Agricultural soils strengthening employing humic acids and its effect on plant growth chilli pepper and eggplant

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ABSTRACT

The Humic substance has been recognized widely as a plant growth promoter because they induce changes in root, architecture and dynamics of growth which result in larger root size. The aim of this study was evaluating the effect Humic acids on the chili pepper (*Capsicum annuum*) and eggplant (*Solanum melongena*) plants growing in soils fertilized with urea. 200 g of soil were incorporated to the fertilizer once and the Humic acid in concentrations of 0.05%, 0.1% and 0.2%. All measurements growths were carried out during 45 days growth. The finding shows that all treatments cause significant increases ($p < 0.05$) in the total area of roots. For the chili pepper, the multiple comparison tests of Tukey and Dunnett showed that there was a statistically significant difference between the control soils and the soils containing 0.1% and 0.2% of Humic acid. By the opposite, no statistical differences ($p > 0.05$) were observed among control soil and those containing 0.05 % of Humic acid. Concerning the eggplant, significant differences were established ($p < 0.05$) between the control and samples incorporated with 0.1% and 0.2% of Humic acid. The Humic acids act as a growth biostimulant in chili pepper plants (*Capsicum annuum*) and eggplant (*Solanum melongena*).

Keywords: Chili Pepper; Coal; Eggplant; Humic Acids; Plant Growth.

INTRODUCTION

Humic acids (HA) are a fraction of the Humic substances (HS), formed by aromatic and polyaromatic nuclei, linked through aliphatic chains. These have a diversity of functional groups, which allow them to perform various functions in the soil-plant relationship (Pantoja et al. 2016, Peña et al. 2005, Canellas et al., 2010). Humic substances (HS) have been widely recognized as a promoter of plant growth mainly by causing changes in the root, in the architecture and growth dynamics, which result in larger root size, better branching and greater density of root hair with the greater surface area. These substances are formed by the decomposition of plant and animal which are deposited in the soil (Wu et al., 2017, Tan et al., 2014). During this process, the organic material is initially degraded and depolymerized by microbiological action to produce new components with a high degree of dark-colored polymerization (Canellas and Olivares, 2014). Humic substances are classified depending on the separation process used; the significant parts of humus are fulvic acids (AF) which are soluble in

acidic medium and HA that are insoluble in acidic medium (Canellas et al., 2015). The structure of the HS (Fig. 1) is highly complex and does not have uniformity in each of its units, which are formed by condensates of aromatic rings containing carboxylic, phenolic, carbonyl, methoxide and aliphatic groups (Gomes de Melo et al., 2016). It is estimated that approximately a quarter of the molecular weight of the HS is due to the oxygenated groups, mainly carboxylic groups that increase with the degree of humification of the organic matter and that can form carboxylates with metals present in the medium. Phenolic groups that are formed in the initial stages of humification and carbonyls groups that by oxidation reactions originate carboxylic groups (Gomes de Melo et al., 2016; Pedrót and Melanie, 2010; Fischer, 2017). The HS play an important role in nature because due to their oxygenated functional groups they participate in cation retention processes that are essential for plants as well as retaining heat on the surface due to their dark color (Pedrót and Melanie, 2010). Current uses of these substances include their activity as fertilizers, their ability to retain useful metals for agriculture and their use

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in the removal of toxic aqueous effluents (Sun et al., 2015; Tang et al., 2014; Kalina et al., 2013). An important source of HS are the low range carbons, which have a high content of oxygenated groups and a part of their structure quite similar to that of HA (Motta & Santana, 2013). This makes possible the extraction with alkaline solutions of the HS for this type of coals. When the mineral carbons are subjected to moderate oxidation reactions, it is possible to increase the content of HS in their structure, leading to higher percentages of extraction of this type of materials (Zhiyuan et al., 2012; Arslan et al., 2010; Novak et al., 2001). In this study, the effect of the HA obtained from low-rank coal from the Montelíbano mine (Córdoba-Colombia) is evaluated in the growth of chili and eggplant plants grown in low fertility soils.

MATERIALS AND METHODS

Mineral material
The starting material in this investigation was coal from the mine located in the Montelibano rural area, in the department of Córdoba-Colombia.

Preparation of the sample
The carbon sample was crushed and sieved to a particle size between 3,1 and 7,1 mm using a sieve with a mesh number of 6. Subsequently, 100 g portions of the material were demineralized for 1h at room temperature with hydrochloric acid, 5 M. Before the oxidation process the material of the carbon sample was subjected to debitumization with an ethanol-benzene mixture (1:1 v/v) under reflux for 24 h. The solvent was removed by distillation in Soxhlet and vacuum filtration with distilled water. The remaining moisture was removed by drying at 80 °C for 12 h (Anillo et al., 2013). Subsequently, oxidation was carried out in an aqueous medium with 30% hydrogen peroxide and concentrated acetic acid. This system was heated to 60 °C and maintained in continuous agitation for 12 h. (Anillo et al., 2013).

