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Evaluation of root growth of coriander (*Coriandrum sativum* L.) by humic substances

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Resumo: *Humic Substances (HS), in addition to providing nutrients to plants, stimulate plant growth, especially due to changes in architecture and root growth, and can increase plant productivity. The interest in the use of HS as agents promoting plant growth has been increasing due to the benefits associated with the physical and chemical structure of the soil, as well as for plant metabolism. The objective of this work was to evaluate the effect of the HS from peat on the promotion of coriander root growth (Coriandrum sativum* L.*). For this, coriander seeds were distributed in germination boxes (Germibox), maintained in a twinning chamber type B.O.D (Biochemical Oxigen Demand) for a period of ten days, at 25 °C. After germinating, the seeds were transferred to a becker containing 50 mL of the different concentrations of HS in each becker (25 mg L^{-1}, 50 mg L^{-1}, 100 mg L^{-1}, 200 mg L^{-1} and 400 mg L^{-1}) and the control (distilled water). After 24 hours, the seeds were transferred to 15 mL tubes with 2 mM calcium chloride solution ($CaCl_2$). The experimental design was completely randomized (DIC) with four replicates per treatment. The results indicate that HS from peat present potential in the initial root growth of coriander with greater root length for the concentration of 100 mg L^{-1} (70.0 mm).*

Palavras-chave: *Coriandrum sativum L. Organic matter. Root growth.*

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I. Introduction

Humic substances (HS) are compounds originated from the deposition of plant and animal waste from the environment, and can be used as input for the management of different crops. They are present in about 85% to 90% of the total organic carbon, can be extracted from different sources and are fractionated in humic acids (HA), fulvic acids (FA) and humin (HUM) [1]. The chemical, physical and microbiological properties of HS may allow an increase in the yield yield of some crops from the various benefits promoted for the chemical and physical structure of the soil, as well as for plant metabolism. Among the main effects promoted by HS in plants are root development, foliar development, increase in nutrient uptake and regulation of enzymes important for plant metabolism [2,3].

Studies related to the characteristics and properties of HS are often performed after the fractionation of organic matter. The International Humic Substances Society [4], in turn, proposes chemical fractionation, which leads to the operational definition for the fractions that make up the humic substances (e.g., FA, HA, HUM). The chemical fractionation of organic matter is based on the solubility characteristics of humic substances and is described in detail on the website of the International Humic Substances Society [4] and Canellas and Santos (2005) [5]. HS, in addition to providing plant nutrients through mineralization, can directly stimulate plant growth and productivity. However, the effects of HS bioactivity on plant development depends both on the source of the organic material from which it was extracted and on the specific physicochemical characteristics of HS to be used, on the concentrations recommended for each species or cultivar used and on the species of the plant studied [6,7].

The stimulation of the root growth promoted by HS has been related to several factors, among them the origin and concentration, besides the plant species used [7]. The stimulation in plant growth is related to the physiological mechanisms, which include the formation of complexes with greater solubility potential with micronutrients [8,9], besides the interaction with enzymatic constituents of the plasma membrane in a way analogous to the action of plant hormones [10].

Coriandrum sativum L. is an annual herbaceous vegetable belonging to the family Apiaceae, which is native to the Mediterranean sea basin [11]. The culture has been used since antiquity, with a record of use for medicinal purposes, with a greater focus on the region of the Mediterranean and Eastern Europe [12]. The culture is still destined to a lesser extent for the production of cosmetics and medicines [13]. In Brazil, coriander is one of the cultivated vegetables, being a very traditional culture of the familiar agriculture, but suffers with the little incentive of organ of research, being very limited the number of works that deal with this culture, both directed to the production by part small farmers, and the food security of the population.

In this way, the economic importance of coriander cultivation is evident, which has become consolidated as the main source of income in several rural communities in Brazil. However, coriander is cultivated in many microregions of Brazil by small farmers, and in many cases, without any technical guidance, which can cause losses due to low production and risk to human health by the chemicals used without proper use. The objective of this work was to evaluate the effects of HS on the promotion of root growth of *Coriandrum sativum* L.

II. Material And Methods

2.1. Humic material

The samples of humic substances were extracted and characterized by Botero et al. 2010 [14]. The samples were derived from peat samples collected in the municipality of Ribeirão Preto - SP (district of Taquaral-SP), at a soil depth of 50 cm, where five simple samples were collected to form a composite sample. These samples were transferred to wood trays and after drying in air for approximately 2 days, were passed in plastic sieves of 2 mm mesh.

The extractions of humic substances (HS) were done according to the procedure adopted by most researchers associated with the International Humic Substances Society (IHSS) with 0.1 mol L^{-1} (NaOH) extractors in the ratio of 1:10 (peat: extractor) under inert atmosphere and agitation for 4 hours. The HS solution had the pH adjusted to 7.0.

