Influence of the application of humic substances on the growth of watermelon and melon seedlings

Influencia de la aplicación de sustancias húmicas en el crecimiento de plántulas de sandía y melón

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ABSTRACT

Growing watermelon (*Citrullus lanatus*) and melon (*Cucumis melo*) is an important activity in the Brazilian Cerrado; however, many factors limit cultivation, including the difficulty of producing high-quality seedlings. In this study, the effect of humic substances (HS) on growth of 'Crimson Sweet' watermelon and 'Yellow' melon seedlings was evaluated. An experiment was carried out in a completely randomized design with five HS treatments applied to the soil: 0 (control), 5, 10, 15, and 20 g L^{-1}. The growth of watermelon and melon seedlings was influenced by the application of humic substances. Use of HS increased plant height, stem diameter, root length, root volume, shoot dry biomass, root dry biomass, and Dickson's quality index. HS boost plant growth, whose improved root system may have provided greater absorption and accumulation of mineral nutrients. Based on the quality of the seedlings (Dickson index), the application of 14 g L^{-1} of HS is recommended for watermelon seedling production and 15 g L^{-1} of HS for melon seedling production.

Key words: humic acids, *Citrullus lanatus* Schrad, *Cucumis melo* L., tropical horticulture, biostimulants, seedling quality.

RESUMEN

El cultivo de sandía (*Citrullus lanatus*) y melón (*Cucumis melo*) tiene gran importancia para los agricultores del Cerrado brasileño. Sin embargo, existen algunas limitaciones de producción, incluida la dificultad en la producción de plántulas de calidad. En este estudio evaluamos la influencia de sustancias húmicas (SH) en el crecimiento de plántulas de sandía 'Crimson Sweet' y melón 'Amarillo'. Para ello, el estudio se configuró en un diseño completamente aleatorio con cinco tratamientos, que consistieron en los siguientes tratamientos de SH aplicados al suelo: 0 (control), 5, 10, 15 y 20 g L^{-1}. El crecimiento de las plántulas de sandía y melón fue influenciado por la aplicación de sustancias húmicas. Hubo un aumento en la altura de las plantas, el diámetro del tallo, la longitud y el volumen de las raíces, así como la biomasa de brotes y raíces y el índice de calidad de Dickson. Las SH impulsaron el crecimiento de las plantas, cuya mejora del sistema radicular puede haber proporcionado una mayor absorción y acumulación de nutrientes. Según la calidad de la plántula (índice de Dickson), se recomienda aplicar 14 g L^{-1} de SH para la producción de plántulas de sandía y 15 g L^{-1} de SH para la producción de plántulas de melón.

Palabras clave: ácidos húmicos, *Citrullus lanatus* Schrad, *Cucumis melo* L., horticultura tropical, bioestimulantes, calidad de plántulas.

Introduction

Cucurbit crops, such as watermelon (*Citrullus lanatus*) and melon (*Cucumis melo*), are of worldwide importance, cultivated in over 3.5 and 1.2 million ha and yielding 101 and 33 million t, respectively (Ebert, 2019). Brazil is the fourth and eleventh largest producer of watermelon and melon, respectively (IBGE, 2020). The Brazilian state of Maranhão stands out, located on the last agricultural frontier of the Brazilian Cerrado. The state has a high fruit-producing potential due to the considerable volume of annual rainfall, the well-distributed quality of the soils ranging from sandy to clayey, and proximity to ports. Therefore, Maranhão can become an important exporter (Caldas *et al*., 2022).

The initial growth of cucurbits is one of the most important phases due to the plant's high nutritional and water demand and sensitivity to biotic and abiotic stresses (Nóbrega *et al*., 2020; Ó *et al*., 2020). Parameters evaluated at the beginning of the growing cycle indicate whether the plant will develop and establish satisfactorily in the field (Phani *et*

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al., 2021; Xanthopoulou *et al*., 2022; Cáceres-Hernandez *et al*., 2023). Good initial development depends on the use of technologies to support plants, especially in the Cerrado, with soils of low natural fertility and high acidity (Dias *et al*., 2019; Lima *et al*., 2019; Procópio & Barreto, 2021) and with a climate classified as humid tropical. In the tropics, finding a good and appropriate seed and a high-quality vegetable seedling has become one of the main key limitations. There is no doubt that the use of good horticultural practices such as a proper substrate and nutrient solution are key factors in achieving the production of vigorous seedlings and, subsequently, obtaining profitable yields (Ramírez-Guerrero *et al*., 2015).

