cm), with a reduction in plant height as the manure doses in the soil increased. When comparing this application association (8 kg ha⁻¹ of HS and 8 Mg ha⁻¹ of CM) with the non-application of HS and the application of the highest dose (12 kg ha⁻¹ of HS) at the same CM dosage, an increase of 21.2% was obtained (Figure 2A). The largest leaf area (90.29 cm²) was obtained when applying 12 kg ha⁻¹ of HS associated with 25.18 Mg ha⁻¹ of CM, resulting in an increase of 58.21%, when compared to the non-application of HS at the same dose of CM (25.18 Mg ha⁻¹). Without the application of HS, the leaf area increased as the CM doses increased, obtaining maximum leaf area (66.99 cm²) applying 38 Mg ha⁻¹ of CM (Figure 2B). Regarding the stem diameter, the maximum value (18.98 mm) was observed applying 26.55 Mg ha⁻¹ of CM (Figure 2C).

The greatest root length was obtained at a dose of 8 kg ha⁻¹ of cattle manure (CM), corresponding to a root measuring 22.45 cm in length. This result was followed by a decrease with increasing CM dosage in the soil (Figure 2D). The maximum value of root dry weight (12.28 g) was obtained when applying 8 kg ha⁻¹ of HS associated with 8 Mg ha⁻¹ of CM, resulting in an increase of 8% compared to the non-application of HS at the same manure dosage of 8 Mg ha⁻¹ (Figure 2E).

3.2. Root nutritional content

There was an interaction between the factors cattle manure and humic substances for the nitrogen content in the roots, while the potassium content in the roots was influenced only by cattle manure, however, no significant effect was observed on the phosphorus content ($p \leq 0.05$).

The highest nitrogen content in the pepper root (26.15 g kg⁻¹) was obtained by applying 38 Mg ha⁻¹ of manure (CM) without the application of humic substances (HS).

However, a similar value of N in the root (25.17 g kg⁻¹) was obtained by applying 8 Mg ha⁻¹ of CM associated with 12 kg ha⁻¹ of HS. When comparing the non-application of HS with the application of the maximum dose (12 kg ha⁻¹) at the same CM dosage (8 Mg ha⁻¹), an increase of 36.05% in the N content was observed. These results highlight the importance of using humic substances in reducing CM, since 30 Mg ha⁻¹ of CM is saved for a difference of only 0.98 g kg⁻¹ in root nitrogen content when associating CM with HS, comparing the maximum (38 Mg ha⁻¹) and minimum (8 Mg ha⁻¹) CM doses (Figure 3A). The potassium content in the root (RK) without application of HS increased from 29.2 g kg⁻¹ to 32.3 (g kg⁻¹) with the application of 8 kg ha⁻¹, with an increase of 10.62% regardless of the CM doses evaluated in this study (Figure 3B).

3.3. Soil fertility and correlation between variables

The results of the chemical analyses of the soil before the experiment was installed and before fruiting are shown in Table 1. No exchangeable aluminum (Al³⁺) was detected in the samples analyzed.

Linear correlations between variables were summarized by principal component analysis (PCA). To describe/select data, 3 principal components were retained following the Kaiser criteria (eigenvalue ≥ 1) and cumulative variances (variance $\geq 60\%$) (Table 2).

Cluster analysis was used to group variables with similar variances. Group 1 was formed close to the vectors root length (CR), carbon (C) and calcium (Ca) concentration, resulting from treatments 1 (CM₈HS₀) and treatment 3 (CM₈HS₁₂) (Figure 4), indicating that root growth in verticality is independent of the application of humic substance, however, it is dependent on the adequate dose of cattle manure (8 Mg ha⁻¹), since treatments 1 and 3 add carbon to the soil 1.11 and 4.54 g kg⁻¹, respectively, when compared to the pre-experimental installation condition, in addition to providing the soil with the adequate amounts of Ca for root development 4.15 and 3.90 cmolc kg⁻¹, respectively (Table 1).

Groups 2 and 3 contain the other treatments, except treatment 9 (CM₂₈HS₁₂). The leaf area (LA) and root phosphorus (RP) vectors are close, indicating that there is a direct relationship between RP accumulation and LA. Furthermore, the LA vector also demonstrates a relationship with soil CEC, demonstrating the importance of generating charges in the soil, retaining cationic nutrients and making them available for plant development and growth (Figure 4). Plant height (AP), fresh root weight (RFW) and dry root weight (RDW) are negatively influenced by exchangeable acidity (H+Al), reducing the availability of macronutrients in the soil. Treatment 2 (CM₈HS₈), which presents the highest scores for RFW and RDW (Figure 4), presents a low value of H+Al (0.73 cmolc kg⁻¹) (Table 1).

