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Roots of ‘BRS Princesa’ banana fertigated with humic substances and saponin-based plant extracts

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ABSTRACT. This study aimed to evaluate the effects of fertigation with humic substances, with and without the addition of plant extracts, on the root system of the 'BRS Princesa' banana cultivar. The banana crop was cultivated at a spacing of 2.0 x 2.5 m and fertigated using a drip system in a dystrocohesive Yellow Latosol. The experimental design was randomized blocks, in a split-split-plot scheme with five replicates, for the following factors: humic substance doses and presence of a plant extract formulation. The treatments consisted of five doses of humic substances, based on a reference dose of 14.14 L ha^{-1} $cycle^{-1}$, in the presence and absence of plant extract. The variable root length (cm) was subjected to variance analyses to evaluate the effect of the humic substances, either isolated or in interaction with plant extract and soil depth. The use of the plant extract increased the effect of the humic substance on root length but overshadowed its effect for the different doses of humic substance. Root length was not sensitive to increasing humic substance dose with plant extract for doses of up to 42.42 L ha^{-1}at 0 – 0.40 m depth.

Keywords: *Musa* spp., root length, humic acid, fulvic acid.

Raízes da bananeira ‘BRS Princesa’ fertirrigada com substâncias húmicas e extratos vegetais a base de saponinas

RESUMO. O trabalho teve como objetivo avaliar efeitos de substância húmica aplicada com e sem adição de extrato vegetal via fertirrigação no sistema radicular da bananeira cultivar ‘BRS Princesa’. A bananeira foi cultivada no espaçamento 2,0 x 2,5 m, fertirrigada por gotejamento em Latossolo Amarelo Distrocoeso. O delineamento experimental foi em blocos casualizados em esquema de parcelas subsubdivididas com cinco repetições, sendo os fatores: doses de substancia húmica, presença de uma formulação de extrato vegetal. Usou-se cinco doses de uma formulação de substâncias húmicas, baseadas na dose de referência correspondente a 14,14 L ha^{-1} $ciclo^{-1}$ de substancias húmicas, na presença e ausência de extrato vegetal. A variável comprimento de raízes (cm) foi submetida a análises de variância para avaliação do efeito isolado da dose de substancia húmica ou em interação com uma formulação de extrato vegetal e com a profundidade do solo. O uso do extrato vegetal potencializou o efeito da substancia húmica no comprimento das raízes, entretanto ofuscou seu efeito para as diferentes doses de substancia húmica. Na profundidade 0 – 0,40 m o comprimento de raízes não foi sensível ao aumento da dose da substancia húmica com extrato vegetal até 42,42 L ha^{-1}.

Palavras-chave: *Musa* spp., comprimento de raiz, ácido húmico, ácido fúlvico.

Introduction

The plant root system plays an important role in the interactions between the soil and the banana crop, and knowledge regarding its distribution in the soil allows better use of cultivation practices, such as irrigation management (Coelho, Oliveira, Araújo, Vasconcelos, & Lima, 2001; Fracaro, & Pereira, 2004).

For crop management, it is important to know various agronomic factors, including the root system development and distribution, as practices such as irrigation and soil management can be better applied to increase crop yield when informed by knowledge on the plant root system (Hughes, Horne, Ross, & Julian, 1992; Fante Júnior, Reichardt, Jorge, & Bacchi, 1999). Studies on root distribution and depth are a source of information for the improvement of culture techniques, such as planting spacing, soil management and fertilization (Castle, Tucker, Krezdorn, & Youtsey, 1989). Knowledge regarding root density and depth allows the distribution of fertilizers at suitable locations, reducing losses and increasing the efficiency of use by the plants (Sousa, Folegatti, Coelho Filho, & Frizzone, 2002).

Coelho et al. (2001) reiterate that knowledge regarding root distribution is also an important tool with respect to irrigation projects, as the amount of water applied must maintain the soil near field capacity at the effective root depth. The distribution of roots in the soil, both horizontally and vertically, is strongly influenced by a series of complex and dynamic processes, which include interactions among the environment, the soil (e.g., soil type, porosity, compaction, water availability) and the plants in full growth (Fante Junior et al., 1999; Robinson, & Galan Sauco, 2010).

Humic substances alter plant development (Nardi, Pizzeghello, Muscolo, & Vianello, 2002; Canellas, Olivares, Okorokova-façanha, & Façanha, 2002; Chen, Clapp, & Magen, 2004) directly affect plant metabolism through their effects on the soil, such as metal complexation, increased cation exchange capacity, nutrient supply and water retention; and influence ion transport, respiratory activity, chlorophyll content, nucleic acid synthesis and the activity of various enzymes (Nannipieri, Muccini, & Ciardi, 1983). The most commonly reported effects of humic acids on plants are related to the root system and involve the formation of lateral roots (Trevisan, Francioso, Quaggiotti, & Nardi, 2010; Baldotto et al., 2011; Jindo et al., 2012; Mora, Baigorri, Bacaicoa, Zamarreño, & García-mina, 2012) and adventitious roots (Baldotto, Baldotto, Soares, Martinez, & Venegas, 2012), root elongation and the formation of root hairs (Canellas et al., 2011; Silva et al., 2011). All these factors increase root mass and surface area, contributing to a greater absorption of water and nutrients (Eyheraguibel, Silvestre, & Morard, 2008). All these advantages support the use of humic substances to improve plant growth and yield.

The banana crop is susceptible to phytonematode attack, with the possibility of damage to the root system and significant yield losses. The most harmful nematodes are involved in the destruction of primary roots and the support system of the plant (Rosa et al., 2009). Saponins belong to the group of glycosidic secondary metabolites, are produced by many plant species, such as *Quillaya Saponaria Molina,* and have been cited as a satisfactory nematode control.

The benefits of humic substances, mainly due to auxin-like activities and ion uptake (Canellas et al., 2002), might be helpful under field conditions for the banana crop. The use of humic substances together with a saponin-based plant extract applied at a specific dose through the irrigation water might improve root growth.

The use of humic substances in Brazil is recent and has followed the recommendations of manufacturers, which are mostly from abroad; this situation has caused users to apply these substances, initially, in the same way as in other countries, followed by empirical adjustments by the farmers. In terms of the recommendations of use, the appropriate dose in Brazilian soils for tropical crops is one of the first questions to be answered. Understanding the effects of the application of these substances on the plant root system through irrigation water will improve the criteria and available information.

This study aimed to evaluate the effect of humic substances applied through fertigation, with and without the addition of plant extracts, on the root system of the 'BRS Princesa' banana.

Material and methods

The study was conducted in the experimental area of Embrapa Cassava and Tropical Fruits, in the municipality of Cruz das Almas, Bahia State, Brazil (12° 48' S; 39° 06' W; 225 m). The climate of the region is classified as humid to sub-humid, with an annual mean rainfall of 1,143 mm. The experiment was conducted in a dystrocohesive Yellow Latosol, with medium texture, which is classified as a sandy clay (Santos et al., 2013). The soil physical, physical-hydraulic and chemical attributes in the subsurface are shown in Table 1.

The banana cultivar 'BRS Princesa' (AAAB Tetraploid; 'Maçã' variety) was used in the study, at a spacing of 2.0 x 2.5 m. Planting and cultivation practices were performed according to the recommendations of Borges and Souza (2004). Top-dressing fertilization was applied through organo-mineral fertigation, with weekly application of urea and potassium chloride as sources of nitrogen and potassium, based on soil chemical analysis, and monthly application of humic substance and plant extract. The humic substance (HS) consisted of a mixture of humic acids (200 g kg^{-1}), fulvic acids (102 g kg^{-1}) and potassium (26.6 g kg^{-1}). The plant extract was based on *Yucca schidigera*, *Quillaja saponaria*, *Tagetes* spp. (93%) and triterpenoid saponins (7%).

Table 1. Attributes of the dystrocohesive Yellow Latosol in the experimental area. Cruz das Almas, Bahia State, 2013.

Layer (m)	Physical and Physical-hydraulic Attributes						
	Total Sand	Silt	Clay	Ds	Water content (cm^3cm^{-3}) under tension (kPa)		
	g kg^{-1}			g cm^{-3}	10	33	1500
0-0.20	732	87	181	1.67	0.1785	0.1761	0.0980
0.20-0.40	629	68	303	1.66	0.1964	0.1936	0.1514
0.40-0.70	600	77	323	1.45	0.1896	0.1637	0.1320

Ds=Soil bulk density.

Layer	Chemical attributes										
	pH in water	P (Mehlich-1)	K	Ca	Mg	Al	Na	H+Al	SB	CEC	V	OM
(m)		mg dm^{-3}					$cmol_c dm^{-3}$				%	g kg^{-1}
0-0.20	6.7	15.6	0.14	2.5	0.9	0.2	0.03	0.7	3.6	4.4	83	7.0
0.20-0.40	6.0	6.0	0.10	1.7	0.7	0.1	0.03	1.4	2.5	4.2	62	5.9
0.40-0.60	5.4	4.6	0.07	1.1	0.5	0.0	0.02	1.9	1.7	3.7	48	5.7

SB (sum of exchangeable bases), CEC (cation exchange capacity), V (base saturation) and OM (organic matter).

A drip irrigation system was used, with one lateral line per row and three emitters per plant, each with a flow rate of 4.0 L h^{-1}, and an irrigation interval of two days. The irrigation depths were calculated based on crop evapotranspiration (ETc) in mm day^{-1}, obtained from the maximum or reference evapotranspiration (ETo), determined according to the modified equation of Penman-Monteith (Allen, Pereira, & Raes, 1998). Fertigation was performed using a Venturi-type injector connected to the controller at the beginning of the experimental area.

The study incorporated two experimental designs in the same experiment. One design was in randomized blocks with five replicates, in a split-split-plot scheme in space, with the HS dose in the plot, the presence/absence of plant extract in the sub-plot, and soil depth as a variable inside the sub-plot. HS doses were established as a function of the reference dose (RD) of a 14.14 L ha^{-1} $cycle^{-1}$, which is equivalent to a 70 L ha^{-1} $cycle^{-1}$ of the commercial product. Thus, the HS doses applied in the experimental plots were 0, 1.5 RD, 2.0 RD and 3.0 RD, equivalent to 0, 21.21, 28.28, and 42.42 L ha^{-1} $cycle^{-1}$, respectively. The following depths were considered: 0.10, 0.20, 0.30, and 0.40 m. The other experimental design was in randomized blocks with five treatments (doses of humic substances) and five replicates, i.e., the sub-plots with the application of humic substances alone were considered as plots in this design. The evaluated dependent variable was root length in the soil profile, considering the sum of root lengths at each distance from the plant and each depth in the soil profile.

Root samplings were performed at positions relative to the pseudostem, along the plant row, following the drip line. Sampling was performed using the monolith method (Bohm, 1979), by collecting 0.10 x 0.10 x 0.10 m samples in two soil pits in the direction of the plant row, one close to the pseudostem of the sub-plot with application of the plant extract and the other close to the pseudostem of the sub-plot without application of the plant extract, i.e., with the application of humic substances only. In the soil pits close to the pseudostems where the plant extract was applied, the samples were collected at distances of 0.25 and 0.50 m and at depths of 0.10, 0.20, 0.30, and 0.40 m. In the soil pits close to pseudostems where only humic substances were applied, the samples were collected at distances of 0.25, 0.50, 0.75, 1.00, and 1.25 m and at depths of 0.10, 0.20, 0.30, 0.40, 0.60, and 0.70 m.

Sample processing involved the separation of roots and the digitalization and determination of root length, according to Santana, