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ORIGINAL ARTICLE

Cattle manure and humic substances improve organic bell pepper production in the Semiarid Region of Brazil[1]

Esterco bovino e substâncias húmicas estimulam a produção de pimentão orgânico no semiárido brasileiro

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HIGHLIGHTS:
Humic substances improve bell pepper production characteristics.
Excess manure reduces crop yield; thus, this study provides valuable information for production cost reduction.
Application of humic substances reduces the need for large quantities of cattle manure when growing organic bell peppers.

ABSTRACT: Bell pepper is a globally important vegetable. The use of humic substances can enhance nutrient absorption efficiency, reducing the amounts of organic residues applied to the soil. Therefore, the objective of this study was to evaluate cattle manure rates combined with humic substance rates to increase bell pepper production. A randomized block design with four replicates was used, in a 4 × 3 factorial arrangement consisted of four cattle manure doses (8, 18, 28, and 38 Mg ha^{-1}) and three humic substance doses (0, 8, and 12 kg ha^{-1}). Humic substances affected bell pepper production characteristics; the highest mean fruit weight per plant (140.02 g) and fruit diameter (75.73 mm) were obtained when applying 8 Mg ha^{-1} of manure combined with 8 kg ha^{-1} of humic substances. Similarly, the greatest fruit length (80.94 mm) was found when applying 8 Mg ha^{-1} of manure combined with 12 kg ha^{-1} of humic substances. The highest yields (1.2 kg per plant and 60.14 Mg ha^{-1}) were obtained when applying 38 Mg ha^{-1} of manure combined with 12 kg ha^{-1} of humic substances. The combination of humic substances and cattle manure increased the organic production of bell peppers under the edaphoclimatic conditions of the Semiarid region of Brazil.

Key words: *Capsicum annuum* L., organic management, leonardite, biostimulant

RESUMO: O pimentão é uma olerícola de importância mundial. O uso de substâncias húmicas pode elevar a eficiência de absorção nutricional reduzindo a quantidade de resíduos orgânicos aplicados no solo. Assim sendo, objetivou-se avaliar doses de esterco bovino associadas a substâncias húmicas para elevar a produção de pimentão. O delineamento em blocos casualizados distribuído em arranjo fatorial com quatro doses de esterco bovino (8, 18, 28 e 38 t ha^{-1}) e três doses de substâncias húmicas (0, 8 e 12 kg ha^{-1}), quatro repetições foi utilizado. As substâncias húmicas influenciaram nas características produtivas, obtendo-se valores máximos de peso médio de fruto (140,02 g), diâmetro de fruto (75,73 mm) aplicando 8 t ha^{-1} de esterco associado a 8 g ha^{-1} de substâncias húmicas, como também, obteve-se valor máximo de comprimento de fruto (80,94 mm) aplicando 8 t ha^{-1} de esterco associado a 12 kg ha^{-1} de substâncias húmicas. A produção máxima de 1,2 kg por planta e 60,14 t ha^{-1}, obteve-se aplicando 38 t ha^{-1} de esterco associado a 12 kg ha^{-1} de substâncias húmicas. A associação de substâncias húmicas com esterco bovino aumentou a produção orgânica de pimentão nas condições edafoclimáticas do Semiárido.

Palavras-chave: *Capsicum annuum* L., manejo orgânico, leonardita, bioestimulante

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Introduction

Bell pepper (*Capsicum annuum* L.) is a vegetable of significant economic importance, ranking among the top ten most grown crops globally (Shahbandeh, 2021). It has a high market expansion and acceptance due to its organoleptic qualities (González-García et al., 2021) and is a source of calcium (Ca), phosphorus (P), iron (Fe), B-complex vitamins, and carotenoids (Trecha et al., 2017).

The success of bell pepper crops is related to cost-effective production techniques, as the application of soil amendments and fertilizers account for an average of 23.4% of the total production cost (Ferreira & Martins, 2021). Consequently, organic residues can be utilized to improve the production of this vegetable. In this context, cattle manure stands out for its ability to enhance soil nutrient availability, structure, aeration, and water retention capacity (Hijami et al., 2022).