Treatment 9 (CM₂₈HS₁₂) formed only group 4, presenting a direct correlation with the root phosphorus (RP) and leaf area (LA) vectors, demonstrating the importance of root phosphorus for the vegetative development of the pepper plant, especially the adequate dose for the association of cattle manure with humic substances (Figure 2B). Furthermore, antagonism of magnesium (Mg) levels with phosphorus (P) levels in the soil was observed (Figure 4).

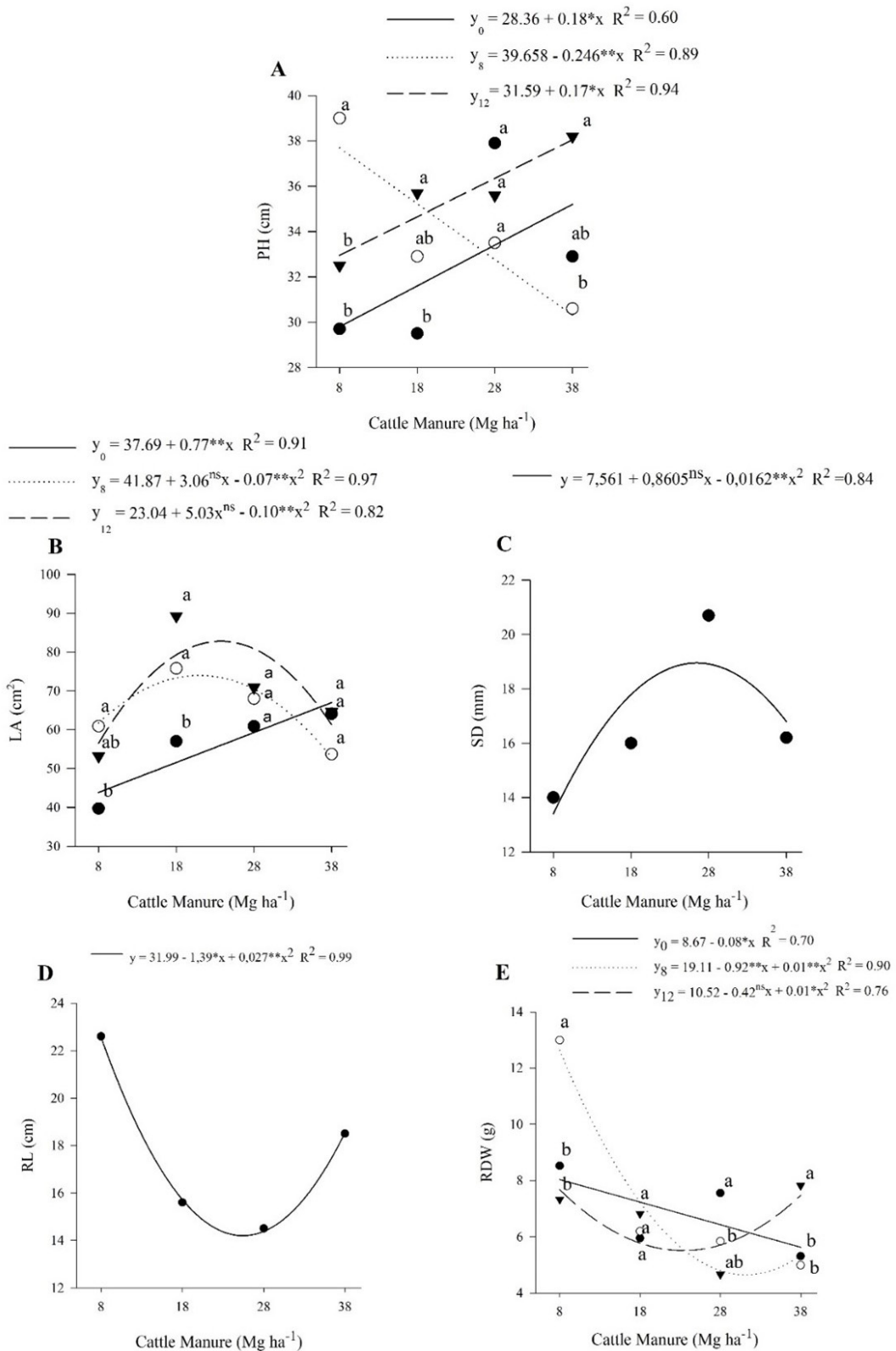


Figure 2. Plant height (A), leaf area (B), stem diameter (C), root length (D) and root dry weight (E) of pepper plants as a function of the application of doses of cattle manure and humic substances. *, **, and ns = significant at $p \leq 0.05$, significant at $p \leq 0.01$, and not significant, respectively, by the F-test; $y_0 = 0 \text{ kg ha}^{-1}$ of humic