Obtaining humic acids
5 g of oxidized carbon was added 100 mL of 0,1 M NaOH, maintaining this system at 60 °C for 1 h in continuous agitation. Subsequently, the solution was filtered under vacuum and 100 mL of 0,1M HCl was added to the filtrate to precipitate the HA. This system was kept at rest for 24 h and was subsequently centrifuged at 3600 rpm for 10 min. The colloid obtained after the centrifugation was washed with portions of ethanol and finally heated at 100 °C for one hour to be subjected to different analyzes. The percentage of HA extracted was quantified in relation to the mass of extracted acids obtained from the initial weight of the oxidized carbon samples (Anillo et al., 2013).

Soil strengthening
This research is used for the cultivation of chili pepper (*Capsicum annuum*) and eggplant (*Solanum melongena*), a soil of low fertility provided by the National Apprenticeship Service (SENA) agribusiness sector, fortified with HS and urea. To 200 g of soil was added directly fertilizer and HA in concentrations of 0.05%, 0.1% and 0.2% with respect to the amount of soil. For comparison purposes, a control sample was used, which contained fertilizer without the addition of HA.

Tests on plants
Seeds of chili pepper (*Capsicum annuum*) and eggplant (*Solanum melongena*) were sown in the different fortified soils and the control sample. From the first day of sowing, growth was measured at the three-day time intervals until 45 days were completed. The soil samples were hydrated by irrigation. To know effect of HA suggestion observation to root characters on chili pepper and eggplant, by side that yield characters is important to study effect HA on plant.

Statistical analysis
For the statistical analysis of the results, the measure of plant growth using urea as fertilizer and HA at different concentrations was used as a response variable. The growth measures were analyzed employing an ANOVA (unidirectional) to determine statistically significant differences ($p < 0.05$) between the samples. The software SPSS (version 17.0 for Windows) and the multiple comparison tests of Dunnett test were used. All the tests were carried out in triplicate. In this methods, describe experimental design used, Analysis statistical and procedure research, measurement, example root length, high plant, etc.

RESULTS AND DISCUSSION

In all the essays, observed the trend of an increase of the root of the plants from the third day of sowings the seeds of the plants (Table 1), being demonstrated that in the case of the plant of chili (*Capsicum annuum)* to the concentration of 0.05% of HA the growth is similar to the control (Fig. 2), whereas to the concentrations of 0.1% and 0.2% the length of the root was the double (Table 1). As the days of growth passed, both the control and the concentration of 0.05%, root growth was not observed, unlike that observed in the cultivation soils using concentrations of 0.1% and 0.2% of HA. As the days of growth were passing both the control and the concentration of 0.05 % they were kept same way, whereas in the concentrations of 0.1% and 0.2% was observed an increase in the length of the root, in the 9th it appreciates a significant increase in the soils of 0.1% and 0.2% accenting as the days pass. In fact, after 24 days of growth, the soil fortified with HA at a concentration of 0.1%, showed a tendency to a steady

Table 1: Growth of the chili pepper plant (*Capsicum annuum*) grown in soils fertilized with urea and different concentrations of HA

Days of growth	Soil fertilized with urea and fortified with HA (%)			
	0.05	0.1	0.2	Control
3	X	X	X	X
6	0,4±0,005[a]	1,0±0,025[b]	1,5±0,050[c]	0,4±0,002[a]
9	2,0±0,002[a]	3,3±0,028[b]	4,9±0,060[c]	1,9±0,050[a]
12	2,5±0,036[a]	4,5±0,023[b]	6,2±0,057[c]	2,5±0,057[a]
15	3,2±0,011[a]	6,3±0,011[b]	6,5±0,115[c]	3,1±0,028[a]
18	3,8±0,011[a]	6,8±0,010[b]	6,8±0,005[b]	3,8±0,005[a]
21	4,3±0,028[a]	7,1±0,011[b]	7,1±0,028[b]	4,4±0,057[b]
24	4,7±0,025[a]	7,5±0,057[b]	7,5±0,017[b]	4,7±0,015[a]
27	4,9±0,015[a]	7,6±,0,086[b]	7,9±0,005[c]	4,7±0,016[d]
30	4,9±0,017[a]	7,8±0,040[b]	8,2±0,017[c]	4,8±0,005[a]
33	4,9±0,022[a]	8,0±0,100[b]	8,5±0,028[c]	4,8±0,005[a]
36	4,9±0,011[a]	8,0±0,100[b]	8,7±0,010[c]	4,8±0,002[a]
39	X	8,0±0,057[a]	9,0±0,028[b]	X
42	X	X	9,3±0,015	X
45	X	X	9,5±0,025	X