Currently, the availability of organic residues on farms is limited, leading to acquisition difficulties and increased prices of agricultural products. Therefore, developing new bioinputs and techniques and management practices for sustainable food production is essential to reduce environmental impact on ecosystems and improve human health (Srivastav et al., 2021).

The combined application of humic substances and cattle manure can enhance nutrient absorption and assimilation efficiency, reducing soil fertilization costs and increasing crop yields. However, the success of agricultural production relies on effects of the biostimulant interaction with application practices and the environment (Cristofano et al., 2021).

Recent research studies have shown positive effects of applying humic substances for growing pepper crops (Antón-Herrero et al., 2022; Kanabar & Nandwani, 2023; Hosseinifarahi et al., 2024), highlighting the potential of using humic substances as plant biostimulants. Therefore, the objective of the present study was to evaluate cattle manure doses combined with humic substance doses to increase bell pepper production.

Material and Methods

The study was conducted from August 2021 to January 2022 at the Canteiro Cheiro Verde, a family-owned agricultural company that was undergoing organic certification audit by the Instituto Biodinâmico (IBD) during the research period. This company is located in Nova Floresta, Curimataú Paraibano microregion, Paraíba state, Brazil (6° 27' 8" S, 36° 12' 26" W, and altitude of 660 m). The region's climate was classified as As, tropical, according to the Köppen classification, with dry summer and rainy winter and autumn (Alvares et al., 2013). The climate data is shown in Figure 1 (AGRITEMPO, 2022).

The soil in the experimental area was classified as Latossolo Amarelo Eutrofico tipico (EMBRAPA, 2018), corresponding to a Typic Hapludox (USDA, 2014). Soil samples were collected from the 0-20 cm layer, sieved through a 2-mm mesh sieve, and air-dried in the shade for 48 hours. Subsequently, the soil was chemically characterized at the Laboratório de Matéria Orgânica do Solo (LABMOS) and physically characterized at the Laboratório de Física do Solo (LABFIS), both laboratories of the Departamento de Solos e Engenharia Rural of the Universidade Federal da Paraíba (DSER – UFPB) (Table 1).

The organic fertilizer source used was cattle manure, presenting 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 = 17409 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; it was composed of humic substances from leonardite, consisting of 70% humic acids, 15% fulvic acid, 14% potassium (K_2O), 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 $cmol_c$ kg^{-1}, and pH of 9.68 in a 1:10 (w v^{-1}) solution.

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 spacing of 0.4 m between rows and plants and 0.6 m between beds, resulting

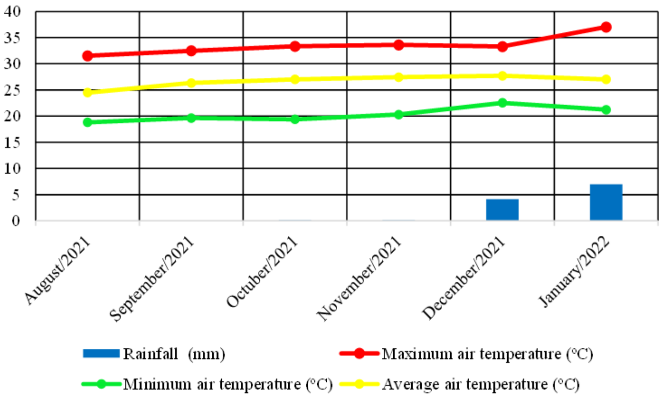


Figure 1. Air temperatures and rainfall depths during the experimental period

Table 1. Chemical and physical characteristics of the soil prior to experiment implementation

Chemical characteristics								
pH	P	K^+	Ca^{2+}	Mg^{2+}	Na^+	Al^{3+}	$H+Al^{3+}$	Organic matter
	(mg kg^{-1})	($cmol_c$ kg^{-1})						(g kg^{-1})
7.4	410.17	0.7	12.24	3.84	2.18	0	0	42
Physical characteristics								
Sand	Silt	Clay	Natural clay	Degree of flocculation	Soil density	Particle density	Total porosity	Texture
(g kg^{-1})					(g cm^{-3})		(m^3 m^{-3})	
727	149	124	26	790	1.53	2.46	0.38	Sandy Loam

Extractors - P, Na, and K – Mehlich 1; H + Al – Calcium acetate 0.5 M, pH 7.0; Al, Ca, and Mg - KCl 1 M in an evaluation area of 0.64 m^2 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 consisted 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.

