ORIGINAL RESEARCH ARTICLE

Application of fulvic acid to seedlings influencing root growth and productivity of iceberg lettuce

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ABSTRACT

Humic substances are used in agriculture as promoters of plant growth, especially of the root system. The objective of the work was to evaluate the effect of the application of different doses of fulvic acid on the growth and productivity of American lettuce, Raider Plus cultivar. The experimental design used was entirely randomized, with five treatments of fulvic acid 0, 1, 2, 4, 8 mL·L⁻¹ and four repetitions, applied at the time of transplanting. Two experiments were conducted simultaneously: one in the greenhouse, where fresh and dry mass of the aboveground and root parts, length and volume of the roots were evaluated; and the other in the field, where, at the end of the cycle, fresh and dry mass of the aboveground parts, number of leaves, stem length and average head circumference were evaluated. The application of different doses of fulvic acid promoted the growth of lettuce plants, especially the root system. The emission of roots, with predominance, of those of smaller diameter, was found in the higher concentrations of fulvic acid. The number of leaves and the average circumference of the head expressed responses in the concentrations of fulvic acid. *Keywords:* Organic System; Humic Substances; *Lactuca sativa* L.

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1. Introduction

The cultivation of lettuce (*Lactuca sativa* L.) is intensive and generally practiced by family farms, being responsible for the generation of five direct jobs per hectare^[1]. The crop responds positively to organic fertilization^[2], when compared to exclusively mineral fertilization, because in the soil organic fertilization exerts multiple direct and indirect actions^[3]. The lettuce cultivated in organic system, when commercialized reaches a differentiated price, if compared to the lettuce produced in the conventional system^[4]. Among the lettuce groups, the American type lettuce, besides being consumed in salads, is used in the minimal processing industry, in fast food chains as an ingredient in sandwiches for its crunchiness, texture and flavor, and has a longer post-harvest life^[5].

The production phase of lettuce seedlings is one of the most important stages in the production process to obtain high standard plants^[6,7], because poorly formed seedlings reduce initial growth of plants in the field, affecting production, and limiting the genetic potential of cultivars^[8]. Furthermore, the seedling production phase represents 60% of the success of a crop, since the final performance of the plants in the production beds depends on the quality of the seedlings^[9].

Thus, techniques compatible with the organic system and that promote adequate root growth and good initial development of lettuce plants become relevant.

In this context, the use of humic substances, as plant growth stimulators at the beginning of the cycle, especially of the root system. The great interest in these substances is due to the benefits associated with them. Humic substances are organic acids, soluble in water, present in different organic sources, such as lignite, leonardite, sewage sludge, organic compost, peat and commercial products, that stimulate the absorption of nutrients, especially cationic ions^[10].

Humic substances are composed of fulvic acids, humic acids and humin, the main components of soil organic matter^[11], whose action on the metabolism and growth of plants has been attributed mainly to the action of fulvic acids, of lower molecular weight^[12], influencing the absorption and transport of nutrients by changing the surface area of the roots^[13,14]. The effects caused by fulvic acid are attributed to its action as auxin^[15–17], a plant hormone related to cell expansion and root initiation, among other physiological effects.

However, the results obtained are variable and depend, besides the species tested, on the humic substances used, concentration, degree of purification of the material and the conditions in which the experiments were performed^[12]. In view of this, the possibility of using these substances in horticulture is evident, with the purpose of promoting the maximum expression of the genetic potential of cultivars. In view of this, the present work aimed to evaluate the effect of the application of fulvic acid, to seedlings, on root growth and productivity of the American lettuce Raider Plus cultivar.

2. Material and methods

Two experiments were implemented simultaneously. One was conducted in the greenhouse of the Department of Plant Science and Plant Health—Agricultural Sciences Sector of the Federal University of Paraná (UFPR) and the other in the organic horticulture area of the Experimental Station Center of Canguirí/UFPR, in Pinhais-PR, located in the physiographic region called Primeira Planalto Paranaense, between coordinates 25°25′ S and 49°08′ W altitude of 930 m, and temperate climate type Cfb by Köppen classification. The soil in the experimental area is classified as Acic Red-Yellow Latosol with a clayey texture and smooth undulated relief^[18], whose chemical analysis in the 0 to 15 cm layer indicated the following average values: pH (CaCl₂) = 6.1; pH SMP = 6.4; Al⁺ = 0; H + Al = 3.7 cmol_c dm⁻³; Ca²⁺ = 7.2 cmol_c dm⁻³; Mg²⁺ = 3.4 cmol_c dm⁻³; K⁺ = 1.44 cmol_c dm⁻³; P =158.4 mg dm⁻³; C = 37.4 g dm⁻³; B = 0.98 mg dm⁻³; V% = 76 and CEC = 15.74 cmol_c dm⁻³.