To overcome such limitations when seeking the production of high-quality seedlings, the use of substances with biostimulant properties is an interesting technology in sustainable agriculture to face problems related to fertilizer use in agricultural crops (Nardi *et al*., 2021). Humic substances (HS) have been used to supply nutrients and/or stimulate the production of phytohormones that lead to root growth that the plant would otherwise not achieve (El-Hai *et al*., 2019; Qin & Leskovar, 2020a; Soteriou *et al*., 2021). Previous studies have demonstrated the effect of humic substances as seedling growth modulators, among other beneficial effects (Gomes Júnior *et al*., 2019; Qin & Leskovar, 2020a; Silva *et al*., 2022; Targino *et al*., 2023).

However, there is much variation in the chemical composition of humic substances, in addition to different effects depending on the crop species (Asadi Aghbolaghi *et al*., 2022; Rostami *et al*., 2022, Sensoy *et al*., 2022). Therefore, it is necessary to evaluate the application of humic substances in short-term cycle fruit species growing under the conditions of the Brazilian Cerrado. This study evaluated how and to what extent the growth of 'Crimson Sweet' watermelon and 'Yellow' melon seedlings can be affected by the application of humic substances at different concentrations.

Material and methods

Study location

The study on the production of cucurbit seedlings was carried out in a greenhouse with 75% shading, from August to September 2017, at the Center for Agricultural and Environmental Sciences (CCAA) of the Federal University of Maranhão (UFMA) (03º44'17" S, 43º20'29" W, altitude 107 m a.s.l.), located in the municipality of Chapadinha, state of Maranhão, Brazil. The region's climate is classified as humid tropical (Aw) (Alvares *et al*., 2013).

Experiment setup

To evaluate the effect of humic substances on watermelon and melon crops, the study was carried out in a completely randomized design with five treatments. Each treatment consisted of the application of a HS rate: 0 (control), 5, 10, 15, and 20 g L^{-1}, with five replicates. Each replicate consisted of five seedlings, totaling 125 seedlings.

The soil was classified as Dystrophic Yellow Latosol according to the Brazilian Soil Classification System (Santos *et al*., 2018). This type of soil is typical in the tropical region of the Brazilian Cerrado. A soil sample was collected (0.0-0.20 m deep) for chemical and granulometric analyses (Teixeira *et al*., 2017) (Tab. 1).

Polystyrene trays having 198 cells, with 18 cm^3 cell volume, were filled with soil. The seeds were obtained from the company Feltrin (Brazil). Two seeds of watermelon cultivar 'Crimson Sweet' and melon cultivar 'Yellow' were sown in each cell at 1.5 cm deep from the soil surface. At 7 d after sowing, thinning was performed to keep one seedling per cell in the tray. The application of humic substances (HS) corresponded to 1 ml per cell of the respective treatments (0, 5, 10, 15, and 20 g L^{-1}) via soil before sowing the seeds. In control treatment (0 g L^{-1}), only water was applied so that the seedlings received the same amount of liquid. The source of HS was the organomineral compound Humitec

TABLE 1. Chemical and granulometric characterization of the soil.

pH	OM	P	K^+	Ca^{2+}	Mg^{2+}	Na^+	Al^{3+}
(H_2O)	g kg^{-1}	mg kg^{-1}	------------cmolc kg^{-1}------------				
5.06	15.4	13	0.07	0.80	0.30	0.31	1.50

$S\text{-}SO_4^{2-}$	H+Al	BS	m	sand	fine sand	silt	clay
cmolc kg^{-1}		---------%--------		------------g kg^{-1}------------			
1.5	7.26	17	50	348	336	112	168

pH = potential of hydrogen; OM = soil organic matter; P = phosphorus; K^+ = potassium; Ca^{2+} = calcium; Mg^{2+} = magnesium; Na^+ = sodium; Al^{+3} = aluminum; H+Al = potential acidity; $S\text{-}SO_4^{2-}$ = sulfur; BS = base saturation; and m = aluminum saturation.