substances; $y_8 = 8 \text{ kg ha}^{-1}$ of humic substances; $y_{12} = 12 \text{ kg ha}^{-1}$ of humic substances; Means with different letters are not significantly different from each other by the Tukey's test ($p \leq 0.05$).

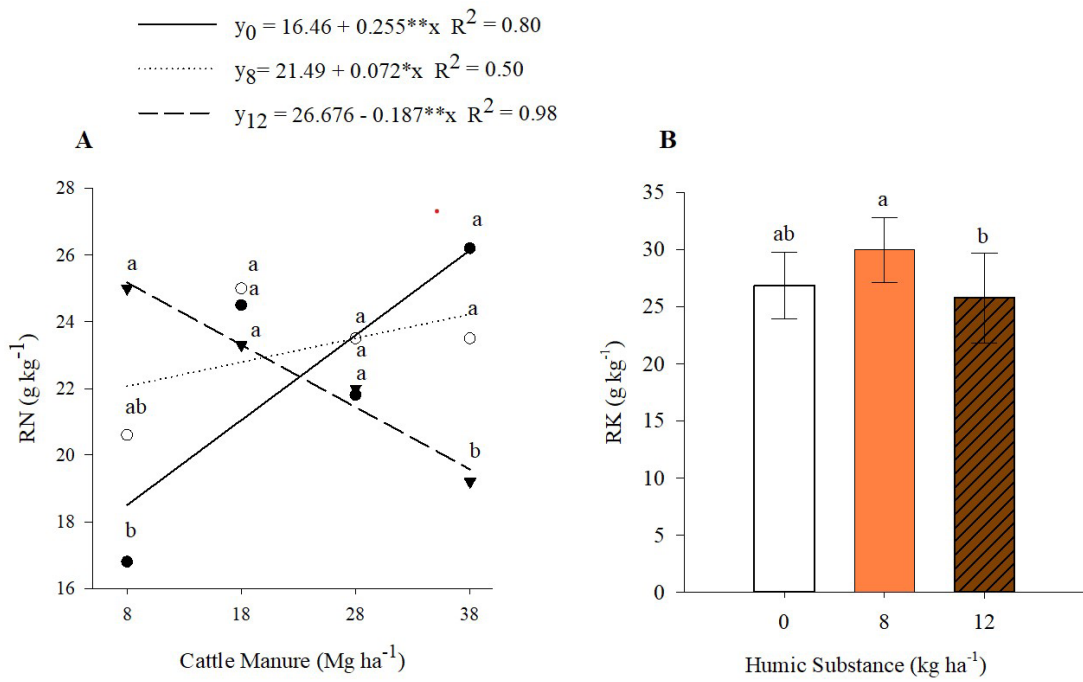


Figure 3. Nutritional contents of nitrogen (A) and potassium (B) in bell pepper roots. *, **, and ns = significant at $p \leq 0.05$, significant at $p \leq 0.01$, and not significant, respectively, by the F-test; y_0 = 0 kg ha⁻¹ of humic substances; y_8 = 8 kg ha⁻¹ of humic substances; y_{12} = 12 kg ha⁻¹ of humic substances; Means with different letters are not significantly different from each other by the Tukey's test ($p \leq 0.05$).

Table 1. Means and standard deviation ($\pm$ SD) of soil chemical elements under application of doses of cattle manure and humic substances.