The Raider Plus cultivar, American type, was sown on September 14, 2010, in five expanded polystyrene trays with 288 cells filled with commercial substrate Plantmax®. After sowing, the seedlings remained in the greenhouse for 30 days, reaching five definitive leaves. One day before the removal of the seedlings, five treatments were established using a solution containing 10% fulvic acid extracted from Leonardite, supplied by the company Nutriplant, diluting the solution in water to establish the concentrations of 0 (control with water application), 1, 2, 4, 8 mL·L⁻¹ of fulvic acid in the solution applied to the lettuce seedlings. Each treatment was applied, one day before transplanting, in each seedling tray using 500 ml of the solutions with the different concentrations, in an entirely randomized design.

To identify the effect of the treatments on the initial growth of lettuce plants, 12 seedlings were randomly collected from each treatment one day after the application of the solutions (1 DAT). Simultaneously, another 80 seedlings from each treatment were individually placed in plastic bags (15 \times 22 cm) filled with Plantmax[®] substrate, placed on benches and kept for 5 days in the greenhouse. This made it possible to evaluate the effect of treatments on initial growth after application, since this occurred when the seedlings presented adequate size for transplanting, thus allowing safe evaluation of root growth. In both collections, that is, one day after the application of the solutions and 5 days after the stay in the greenhouse (1 and 5 DAT), the roots of the plants were washed in running water

until complete removal of the substrate and scanned to determine the length and root volume, using the program Whin Rhizo brand LA 1600, version 98-2003. In addition, the fresh and dry mass of the aerial part and roots were quantified. To obtain the dry mass, the plants were kept in an oven at 65 °C until they reached a constant weight.

Simultaneously to the implementation of the seedlings in the greenhouse, the field experiment was installed, in which the plants were arranged in beds covered with black plastic film, spaced 30×30 cm, distributed in four rows. The treatments were distributed in an entirely randomized design with four repetitions, each repetition consisting of 28 plants. The treatments were applied one day before transplanting, at the established concentrations. Because it is an area of good fertility, according to the values identified by chemical analysis and conducted in the organic system, we applied $10 \text{ t} \cdot \text{ha}^{-1}$ of organic compost, made with sheep manure and biomass of elephant grass, applied at the time of making the beds.

At the end of the cultivation cycle, 50 days after planting the seedlings, three plants per repetition were collected from the central rows, considering that the area was homogeneous. The following were determined: the total fresh mass, with the plants cut close to the ground; the commercial fresh mass, referring to the mass of the plants, discarding the basal leaves because it was an American type lettuce; the average circumference of the head, obtained after the removal of the non-commercial outer leaves, measured with a tape measure from one end of the head to the other; the length of the stem, after the removal of the leaves, using a ruler and the number of leaves on the heads. Afterwards, the samples were kept in an oven at 65 °C until they reached a constant weight for subsequent determination of the dry mass of the aerial part.

The plants collected in this evaluation were classified according to the standards of the Brazilian program for standardization of horticulture^[19], according to the lower and upper limit of mass in grams per plant (classes $5 \le 100$ g; 10 = 100-150 g; 15 = 150-200 g; the classes follow up to $100 \ge 1,000$ g).

Each experiment was analyzed individually. In the vegetation house stage, five doses of fulvic acid \times two collection periods were analyzed, and the factorial scheme or subdivided plot in time was adequate for the data analysis. In the field stage, regressions were performed as a function of fulvic acid concentrations, when identified as significant by variance analysis. The mean root diameters and biometric parameters evaluated in the field experiment were submitted to analysis of variance and comparison of means by the Tukey test at 1% significance level. The statistical software ASSIS-TAT 7.6 beta^[20] was used.