2.1.1. Characterization of humic substances

2.1.1.1. Elemental analysis

Samples of HS were characterized by elemental analysis (C, H, N, O, S) in ThermoFiningan Flash EA1112 equipment. Standards used were: cystine, BBOT (2, 5-bis(5-tercbutyl-2-benzoxazol-2-yl) tiophen; methionine; sulfanilamide. All samples were analyzed in triplicate. The atomic ratios H/C and C/O were calculated from the data obtained in the elemental analysis.

2.1.1.2. Nuclear magnetic resonance of carbon 13 (NMR ^{13}C)

The functional groups content in the samples of humic substances were determined by Nuclear magnetic resonance of carbon 13. The NMR^{13}C experiments with cross-polarization (CP) and magic-angle spinning (MAS) with variable amplitude (VA) were carried out in a Varian spectrometer (model Unity Inova 400). The samples were arranged in a cylindrical 5-mm diameter zirconium rotor (Doty Supersonic) spinning at 6 kHz in a Doty Supersonic probe for solid tests. TheNMR13CVACP/ MAS spectra were obtained under the following experimental conditions: resonance frequency of 100.05 MHz for 13C, spectral band of 20 kHz for cross-polarization, proton preparation pulse of 3.8 μs, contact time of 1 ms, acquisition time of 12.8 ms, and wait time of 500 ms for relaxation. The chemical shift values were referenced to hexamethyl benzene (HMB), which has a well-defined line at 17.2 ppm.

2.1.1.3. UV–vis spectroscopy: determination of the E_4/E_6 ratio

The E_4/E_6 ratio was ascertained by measuring approximately 2.0 mg of HA and HS samples in 10 mL of a NaHCO 3 0.05 mol L^{-1} solution and later reading the absorbance at 465 and 665nm on a DR 3900 spectrometer [15].

2.4 Promotion of root growth of cilantro seedlings treated with HS

The experiment was conducted at the Agreste Environmental Sciences Laboratory (AESL) and Laboratory of Plant Physiology (LABPP) of the Federal University of Alagoas, Arapiraca city. The experimental design was completely randomized (DIC) with 4 replicates per treatment. Coriander seeds of the "Verdão" variety were disinfested in 2.5% sodium hypochlorite solution for 10 minutes followed by washing with distilled water. The seeds were then distributed in germination boxes (Germibox) on two sheets of blotting paper moistened with distilled water 2.5 times the dry paper weight, kept in a Biochemical Oxigen Demand (BOD) type twinning chamber for a period of ten days at 25 °C in a 12h photoperiod. After this period the germinated seeds, that is, with the protrusion of the radicle, were placed in 50 mL HS with different concentrations (25 mg L^{-1}, 50 mg L^{-1}, 100 mg L^{-1}, 200 mg L^{-1} and 400 mg L^{-1}) and the control (distilled water) in each Becker for 24 hours. Then, they were transferred to a system adapted with 15 mL Falcon-type tubes covered with aluminum foil to avoid light passing to the roots, containing a 2 mM calcium chloride solution ($CaCl_2$), for vertical root growth. In each tube a layer of aluminum foil was used on the surface with a hole where the seeds were positioned with the radicle in contact with the solution, for a period of ten days.

After this period, the seedlings were removed and the root length (RL) was evaluated using a millimeter ruler, with the criterion being the distance between the plant collar and the root end.

The experiment was conducted in the laboratory of environmental sciences of the agreste and in the laboratory of plant physiology of the Federal University of Alagoas, Campus Arapiraca. The experimental design was completely randomized (DIC) with 4 (four) replicates per treatment.

2.5 Análise estatística

Statistical analyzes were carried out using the statistical program SISVAR® version 5.6 [16]. The data were submitted to analysis of variance and the means were compared by Tukey test at 5% probability level ($p < 0.05$) and adjusted by linear or polynomial regression ($p \leq 5\%$). The LibreOffice Calc® version 5.4 software was used to build the graphics and the tables were organized using LibreOffice Writer® software version 5.4.

III. Result

The results of characterization of HS samples are shown in Table 1. These samples were characterized by Botero et al. 2010 [14]. The results show high levels of aliphatic, aromatic and carboxylic groups.

Table 1: Characterization of humic substances (HS) samples extracted from peat for the atomic reasons H/C and O/C, content of functional groups and E_4 / E_6 ratio [14].