Experimental pre-installation									
pH	P	K ⁺	Ca ²⁺	Mg ²⁺	Na ⁺	H+Al	CEC	C	
	mg kg ⁻¹	----- cmolc kg ⁻¹ -----						g kg ⁻¹	
6.2	95.52	10.5	4.50	1.70	2.43	1.4	20.53	20.53	
Tratat. Pre-fruiting*									
1	6.3 ± 0.04	134.08 ± 23.41	9.19 ± 4.49	4.15 ± 0.13	0.85 ± 0.68	12.30 ± 6.37	0.48 ± 0.33	32.84 ± 11.22	21.64 ± 1.99
2	6.5 ± 0.02	114.25 ± 13.89	9.98 ± 3.59	3.93 ± 0.49	2.13 ± 1.03	11.50 ± 3.54	0.73 ± 0.37	34.04 ± 6.84	19.93 ± 2.03
3	7.3 ± 0.95	86.45 ± 7.77	7.87 ± 13.35	3.90 ± 0.43	2.53 ± 0.79	10.17 ± 3.58	0.28 ± 0.42	25.46 ± 4.18	25.07 ± 1.57
4	6.5 ± 0.23	76.43 ± 25.93	8.66 ± 2.63	2.78 ± 0.17	3.08 ± 0.41	2.00 ± 1.38	1.10 ± 0.16	23.01 ± 4.04	13.98 ± 2.80
5	6.4 ± 0.11	74.58 ± 1.98	10.24 ± 2.63	3.20 ± 0.29	1.88 ± 1.04	12.31 ± 5.40	1.10 ± 0.11	34.01 ± 7.00	19.61 ± 1.62
6	6.6 ± 0.44	78.02 ± 17.40	9.72 ± 1.58	3.53 ± 0.33	3.58 ± 1.58	10.26 ± 1.82	1.20 ± 0.73	33.65 ± 3.07	18.80 ± 1.99
7	6.8 ± 0.21	72.06 ± 37.77	7.87 ± 2.19	3.40 ± 0.42	3.30 ± 1.79	11.70 ± 8.99	0.28 ± 0.20	33.05 ± 10.70	22.18 ± 1.34
8	6.4 ± 0.13	86.37 ± 19.34	8.66 ± 2.17	3.43 ± 0.25	2.43 ± 0.48	7.69 ± 5.30	0.95 ± 0.46	28.65 ± 9.46	22.82 ± 1.70
9	6.5 ± 0.17	118.45 ± 19.00	9.19 ± 2.77	0.85 ± 0.39	2.58 ± 0.32	9.90 ± 5.30	0.85 ± 0.31	31.44 ± 6.29	14.30 ± 1.43
10	6.7 ± 0.30	102.28 ± 29.00	12.61 ± 2.77	3.91 ± 0.26	2.52 ± 0.68	2.43 ± 1.51	0.74 ± 0.40	24.24 ± 2.79	22.08 ± 2.83
11	6.7 ± 0.69	111.99 ± 7.49	10.15 ± 2.78	3.97 ± 0.17	2.97 ± 0.64	0.22 ± 0.07	0.93 ± 0.49	24.78 ± 3.14	21.86 ± 2.62
12	6.4 ± 0.19	93.28 ± 32.16	14.71 ± 7.92	3.70 ± 0.65	2.43 ± 0.40	7.57 ± 0.28	0.63 ± 0.27	34.84 ± 7.39	20.73 ± 1.92

Trataments: (1) CM₀HS₀; (2) CM₈HS₈; (3) CM₁₂HS₁₂; (4) CM₁₈HS₀; (5) CM₁₈HS₈; (6) CM₁₈HS₁₂; (7) CM₂₈HS₀; (8) CM₂₈HS₈; (9) CM₃₈HS₀; (10) CM₃₈HS₈; (11) CM₃₈HS₁₂; (12) CM₃₈HS₁₂. CM = Mg ha⁻¹; HS = kg ha⁻¹. *Stage characterized by the fertilization of flowers, immediately before the formation of the first fruits.

Table 2. Summary of principal component analysis with variable scores ($p \leq 0.05$) between components (λ_i) 1, 2 and 3.