WG® Tradecorp company (Brazil), consisting of 17% K_2O, 31% organic carbon, 68% total humic extract, 52% humic acids, and 16% fulvic acids. No fertilization other than HS was applied. Irrigation was performed daily to maintain soil moisture at 70% of soil water retention capacity. To ensure that there was no excess or shortage of water for the seedlings, the lysimeter weighing method was applied. Substrate humidity was monitored daily, and water lost through evapotranspiration was replaced with the aid of a beaker graduate.

Variable analyzed

Evaluations of the effects of HS on the production of seedlings were performed 20 d after sowing, which is the length of the production phase (growth and development) of the seedlings. Number of leaves (NL) was determined from direct count in each seedling; plant height (PH) was measured from the collar to the apex of the seedling using a millimeter ruler; stem diameter (SD) was obtained with a digital caliper (Digimess®); root length (RL) was measured using a ruler graduated in mm; root volume (RV) was determined by measuring the displacement of a water column in a graduated cylinder according to Harrington *et al*. (1994); shoot dry mass (SDM) and root dry mass (RDM) were determined by weighing on a scale with precision of 0.01 g the respective dry plant biomass. To obtain dry plant biomass, the plant material was dried in a forced-air oven at 65°C until constant weight. The quality of the seedlings was determined using the Dickson quality index (DQI) (Dickson *et al*., 1960).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) by the F test for detection of significant effect using the Infostat® software (Di Rienzo *et al*., 2020) and the data explored by quantitative regression analysis when a significant effect was found ($P<0.05$). Multivariate analysis was also carried out with principal component analysis (PCA) using the SAS software (SAS® Inst. Inc., Cary, NC) to evaluate the relationships and determining factors.

Results

The growth of watermelon and melon seedlings was influenced by the application of humic substances (HS). Except for number of leaves (NL), the other variables of size, biomass, and quality of watermelon seedlings were influenced by the application of HS ($P<0.05$). Similarly, there was no effect of HS application on NL of melon. On the other hand, there was influence ($P<0.05$) of HS on the biometric and biomass responses of melon seedlings, including seedling quality index (Tab. 2).

Increasing HS rate in soil promoted a significant increase in watermelon seedling height of 25%. Height response of melon seedlings was consistent with a second-degree curve, in which there was a maximum increase of 6.9 cm in height at the HS rate of 9.6 g L^{-1}, followed by a decrease in height of the melon plants (Fig. 1A). There was a different response regarding stem diameter for each cucurbit species (Fig. 1B). Higher HS rate (20 g L^{-1} of HS) was necessary for the melon plants to obtain a greater increase in stem diameter, while watermelon seedlings obtained the maximum stem (2.3 mm) increment with the application of only 11.2 g L^{-1} of HS.

The HS rate of 18.2 g L^{-1} promoted maximum root length of watermelon seedlings by increasing root growth by 31% (Fig. 1C), which validates how watermelon is responsive to HS in the initial growth phase. Despite the lack of response of root length in melon seedlings to HS application, root volume (development phase) of melon seedlings was strongly influenced by HS, with an increase of up to 0.5 cm^3 at 11.8 g L^{-1} of HS. This increase is 38% more than that of untreated plants (0 g L^{-1} of HS). Watermelon root volume (development phase) was also strongly influenced

TABLE 2. Growth of watermelon and melon seedlings in response to doses of humic substances.

Watermelon	NL	PH	SD	RL	RV	SDM	RDM	DQI
P-value	0.17	<0.001	<0.001	0.001	0.008	<0.001	0.02	0.01
CV%	6.12	5.50	7.30	9.48	18.22	9.26	33.75	22.84
Melon	NL	PH	SD	RL	RV	SDM	RDM	DI
P-value	0.09	<0.001	<0.001	0.07	0.04	<0.001	<0.001	<0.001
CV%	3.09	8.72	10.13	9.27	27.97	22.15	22.75	20.76

NL: number of leaves, PH: plant height, SD: stem diameter, RL: root length, RV: root volume, SDM: shoot dry mass, RDM: root dry mass, and DQI: Dickson quality index. CV: coefficient of variation; *P*-value < 0.05: significant at the 0.05 level of probability according to the F-test.