The manure was applied as a basal dressing and broadcast on the experimental plots after soil preparation. 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 at harvest (87 DAT).

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 a 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, which was 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 at depths of 0.20 and 0.40 m 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. Staking of plants was not necessary, and no significant pest or disease incidences were observed throughout the crop cycle.

The following production variables were assessed: number of fruits per plant, total fruit weight per plant, mean fruit weight per plant, fruit diameter and length, and commercial fruit weight per hectare. These variables were calculated by summing production data of eleven harvests, conducted weekly from 63 to 134 DAT. Only commercial fruits were evaluated, excluding those with severe damage, such as wilting, rot, sunburn, and unhealed lesions, according to the classification standards of the Canteiro Cheiro Verde company.

The obtained data were subjected to analysis of variance (ANOVA; $p \leq 0.05$). Polynomial regression models were fitted to analyze the effects of cattle manure doses, and the Tukey's test was used to compared means for the humic substance doses ($p \leq 0.05$). Principal component and cluster analyses were performed to analyze the data from production variables. These analyses were conducted using the R 4.3.3 statistical software, whereas multivariate analyses were conducted using the FactoMineR 2.4 package (R Core Team, 2023). Figures were generated using SigmaPlot® 12.5 software.

Results and Discussion

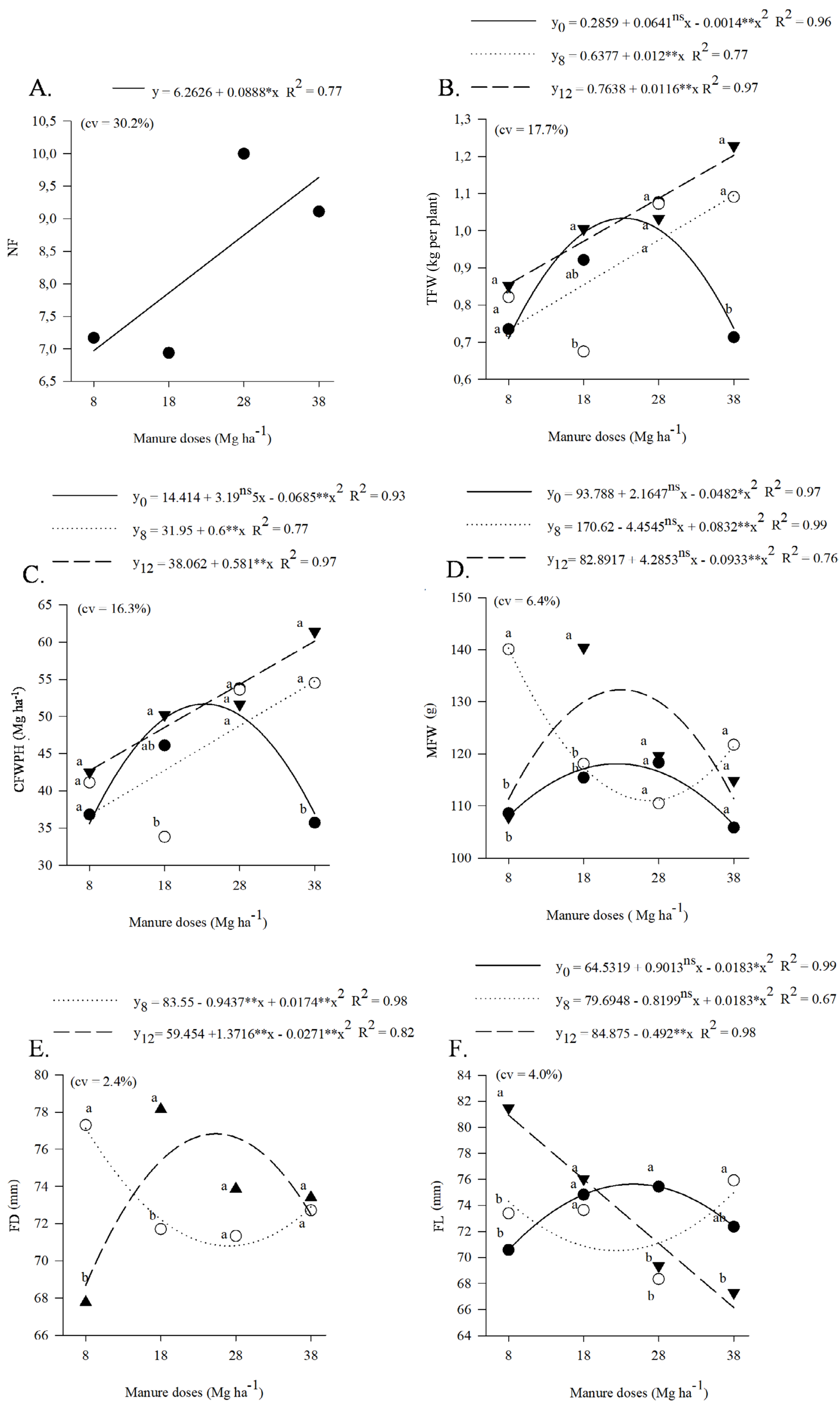
The following production variables were affected by the interaction between cattle manure and humic substances: mean fruit weight per plant, fruit diameter and length, total fruit weight per plant, and commercial fruit weight per hectare. The number of fruits per plant was affected only by the cattle manure factor; however, the humic substance factor had no effect on production variables ($p \leq 0.05$).