3. Results and discussion

In an experiment carried out in the greenhouse it was observed that the application of fulvic acid to the seedlings affected the initial growth of the lettuce plants, altering the fresh and dry mass of the leaves and roots, as well as the length and root volume. The significant differences in leaf and root fresh mass occurred at the two evaluation dates, i.e., 1 DAT and at 5 DAT. The analysis of variance also detected the interaction between treatments and evaluation dates for root fresh and dry mass (**Table 1**).

 Table 1. F values for the variables analyzed in lettuce plants treated with different doses of fulvic acid in a greenhouse experiment analyzed one and five days after transplanting (1 and 5 DAT)

			8				
Source of variat	tion GL	FLM (g·plant ⁻¹)	FRM (g·plant ⁻¹)	DLM (g·plant ⁻¹)	DRM (g·plant ⁻¹)	RL (cm)	VR (cm ³)
Treatments	4	4.94**	18.94**	13.65**	15.78**	10.30**	11.23**
Dates	1	125.64**	128.36**	169.42**	70.08**	53.92**	84.58**
$\mathbf{T} \times \mathbf{D}$	4	0.639 ^{ns}	3.53**	1.31 ^{ns}	4.57**	2.48	1.55 ^{ns}
CV (%)		21.7	21.3	17.7	22.3	21.2	24.1
TT () D							$(1, d(\cdot))$

T: Treatments; D: Dates; FLM: fresh leaf mass; FRM: fresh root mass; DLM: dry leaf mass; DRM: dry root mass; RL: root length (cm); RV: root volume (cm³); *: significant 5% probability; **: significant 1% probability; ns: not significant.

The effect of the different doses of fulvic acid was observed with an increase in the fresh and dry

mass of the leaves, up to a concentration of 6 $mL \cdot L^{-1}$ (Figures 1(a) and 1(b)), indicating that ful-

vic acid rapidly stimulated the growth of the seedlings. These results corroborate those obtained by Rosa et al.^[21] who, when evaluating bean plants at 28 days of age, obtained a quadratic response to the addition of humic substances for the aerial part dry mass variable.



Figure 1. Fresh leaf mass (a) and dry leaf mass (b) of lettuce plants treated with different doses of fulvic acid in a greenhouse experiment.

: significant 1% probability.

This effect may be associated with the presence of auxin-like compounds in humic substances, contributing to plant growth, especially of the root system, as reported by Sediyama et al.^[22], Canellas et al.^[23], Façanha et al.^[14] Nardi et al.^[12]; Zandonadi et al.^[24]; Trevisan et al.^[17].

For fresh root mass, it was observed that in both evaluation dates, the growth was stimulated starting at the dose of 2 mL \cdot L⁻¹ and increasing until the highest doses (Figures 2(a) and 2(b)). Dry root mass showed only a quadratic response at 5 DAT.

These results are in agreement with those found by Silva et al.^[25] who also observed greater root growth when humic substances were used.

The plants that were subjected to the application of different doses of fulvic acid showed an increasing response, both for root length (Figure 3(a)) and root volume (Figure 3(b)). It is worth noting that root volume is an important parameter, because it is directly related to the volume of soil explored by the roots.



Figure 2. Root production in lettuce plants one day (1 DAT) and five days (5 DAT) after transplanting from seedlings treated with different doses of fulvic acid under greenhouse conditions. Production of fresh root mass (a) and production of dry root mass (b).



Figure 3. Root length (a) and root volume (b) of plants from the experiment conducted in the greenhouse. *: significant 1% probability

Because there was no interaction between treatments and dates, the data of average root diameter are presented as the average of the treatments of the two evaluations at 1 and 5 DAT (**Table 2**). Taking into account that the greater the number and thinner the roots, the greater the efficiency of water and ion absorption^[26], the average root diameter was quantified as a function of the treatments. In

this sense, it is noteworthy that the application of fulvic acid stimulated the emission of roots, with the predominance of smaller diameter roots being evident, especially in the highest concentrations of fulvic acid (**Table 2**). The treatment containing 8 mL·L⁻¹, that is, the highest dose, promoted an increase in the number of roots in all diameters analyzed, when compared to the control.