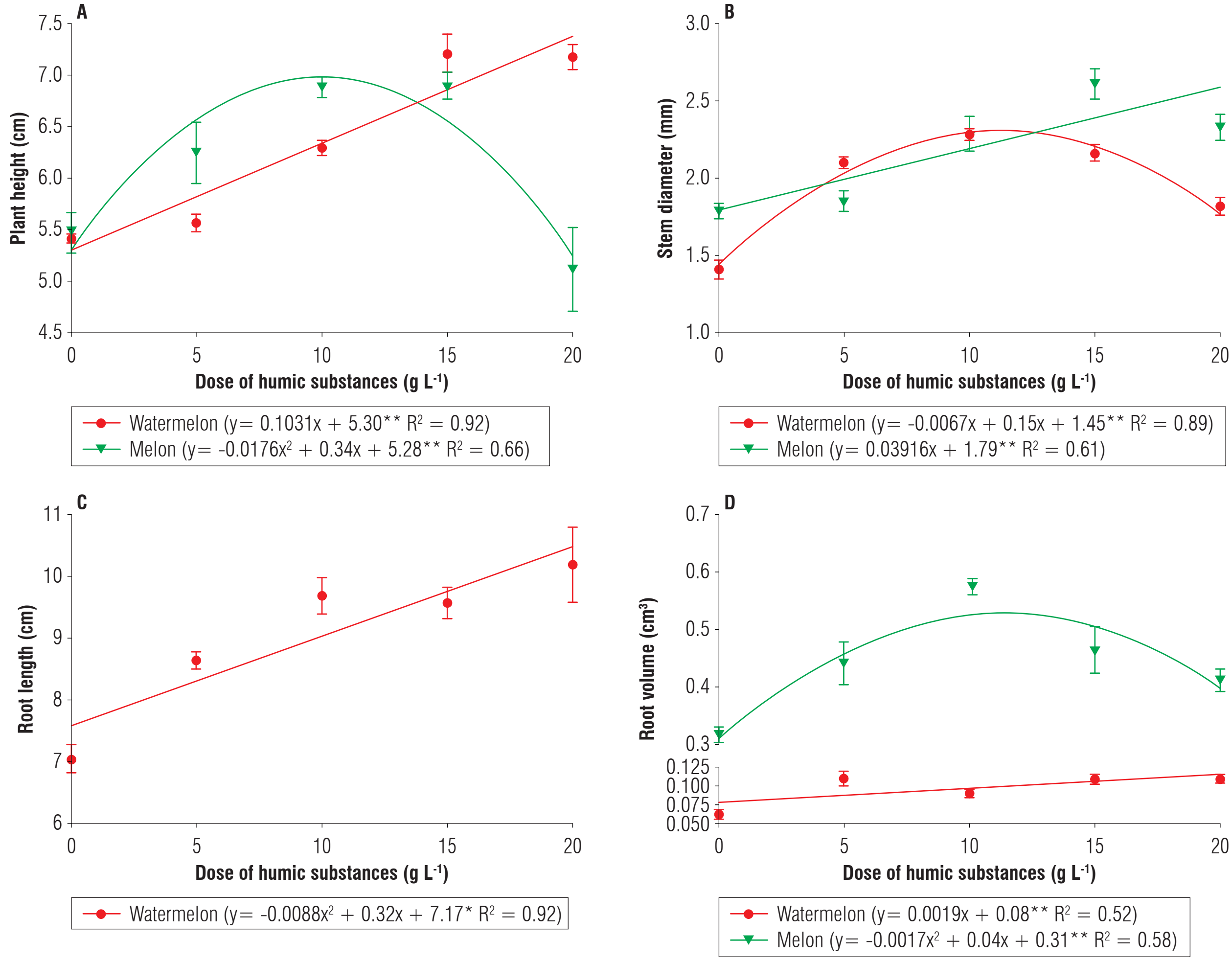

FIGURE 1. Responses of plant height (A), stem diameter (B), root length (C) and root volume (D) of watermelon and melon seedlings to application of humic substances. Mean (●▼) ± standard error. * and ** are significant at the 0.05 and 0.01 level of probability, respectively.

by HS, with an increase of 43% for the maximum rate (20 g L^{-1} of HS) (Fig. 1D).