A linear positive effect on the number of fruits per plant (NF) was found, denoting that increasing manure doses increased the availability of nutrients for the plants through mineralization, contributing to flowering and fruiting stages. The highest NF (9.31) was achieved when applying the highest cattle manure doses (38 Mg ha^{-1}), representing a 27% increase compared to the lowest evaluated manure doses (8 Mg ha^{-1}) (Figure 2A).

The increased NF as the cattle manure doses was increased can be attributed to the activation of soil microbiota through manure addition, accelerating biochemical mineralization processes. This is due to the low C to N ratio (1.2) of the manure, allowing for a greater nutrient availability for the bell pepper plants. Supplementation with exogenous organic materials, such as manure, results in an interaction with the native soil matter, a biological interaction known as the priming effect, which accelerates mineralization processes (Wu et al., 2021). Additionally, the residual effect of organic succession management practices adopted in the production area contributed to the maintenance of a high soil organic matter content (42 g kg^{-1}) in the pre-planting period (Table 1).

Manure application to the soil provides essential macronutrients, such as N, P, and K, for plant growth and fruit development, especially K, which accumulates in the soil with continuous manure application. This explains the increased NF with application of higher manure doses (Figure 2A) (Camargo, 1984). Additionally, genes encoding the enzyme sucrose phosphate synthase (SPS), which contains phosphorus, are involved in the regulation of the flowering stage, number of flowers, pollen formation, and fruit development (Duan et al., 2021).

Applying a combination of organic manure (5 Mg ha^{-1}), mineral fertilizer (100:75:505 kg ha^{-1}), growth-promoting bacteria (*Azospirillum*; 4 Mg ha^{-1}), and phosphorus-solubilizing bacteria (4 Mg ha^{-1}) for growing bell peppers resulted in the



*, **, 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; and y_{12} - 12 kg ha^{-1} of humic substances. Means followed by different letters are not significantly different from each other by the Tukey's test ($p \leq 0.05$)

Figure 2. Number of fruits per plant (A), total fruit weight per plant (B), commercial fruit weight per hectare (C), mean fruit weight per plant (D), fruit diameter (E), and fruit length (F) as a function of application of cattle manure doses and three humic substance doses

highest NF (9.39) (Chandan et al., 2022). The comparison of this highest NF with that found in the present study (9.31) with the highest manure doses (Figure 2A) denotes the effectiveness of cattle manure in promoting fruit development, as this organic fertilizer management met 100% of the bell pepper plants' nutritional requirements. Foliar application of humic substances (30 mL L^{-1}) increased the number of fruits in two bell pepper hybrids by 16.18% (Saeid & Aswad., 2021).