Doses of A (mL [·] L ^{·1})	Average root diameter range (mm)						
()	0-0.5	0.5-1.0	1.0-1.5	1.5-2.0	2.0-2.5	2.5-3.0	
0	70.90 d	14.1 c	4.20 d	0.45 d	0.31 c	0.023 c	
1	108.2 bc	22.9 с	8.20 cd	1.30 c	0.93 c	0.113 c	
2	87.20 cd	22.1 c	10.65 bc	2.01 c	2.13 b	0.41 b	
4	132.70 b	34.1 b	14.80 b	2.95 b	3.03 b	0.48 b	
8	167.47 a	43.9 a	20.70 a	4.20 a	4.60 a	0.85 a	
CV (%)	20.7	23.7	26.9	26.7	37.3	42.4	

 Table 2. Intervals between the average diameter of roots of lettuce treated with different doses of fulvic acid (FA) in the greenhouse

Means followed by the same letter in the column do not differ, Tukey test, p < 0.05

According to Rima *et al.*^[27] the greater number of physiologically active roots concomitant with the greater root surface area, reflects in the increase of the volume of soil explored, representing a benefit of the treatment with humic substances. The increase in lateral roots can have positive effects on production, due to the greater ability of plants to adapt to the environment under adverse conditions, as well as for increased nutrient uptake^[28].

The results obtained in the present work indicate the stimulatory effect of fulvic acid on lateral root development, mainly at the beginning of plant growth, when cells are in division and differentiation, processes modulated by auxins, as demonstrated by Trevisan *et al.*^[17], in studies with *Arabidopsis* sp. The stimulation of root growth may reflect in higher production at the end of the cycle due to the larger volume of soil explored^[29], as observed in the experiment conducted in the field, in which solutions containing fulvic acid, applied to lettuce seedlings, promoted an increase in the number of leaves at the concentrations of 1, 2, and 4 mL·L⁻¹ and in the circumference of the heads of American lettuce Raider Plus cultivar in all doses used (**Table 3**).

The highest average values of fresh plant mass obtained in this work were 556 to 655 g·plant⁻¹, being similar to those found by Yuri *et al.*^[30] for the same cultivar, obtaining the highest fresh mass of 634 g·plant⁻¹, in conventional cultivation with the addition of 56 t·ha⁻¹ of organic compost, much higher than that used in the present research.

Doses and	SFM ^{ns} (g·plant ⁻¹)	LN	FRM ^{ns} (g·plant ⁻¹)) SL ^{ns} (cm)	HC (cm)	DLM ^{ns} (g·plant ⁻	¹) DSM ^{ns} (g·plant ⁻¹)
AF (mL·L ⁻¹)						
0	591	28.25 с	21.92	5.61	47.58 b	16.18	1.09
1	617	30.90 ab	22.31	5.58	52.91 a	16.16	1.05
2	655	32.58 a	23.88	6.00	53.00 a	16.48	1.10
4	556	30.58 abc	19.74	5.42	52.83 a	15.35	0.88
8	627	28.83 bc	22.00	5.37	52.83 a	16.53	1.04
CV (%)	8.25	3.55	12.10	10.4	2.27	8.22	14.32

 Table 3. Fresh leaf mass (SFM), leaf number (LN), fresh stem mass (FRM), stem length (SL), head circumference (HC), dry leaf dry

 mass (DLM) and dry stem mass (DSM) of lettuce plants treated with different doses of fulvic acid (FA) in a field experiment

Means followed by the same letter in the column do not differ, Tukey test, p < 0.05. ^{ns}: not significant.

According to the norms of the Brazilian program for standardization of horticulture^[19], the plants obtained in this experiment can be classified between classes 55 and 65, which is considered a good commercial standard, reflecting the good fertility of the experimental area and adequate organic management. It is noteworthy that even under these conditions, the use of fulvic acid in lettuce seedlings promoted an increase in the circumference of the heads of "Raider Plus" lettuce at all doses used.

The research aimed to identify the effect of fulvic acid in the production of seedlings and its unfolding in the final production of lettuce. Most of the works with this profile stop at the seedling production stage, without discussing the economic implications of the techniques tested. In the present work, the authors went further, evaluating the effect of the treatments performed on seedlings in the final production. It was not the objective of this research to discuss the cost of the technique.

4. Conclusions

Fulvic acid applied to the seedlings, in its different doses, stimulated plant growth at the beginning of the cycle, especially root growth, and promoted the greatest circumference of the heads of 'Raider Plus' American lettuce.

Conflict of interest

The authors declare that they have no conflict of interest.

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