The HS rate of 20 g L^{-1} led to an increase in shoot dry mass of watermelon seedlings, with an increase of 42% compared to that of plants under 0 g L^{-1} of HS. A lower HS rate (12 g L^{-1}) increased shoot dry mass of melon seedlings, with a maximum of 0.07 g of shoot biomass per seedling (Fig. 2A). Root dry mass of watermelon seedlings increased linearly, with an increase of 53% at 20 g L^{-1} of HS when compared to the untreated treatment (0 g L^{-1}). Root dry mass of melon seedlings, however, fitted to a quadratic model, in which a maximum increase in root biomass was observed at a rate of 13 g L^{-1} HS, when the seedlings had 0.05 g of root biomass (Fig. 2B).

Improved quality of cucurbit seedlings was observed with the use of HS. The Dickson quality index suggests that a HS rate of 14 g L^{-1} promoted better seedling quality with a value of 0.020, that is, 45% higher than that of the control treatment (0 g L^{-1} of HS). Melon seedlings required a higher HS input (15 g L^{-1} of HS) to obtain a better response regarding seedling quality, with a value of 0.024, which represents an increase of 57% in relation to that of the control treatment (Fig. 3).

Principal component analysis shows the correlation between rates (0, 5, 10, 15, and 20 g L^{-1}) of HS and growth and development responses, biomass production and seedling quality of watermelon (Fig. 4A) and melon (Fig. 4B). With regard to the production of watermelon seedlings, HS rates of 10 and 15 g L^{-1} resulted in greater effects on SDM, PH, SD, and RL. Humic substances rates of 5 and 20 g L^{-1} were highly correlated with the variables DQI, NL, RDM, and RV. The absence of HS (0 g L^{-1} of HS) was associated with low values of watermelon seedling production variables.

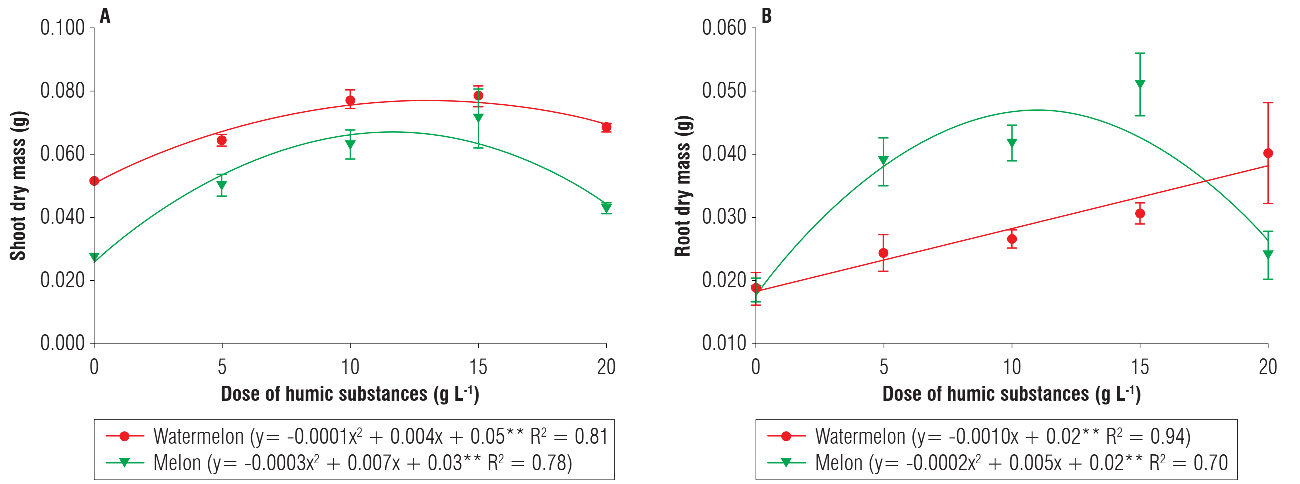

FIGURE 2. Responses of shoot dry mass (A) and root dry mass (B) of watermelon and melon seedlings to application of humic substances. Mean (●▼) ± standard error. * and ** are significant at the 0.05 and 0.01 level of probability, respectively.