Total fruit weight per plant and fruit weight per hectare showed quadratic responses to application of manure alone, with the highest means (1.09 kg per plant and 51.65 Mg ha^{-1}, respectively) found for the manure doses of 22.9 Mg ha^{-1} (Figures 2B and C). The means of these variables did not differ significantly when the highest cattle manure doses (38 Mg ha^{-1}) was combined with the tested humic substance doses (8 and 12 kg ha^{-1}), according to Tukey's test ($p \leq 0.05$); however, a significant difference was found when compared to the treatment fertilized only with manure.

Combining the highest cattle manure doses (38 Mg ha^{-1}) with the 12 kg ha^{-1} of humic substances resulted in means of 1.2 kg per plant and 60.14 Mg ha^{-1}, corresponding to increases of 10 and 16.43%, respectively, compared to those found when applying only manure (Figures 2B and C). Foliar application of humic substances (30 mL L^{-1}) to bell pepper plants of two different hybrids resulted in increased fruit yield, with the highest yield (48.1 Mg ha^{-1}) representing a 31.01% increase compared to the control (Saeid & Aswad, 2021). Contrastingly, humic substances did not affect fruiting of bell pepper plants in the present study; this may be due to the genetic characteristic of the hybrid used (Kolima), which may lack genes responsive to the flowering-inducing and fruiting-promoting effects of humic substances.

This greater effect of humic substances on crop yield, compared to that found in the present study (16.43% increase), may be attributed to the application method used (foliar application), which differed from that used in the present study (soil application). Yield gains due to application of humic substances depend on the interaction between management practices and environmental growing conditions, especially soil structure characteristics. In this context, the clay content of 124 g kg^{-1} (Table 1) may have potentiated the beneficial effects of humic substances in the soil. The interaction of organic matter with clay can lead to the formation of clay-organic complexes, aggregating colloids and structuring aggregates. Consequently, this promotes root development and increases water retention and uptake in the soil (Bertoni & Lombardi, 2017).

The increase in fruit yield due to application of humic substances can be attributed to anatomical, biochemical, and molecular changes in the roots, which can lead to root elongation and early differentiation in the apical and primary zones (Pizzeghello et al., 2020; Nardi et al., 2021), resulting in increased nutrient uptake by roots, enabling them to explore larger soil volumes (Nardi et al., 2021).

Soil fertilization with cattle manure and foliar application of biofertilizers (500 mL planting $hole^{-1}$ at 20%) to bell pepper plants grown in the Semiarid region of Brazil resulted in the highest fruit yields (0.485 kg per plant and 9.6 Mg ha^{-1}) when applying 14.5 and 14.0 Mg ha^{-1} of manure, respectively (Araújo et al., 2007); the yield per hectare found in the present study was approximately 6.5 times higher (60.14 Mg ha^{-1}). This denotes a greater effectiveness of the biostimulant compared to the biofertilizer. The number of fruits per plant was affected only by manure application, differing from the other production variables, which were affected by the interaction between cattle manure and humic substances; this may be attributed to the initial soil conditions, with a high organic matter content, 42 g kg^{-1} (Table 1), which probably suppressed the flowering-inducing effect of humic substances, consequently reducing their effect on fruiting.

Humic substances significantly affected the mean fruit weight per plant (MFW), fruit diameter (FD), and fruit length (FL) ($p \leq 0.05$). Regarding the lowest manure doses (8 Mg ha^{-1}), combining it with 8 kg ha^{-1} of humic substances resulted in significant difference in MFW and FD, whereas combining it with 12 kg ha^{-1} of humic substances resulted in significant difference in FL compared to the other humic substances treatments (Figures 2D, E, and F).

Applying 8 Mg ha^{-1} of manure resulted in the highest MFW (140.02 g) and FD (75.73 mm) when combined with 8 kg ha^{-1} of humic substances, whereas its combination with 12 kg ha^{-1} of humic substances resulted in the highest FL (80.94 mm). Manure application alone did not allow for fitting the equation ($y = 68.5214+0.0844^{ns} \times R^2 = 0.58$) for FD; however, the highest MFW (118.14 g) and FL (75.62 mm) were obtained with manure doses of 22.48 and 24.62 Mg ha^{-1}, respectively. The highest MFW and FL obtained with application of humic substances were 18.52 and 7.03% higher, respectively. The treatment with 8 kg ha^{-1} of humic substances combined with the lowest manure doses (8 Mg ha^{-1}) resulted in increases of 13.12, 5.63, 7.03% in mean fruit weight per plant, fruit diameter, and fruit length, respectively, compared to the maximum values found when applying only manure, enabling reductions in the amount of manure applied by 14.48, 30.00, and 16.62 Mg $ha^{-1,}$ respectively. Humic substances affect commercial fruit characteristics by interacting with cell wall components and membrane receptors, activating specific internal genes and triggering transduction cascades (Muscolo et al., 1998; Nardi et al., 2021).