Variance components	Principal component		
	1	2	3
Eigenvalues λ_i	5.200	3.714	3.117
Variance %	27.36	19.54	16.40
Cumulative of variance %	27.36	46.91	63.32
Variables	Correlation with componentes and (P -value)		
LA	0.76 (0.000)		
CEC	0.70 (0.009)		
PR	0.67 (0.015)		
C	-0.61 (0.032)		
Ca	-0.72 (0.008)		
RL	-0.74 (0.004)		
RFW		0.88 (0.000)	
RDW		0.79 (0.001)	
PH		0.68 (0.015)	
H+Al		-0.65 (0.020)	
P			0.69 (0.013)
Mg			-0.68 (0.014)

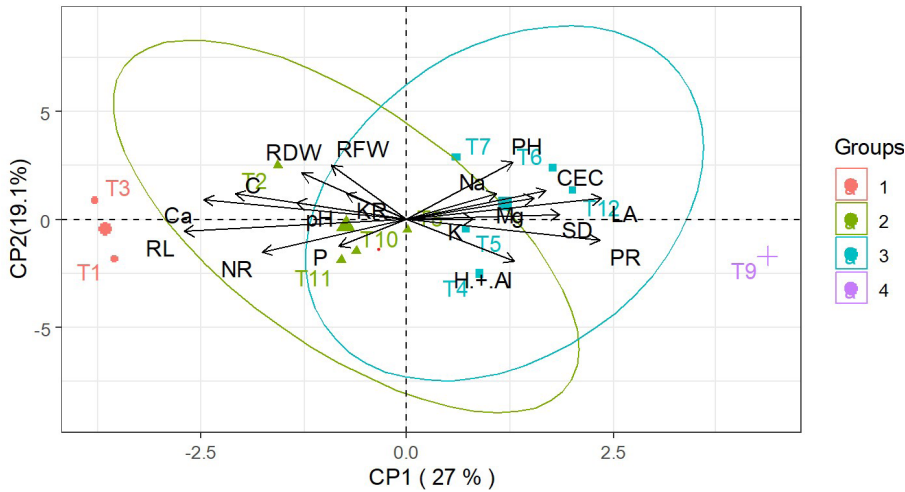


Figure 4. Principal component analysis (ACP) for growth, root nutrition and soil elements of pepper plants under application of cattle manure associated with humic substances. Tratamentos: (1) CM_8HS_0 ; (2) CM_8HS_8 ; (3) CM_8HS_{12} ; (4) $CM_{18}HS_0$; (5) $CM_{18}HS_8$; (6) $CM_{18}HS_{12}$; (7) $CM_{28}HS_0$; (8) $CM_{28}HS_8$; (9) $CM_{28}HS_{12}$; (10) $CM_{38}HS_0$; (11) $CM_{38}HS_8$; (12) $CM_{38}HS_{12}$. CM = $Mg\ ha^{-1}$; HS = $kg\ ha^{-1}$.

4. Discussion

The bell pepper is a tropical crop with thermal requirements ranging from 20 to 30 °C for its complete development, the plant needs milder temperatures (20 to 25 °C) during the flowering and fruiting stages (Blat and Costa, 2007). Based on this information, it is observed that the average air temperature (26.6 °C) was adequate during

the crop cycle (Figure 1), however, all months exceeded the maximum air temperature tolerance limit (30 °C), especially from october onwards, when the flowering stage began and then fruiting, given that these stages require milder temperatures (air temperature ≤ 25 °C), directly influencing production.

The high maximum temperatures recorded during the crop cycle did not significantly affect the plants, possibly due

to the high water supply (7.5 mm day^{-1}), the water demand of peppers can vary from 2.5 to 5.0 mm day^{-1} depending on the cultivation location (Caixeta, 1984). Furthermore, there was no significant rainfall during the cycle, recording only 4.15 and 6.99 mm per month in the months of december and january, respectively (Figure 1).

The humic substances (HS) are excellent promoters of plant growth, the increase in growth is due to several metabolic and physiological processes such as increased permeability of cell membranes, intracellular oxygen content, phosphorus absorption, respiration, photosynthesis and root cell growth (Alsudays et al., 2024). The application of HS has a non-linear behavior in the plant growth response, as the response curve shows a positive correlation of growth as a function of increasing HS concentrations, followed by a reduction in growth under high HS concentrations (Pizzeghello et al., 2020). This behavior was observed in the aerial part of the pepper, more specifically in the plant height, where the highest plant heights (37.69 cm) were observed when applying the intermediate dose of HS (8 kg ha^{-1}), with a reduction in the highest dose of 12 kg ha^{-1} .