Parameter	HS
H/C	1,16
O/C	1,24
Aliphatic (%)	23,7
Ether (%)	4,2
Aromatic (%)	61,6
Carboxylics, ethers and amide (%)	10,5
E_4/E_6	3,47

Different concentrations of HS influenced significantly the root growth of coriander plants (Figure 1 and Table 2) in relation to the control. The treatment root growth that received the dose of HS 100.0 mg L^{-1} was superior when compared to the other treatments, but did not statistically defer treatment of the dose of 50.0 mg L^{-1}. This treatment (50.0 mg L^{-1}), although promoting root growth, did not statistically differ from the 25.0 mg L^{-1} dose. The root growth of seedlings submitted to treatments with concentrations of 25.0 mg L^{-1}, 200.0 mg L^{-1} and 400.0 mg L^{-1} of HS did not differ statistically from each other. The control treatment obtained the lowest root growth in relation to the other treatments.

Table 2: Summary of the analysis of variance and mean length of seedling radicular length (SRL) of *Coriandrum sativum* L. at 10 days after the imbibition period in 24 hours submitted to different HS concentrations.

Source of Variation	LG	Sum of the Average Squares
		SRL
Concentration	5	14.21**
Linear Regression	1	0.88^{NS}
Quadratic Regression	1	19.47**
Cubic Regression	1	49.76**
Deviation Regression	2	0.46^{NS}
Residue	18	0.72
CV (%)		19.16

* e ** significant at 5 and 1% probability, respectively, ^{NS} not significant.

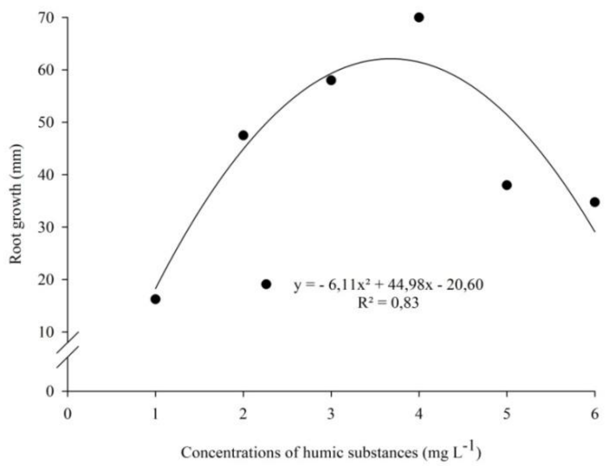

Figure 1: Seedling radish length (mm) of *Coriandrum sativum* L. at 10 days after the soaking period in 24 hours submitted to different concentrations of HS (mg L^{-1}).

IV. Discussion

According to Ayuso et al. (1996) [17] the characteristics of organic matter can influence both seed germination and initial plant growth. The atomic ratios determined by elemental analysis provide evidence of the structural characteristics of humic substances. A lower H/C ratio indicates a higher aromaticity and a lower O/C ratio indicates a lower degree of humification of the soil organic matter fractions [14].

The ratio E_4/E_6 is an important indication of the degree of condensation of the humic macromolecule which is generally associated with its aromaticity. The increase in the ratio indicates structure with lower aromaticity and the reduction of the higher aromaticity ratio. Reasons below 4 indicate higher presence of condensed aromatic structures, while values higher than 4, presence of few condensed aromatic structures [18]. Thus, the results of the present work show a greater presence of the condensed aromatic structures, since it obtained the determined value of 3.47 for the ratio E_4/E_6.

These results related to the use of different concentrations of HS to promote root growth may be related to the bioactivity of the HS, promoting an increase in the electrochemical H^+ gradient, causing acidification of the apoplasma that leads to the rupture of cell wall bonds, promoting its elasticity, which will contribute to cell growth [19, 5].

The negative effect on root growth parameters observed as the HS concentration increases at 200.0 mg L^{-1} and 400 mg L^{-1}, shows that the ideal HS dose is from 50.0 mg L^{-1} and below 200.0 mg L^{-1}. The best dose response was 100.0 mg L^{-1} although this result did not differ statistically from the 50 mg L^{-1} dose treatment. This negative result from the concentration of 200.0 mg L^{-1} can be explained by Camargo et al. (2001) [20] who claim that some organic acids can cause phytotoxicity by limiting the germination and development of plants. According to these authors, phytotoxicity is directly related to pH, carbon chain length and concentration of acids in the environment in which they are found. These effects are associated with the inhibition of germination and root expansion, as a result of lesions caused to the meristematic tissue of the radicle or limitations to respiration, leading to inhibition of cell division.

Thus, it is emphasized that the application of HS stimulated the development and growth of the roots at the beginning of the growth of coriander plants, phase in which the cells are in constant division and cell differentiation. This stimulus may reflect higher production at the end of the cycle due to the greater volume of soil that can be exploited [21].

V. Conclusion

Humic substances from peat stimulate root growth of *Coriandrum sativum* L. in the initial growth phase of plants. The highest root lengths were found at concentrations 50 mg L^{-1} and 100 mg L^{-1}, with negative effects at higher concentrations. Thus, the use of natural organic matter as a promoter of root growth must be done consciously and based on scientific data, thus avoiding waste and harmful actions to the plant and the environment.

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