Different letter in the same row differ significantly (*p*<0.05) (*Tukey* test). The values represent the mean±S.E. (*n*=3). The growth of the plant is expressed in centimeters (Cm). The X denotes absence of growth
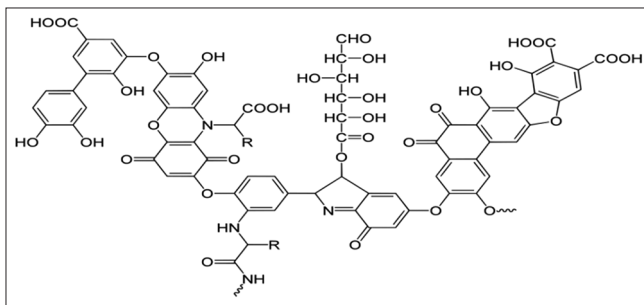

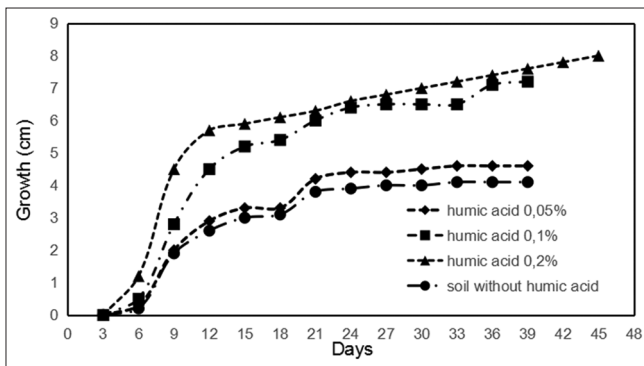**Fig 1.** Structure of humic substance.

Fig 2. Growth rate of Capsicum annuum (chili pepper) using different concentrations of Humic acid in the soil.

state behaving at that time similar to the control and soil fortified with 0.05% HA, while growth in soil fortified with 0.2% HA increased exponentially (Fig. 2).

The Analysis of variance (ANOVA) showed that there are statistically significant differences in the growth trend of the pepper plant (p < 0.05). The multiple comparison

tests of Dunnett (Fig. 4a) showed that there is a statistically significant difference (p < 0.05) between the control and the concentrations of 0.1% and 0.2% of HA. Likewise, the control and the concentration of 0.05 % there was no statistically significant difference (p < 0.05). From the 27th day of growth, the chili pepper plant (*Capsicum annuum*) cultivated on soils fertilized with urea and fortified with HA at a concentration of 0.05% presents. In general terms, slightly higher growth than the plant cultivated in the soil without the addition of HA used as the control.

When each of the concentrations is compared with the control, significant differences are established between this and their concentrations of 0.1% and 0.2%, while with the concentration of 0.05%, there are no statistically significant differences (p < 0.05) as shows the Dunnett test. In the case of the eggplant (*Solanum melongena),* similar behavior presented in the chili pepper plant, as shown in the growth curve of this plant (Table 2). In the same way, statistically significant differences are presented as shown by the analysis of variance (p > 0.05) in the growth of the root of the eggplant. When each of the concentrations is compared, significant differences are established between the control and concentrations of 0.1% and 0.2%, while with the 0.05% concentration there are no statistically significant differences (p < 0.05) until the ninth (9) day of growth as shown by the Dunnett test (Fig. 4b). But from day twelve (12) of growth if there are statistically significant differences (p < 0.05) between them. The results of the HA application test show that all treatments cause significant increases in the total surface area of roots. The increase in leaf area and total root surface, caused by treatments with different types of HA, contributes to the hypothesis of a biostimulant action in plant development (Nebiosso and Picolo, 2012).

According to Barros et al. (2010), HA influence the proliferation of secondary roots and the absorption of nutrients. Besides, constitute an essential factor in the establishment and adaptation of plants in the soil under adverse conditions. Many authors stated that HA in contact with root cells could stimulate the plant development through the mechanism known as acid root growth. The increase of H^+ ions pumped to the apoplast through the H^+ ATPase enzyme acidifies the cell wall, making it more flexible.

This behavior facilitates the elongation of the roots and the appearance of active mitosis points, which become emergency points radicular (Canellas and Olivares, 2014; Canellas et al., 2015; Nardi et al., 2016).