When cultivating two pepper hybrids (Barbarian and Kizil) and applying 2 g L^{-1} of humic acid via soil, an average increase of 19.83% was observed when compared to control plants (Fadala et al., 2023), a similar increase obtained in this study 21.2% (Figure 2A). The combination of humic acid with amino acids in the pepper variety NS1701DG, obtained a 50.5% increase in plant height by applying, via foliar application, 3 mL L^{-1} of the biostimulant. This response in plant size is due to the increase in metabolic activities, which produce metabolites responsible for cell division and acceleration in the multiplication of shoot cells in the apical zones, mediated by auxin and stimulated by humic acid in plant growth (Suhaini et al., 2023).

The increase in leaf area is directly related to the increase in chlorophyll and photosynthetic activity. Silva et al. (2024) obtained an increase of 3.35% and 34.2% in chlorophyll and net photosynthesis contents, respectively, applying 12 kg ha^{-1} of humic substances under the same experimental conditions. Corroborating the leaf area results obtained in this study, the combination of humic acid and NPK mineral fertilizer (15:15:15) at doses of 25% humic acid + 75% NPK in pepper planting compared to the control, obtained a 21.96% increase in leaf area (Ichwan et al., 2020). In this sense, organic fertilization, when well performed, can be used as an alternative to mineral fertilization management.

The increase in stem diameter is explained by the adequate dosage of cattle manure, since the manure used in the present study has a residual effect, since the producer uses organic succession management, maintaining a high level of soil organic carbon 20.53 g kg^{-1} (Table 1). In addition, the manure has a low C/N ratio (1.2) and low humidity (9.9%), factors that allow an accelerated mineralization process and greater availability of macro and micronutrients, which can meet the nutritional needs of the bell pepper (Malavolta et al., 1997).

Humic substances (HS) induce the accumulation of auxin in root epidermal cells, resulting in increased root hair formation and cell elongation, which can lead to an increase in root biomass (Nardi et al., 2021). The activation of root ATPases by HS can cause the biological phenomenon of acid growth,

where there is loosening of the cell wall by pumping protons (H^+), making it acidic and favoring growth. This mechanism is induced by indoleacetic acid (Olaetxea et al., 2019). In pepper seedlings, Pandya et al., (2019) found that treatments with humic acids had greater gains in total biomass, demonstrating the beneficial effects of HS on pepper roots.

The morphological and biochemical modifications in the roots promoted by humic substances (HS) are mediated by the polar flow of auxin and nitric oxide (NO) signaling (Nardi et al., 2021). When HS was applied to corn planting, an increase in the enzymatic activity of nitrate reductase was observed, a fact that may indicate greater assimilation of nitrogen (N) and consequent greater synthesis of NO in the root (Vujinović et al. 2020). Potassium (K) is the most required element in pepper crops, as it promotes the translocation of sugars produced in the producing organs to fruits, seeds, and roots, in addition to being associated with cell expansion (Oliveira Filho et al., 2018).

The greater accumulation of potassium (K) in the root can enable better management of cell expansion, promoting greater elongation and more efficient translocation of nutrients, as K causes water retention in the cells, increasing turgor pressure, promoting cell expansion (Menegatti et al., 2019).

The calcium ion (Ca^{2+}) is an important molecular signaler; its adequate availability in the soil promotes satisfactory root growth, since specific proteins sensitive to calcium sensors present in the plasma membranes of the roots regulate nitrate absorption, affecting the general patterns of phosphorylation and the distribution of proteins in the microdomain, promoting primary root growth (Chu et al., 2021).

Phosphorus is a structural component of membrane ATPases, humic acids promote the elevation of H^+ protons from the plasma membrane, activating ATPases, therefore acidifying the apoplast and decreasing the physical resistance of the cell wall, which allows the elongation of root cells and expands the surface of the root area, increasing the electrochemical gradient of protons, boosting the transport of ions across cell membranes, thus improving nutrient absorption (Purwanto et al., 2021).

Soil acidity reduces the availability of nutrients, especially essential macronutrients. The concentration of hydrogen ions resulting from potential acidity is small. However, this form of soil acidity can affect the environment to which the plant is exposed, limiting its growth and development (Dejene et al., 2023).

The application of humic substances increases nitrogen and potassium contents in the roots. The availability of calcium in the soil regulates primary root growth. The concentration of root phosphorus is directly related to the size of pepper plants. Humic substances associated with cattle manure promote increased aerial growth and root dry weight in pepper plants.

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