Foliar application of a biostimulant composed of glycine betaine, alginic acid, and a vitamin K1 derivative (0.5 mL per plant) to tomato plants in the field resulted in a 28.4% increase in fruit yield, attributed to a 5.25% increase in mean fruit weight (Zucatti et al., 2020); however, application of a biostimulant composed of humic acids, polysaccharides, polypeptides, amino acids, hormone precursors, and vitamin complexes had no effect on fruit yield and mean fruit weight. The effects of humic substances on plant growth and crop yield depend on the method of application to the plants, content of bioactive molecules, source, application doses, molecular weight of the humic fraction, and plant species (Nardi et al., 2021).

Linear correlations between production variables were summarized using principal component analysis. Two principal components (PC) were retained based on Kaiser's criterion (eigenvalue > 1) and cumulative variances: PC1 and PC2 accounting for 47.3 and 28.5% of the variance of the data, respectively. Although the PC2 presented a value considered low, the sum of the variances of PC1 and PC2 accounts for 75.8% of the total variance, meeting the criterion of accumulated variance to explain the data (variance > 60%) (Figure 3).

PC1 separates the variables number of fruits per plant (NF), total fruit weight per plant (TFW), commercial fruit weight per hectare (CFWH), and fruit diameter (DF), whereas PC2 separates mean fruit weight per plant (MFW) and fruit length (FL). TFW and CFWH have a positive correlation with NF. However, NF had a negative correlation with FL, MFW, and FD.

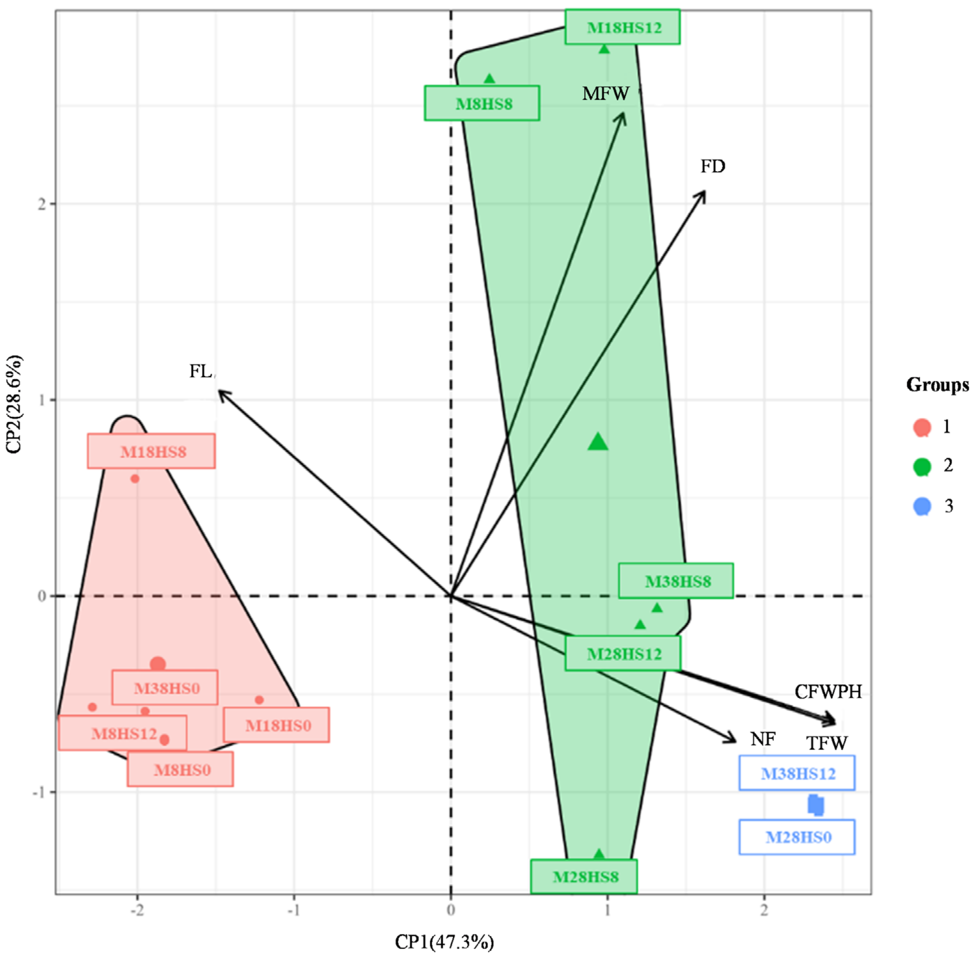
Regarding the cluster analysis for production variables, Group 1 was formed close to the FL vector (Figure 3) and was characterized by treatments with lower cattle manure doses (8 and 18 Mg ha^{-1}) combined with an intermediate humic substance doses (8 kg ha^{-1}). Group 2 was formed by treatments with higher manure doses combined with humic substance doses (8 and 12 Mg ha^{-1}), which resulted in the highest NF, showing direct correlation with CFWH and TFW, resulting in higher yields.

Additionally, Group 3 was formed near the TFW and CFWH vectors, characterized by treatments with only manure application at 28 Mg ha^{-1} or the combination of manure at 38 Mg ha^{-1} and humic substances at 12 kg ha^{-1} (Figure 2), showing the highest means for TFW and CFWH. The observed correlations denote the effect of fertilization management practices on bell pepper production and quality.

The observed decreasing trend in production characteristics with increasing manure doses can be attributed to the linear increase in the number of fruits per plant obtained at higher manure doses. A greater number of fruits indicates the need for a higher quantity of photosynthates, which are translocated to the developing fruits. Contrastingly, a lower number of fruits per plant indicates a concentration of metabolic energy and photosynthates, resulting in larger, more robust, and heavier fruits. Similarly, Shirahige et al. (2010) found a direct correlation between the number of fruits and the mean fruit weight when evaluating hybrid tomato plants; decrease in the number of fruits resulted in an increase in mean fruit weight, indicating a shift in the source-sink relationship, concentrating photosynthates in fruits of plants with fewer fruits.

The dynamics of plant metabolism involve competition for photosynthates among various sources and sink organs (leaves, fruits, and new shoots). Fruit size and weight tend to decrease as the number of fruits increases due to a higher competition for photosynthates. This is due to the plant's need to translocate photosynthates, distributing them to a larger number of fruits (Nascimento et al., 2020).

The increased fruit yield found in the present study with combined application of cattle manure and humic substances



M - 8, 18, 28, and 38 Mg ha^{-1} of cattle manure; HS - 0, 8, and 12 kg ha^{-1} of humic substances

Figure 3. Principal component analysis for production variables: number of fruits per plant (NF), total fruit weight per plant (TFW), commercial fruit weight per hectare (CFWPH), mean fruit weight per plant (MFW), fruit diameter (FD), and fruit length (FL)

is consistent with the study of Silva et al. (2024), who reported positive responses in morphophysiology of bell pepper plants grown under the same experimental conditions.

Conclusions

1. The treatment with 8 kg ha^{-1} of humic substances combined with the lowest manure doses (8 Mg ha^{-1}) resulted in increases of 13.12, 5.63, 7.03% in mean fruit weight per plant, fruit diameter, and fruit length, respectively, compared to the maximum values found when applying only manure, enabling reductions in the amount of manure applied by 14.48, 30.00, and 16.62 Mg ha^{-1}, respectively. Therefore, using humic substances as plant biostimulants can contribute to reductions in cattle manure application rates.

2. The highest bell pepper yields (1.2 kg per plant and 60.14 Mg ha^{-1}) was found when combining the application of 38 Mg ha^{-1} of cattle manure and 12 kg ha^{-1} of humic substances.

3. The combination of humic substances and cattle manure improved the organic production of bell peppers under the edaphoclimatic conditions of the Semiarid region of Brazil.

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