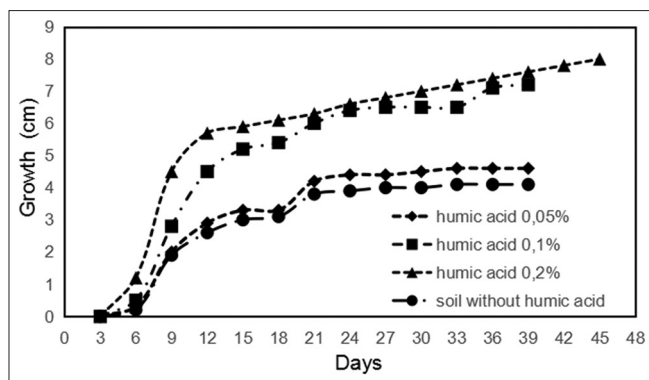Humic substances induce H^+-ATPase activity which, in turn, can energize secondary ion transporters and promote

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Fig 3. Growth rate of Solanum melongena (Eggplant) using different concentrations of Humic acid in the soil.
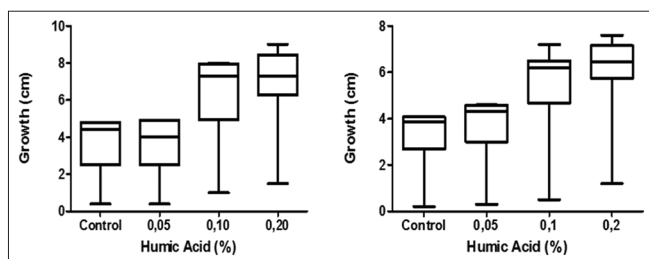

Fig 4. Variance analysis of the growth measurements in the presence of different concentrations of Humic acids compared to the control (without Humic acid). a) Capsicum annuum (chili pepper) and b) Solanum melongena (eggplant).

Table 2: Growth of eggplant plant (*Solanum melongena*) grown in soils fertilized with urea and different Humic acid concentrations

Days of growth	Soil fertilized with urea and fortified with HA (%)			
	0.05	0.1	0,2	Control
3	X	X	X	X
6	0,3±0,011[a]	0,5±0,025[b]	1,2±0,023[c]	0,2±0,005[a]
9	2,0±0,057[a]	2,8±0,011[b]	4,5±0,015[c]	1,9±0,010[a]
12	2,9±0,017[a]	4,5±0,028[b]	5,7±0,010[c]	2,6±0,026[d]
15	3,3±0,025[a]	5,2±0,080[b]	5,9±0,057[c]	3,0±0,025[d]
18	3,3±0,020[a]	5,4±0,057[b]	6,1±0,044[c]	3,1±0,050[d]
21	4,2±0,086[a]	6,0±0,100[b]	6,3±0,010[c]	3,8±0,015[d]
24	4,4±0,057[a]	6,4±0,056[b]	6,6±0,014[c]	3,9±0,055[d]
27	4,4±0,050[a]	6,5±0,005[b]	6,8±0,005[c]	4,0±0,100[d]
30	4,5±0,015[a]	6,5±0,078[b]	7,0±0,100[c]	4,0±0,050[d]
33	4,6±0,005[a]	6,5±0,005[b]	7,2±0,172[c]	4,1±0,023[d]
36	4,6±0,000[a]	7,1±0,057[b]	7,4±0,005[c]	4,1±0,020[d]
39	4,6±0,005[a]	7,2±0,023[b]	7,6±0,003[c]	4,1±0,010[d]
42	X	X	7,8±0,100	X
45	X	X	8,0±0,005	X

Different letters in the same row differ significantly ($p < 0.05$) (*Tukey* test). The values represent the mean±S.E. ($n=3$). The growth of the plant is expressed in centimeters (Cm). The X denotes absence of growth

the absorption of nutrients. The transport of nitrate through the plasma membrane is facilitated by ion channels, that is, a secondary active transport, which requires an electrochemical proton gradient generated by the induction of H^+-ATPase (primary transport).

CONCLUSIONS

The critical influence of HA on the physical, chemical and biological properties of the soil has been amply demonstrated and its role in maintaining the growth of plants is recognized. According to the results obtained, HA act as growth biostimulant in pepper plants (*Capsicum annuum*) and eggplant (*Solanum melongena*), these changes can be observed in the morphology of the plant. The contact of seeds with humic acid triggers reactions, which lead to increased plant development. This type of research can help overcome the complexity of whether a HS influence plant biology and allows the development of new technologies to increase the growth of plants based on humic matter.

ACKNOWLEDGEMENT

DECLARATIONS OF CONFLICT OF INTEREST

The authors report no declarations of conflict of interest.

Authors' Contributions

All the authors contributed to the work presented in this article. Fredy COLPAS and Arnulfo TARON carried out the experiments, designed the research plan, organized the study and contributed to the writing of the manuscript. Jairo MERCADO performed statistical analysis and helped in interpretation of data and discussion of results.

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