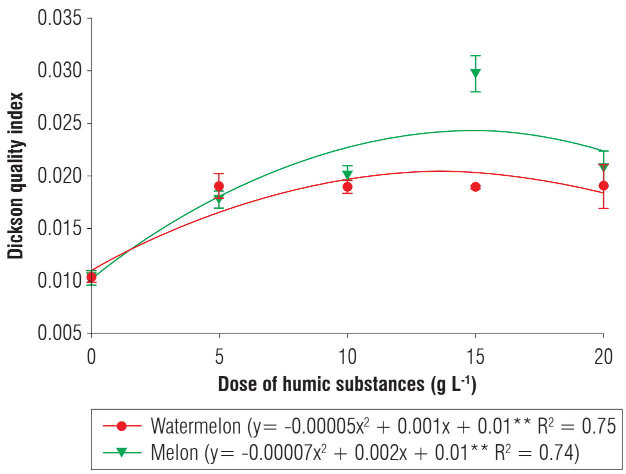

FIGURE 3. Responses of watermelon and melon seedlings to application of humic substances as measured by the Dickson quality index. Mean (●▼) ± standard error. * and ** are significant at the 0.05 and 0.01 level of probability, respectively.

In the production of melon seedlings, there was an overlap of the rate of 0 and 20 g L^{-1} of HS, with a high mean value of PH, but low values of DQI, SDM, and SD. These variable responses are highly correlated with HS rates of 5 and 15 g L^{-1}. The HS rate of 10 g L^{-1} is associated with high values of RL, NL, RDM, and RV.

Discussion

The use of humic substances in tropical Cerrado soil improves growth and development, biomass and quality of watermelon and melon seedlings. Reports have demonstrated that HS are able to regulate plant growth due to their biostimulation effects similar to auxins (Zandonadi *et al*., 2007; Canellas & Olivares, 2014; Olaetxea *et al*., 2015; Olivares *et al*., 2017; Qin & Leskovar, 2020b), with structural and physiological changes in roots and shoots

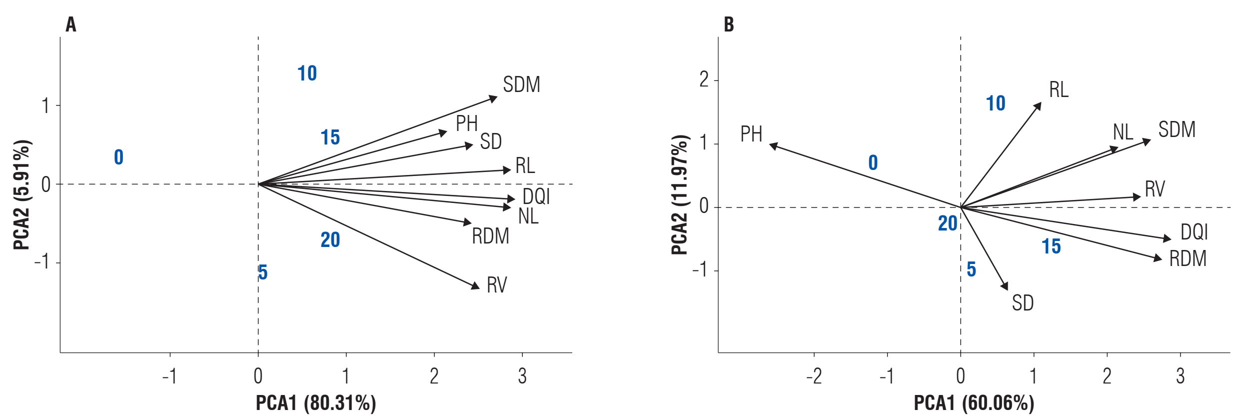

FIGURE 4. Principal component analysis of (A) watermelon and (B) melon seedling growth under application of humic substances. NL: number of leaves, PH: plant height, SD: stem diameter, RL: root length, RV: root volume, SDM: shoot dry mass, RDM: root dry mass, and DQI: Dickson quality index.

(Tarón Dunoyer *et al*., 2022). This fact corroborates the results of increased plant height and stem diameter reported herein, in which the magnitude of the effects on seedling growth and development are induced by the contribution of HS. Several authors described that the HS-mediated auxin response was connected with the response in the H^+-ATPase activity of the root plasma membrane (Ramos *et al*., 2015; Olaetxea *et al*., 2019). In our experiments, we did not measure this parameter, but it likely is one of the first responses in the HS-mediated action in plant growth.

The increase in plant biomass of melon and watermelon seedlings with the use of HS may be related to the effect of these substances in increasing the uptake and accumulation of nutrients. Seedlings assimilate the absorbed nutrients and use them for their metabolism and biomass production. Some changes induced by HS in the nutritional status of plants include the up-regulation of the gene expression of some of the nutrient transporters located in the plasma membrane, which promote the absorption of secondary ions and nutrients (Olaetxea *et al*., 2019; Pizzeghello *et al*., 2020), thereby increasing nutrient use efficiency (Tarón Dunoyer *et al*., 2022). Jing *et al*. (2020) have also reported improved growth of maize seedlings with the use of HS. The authors reported that with the increase in HS, there was greater absorption of essential elements and, with this, stimulation of the growth and biomass of corn seedlings. But there was growth inhibition with a high dose of HS. Improved nutrient use efficiency with HS was also observed by Nardi *et al*. (2017). These authors, in turn, highlighted the fundamental function of HS in increasing the uptake and transport of nutrients by maize seedlings, as well as stimulating the solubilization of adsorbed or precipitated cations with the secretion of organic anions by the roots of seedlings.

A prominent effect of using HS is root development, as verified in this study. Jindo, Olivares *et al*. (2020) reported beneficial effects of HS on formation of lateral roots, which corroborates our observation of expressive increase in root volume of watermelon and melon seedlings. Conselvan *et al*. (2017) and Šerá and Novák (2022) have also found that HS stimulate elongation and proliferation of secondary roots in in maize and poppy (*Papaver somniferum*) seedlings, respectively, which influences water and nutrient uptake, resulting in greater growth and development of the shoot in seedlings.

Increased growth and development in shoots and roots can also be related to greater nutrient availability due to increased production of exudates in the rhizosphere in response to HS (Canellas *et al*., 2019). Furthermore, HS stimulate auxin synthesis in roots, which has the function of stimulating lateral and adventitious root formation (Müller *et al*., 1998). A well-developed root system during the seedling production phase is essential for seedling establishment in adverse field conditions. The results obtained here clearly demonstrate the importance of HS during early development of watermelon and melon seedlings.

Although using HS favors plant growth and development, dosage is an important criterion to be defined for each plant species (Jindo*et al*., 2020). The results in this study indicate species-specific effects of HS on seedling growth. This is due to a relationship between HS-induced root exudation of organic and functional groups of these HS, which can cause different responses depending on the species (Rose *et al*., 2014). Thus, it is important to define the best dose for a desirable response, such as greater growth of watermelon and melon seedlings. Such dose-response assessment is even more important when it is evident that crop species may play a major role in the dose-response relationship.

Humic substances normally do not impact growth linearly, with decline in plant growth rates at high HS concentrations (Pizzeghello *et al*., 2020). These studies have also showed a decrease in SH, SD, RV, SDM, RDM, and DQI of melon seedlings as HS rates increased, and, for watermelon seedlings, a decline was observed in SD, RL, SDM, and DQI under high HS rates. This suggests watermelon seedlings are more responsive to HS than melon seedlings. Likewise, Jing *et al*. (2020) found a reduction in maize seedling growth with a high increase in HS concentration, while low concentrations resulted in the opposite effect on growth, thereby showing low HS concentrations are enough to obtain better production responses from melon seedlings.

Tropical Cerrado soils are predominantly sandy, meaning that a greater portion of nutrients and water added to soil are not used by crops in contrast to clayey soils. Most variables increased in value with the addition of HS, probably due to the central role that HS has in the formation and stabilization of soil aggregates (Swift, 1991; Mamedov *et al*., 2014), causing more available water and nutrients to retain in soil.

Using HS in the initial growth and development phase of cucurbits is an interesting technology for farmers who lack technical information on melon and watermelon cultivation. The positive results obtained here when using HS are evident. Such results identify HS as a promising tool in the rapid development of seedlings, subsequently contributing to increased yield in the field (Rodrigues *et al*., 2017).

Conclusions

The use of humic substances is beneficial in the initial growth of watermelon and melon seedlings. It is an alternative for vegetable growers who cultivate in low-fertility tropical soils in the Cerrado. Based on seedling quality, 14 g L^{-1} of HS is best for watermelon seedlings and 15 g L^{-1} of HS for melon seedlings.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

HAFA and RRSSM designed the experiment and formulated the research goal. RRSSM developed the methodology and research activity planning. HAFA, EDON, FFPJ and LMM carried out the greenhouse experiment and the data collection. NAFM, HAFA and FFPJ contributed to the data analysis. HAFA wrote the initial draft. All authors reviewed the final version of the manuscript.

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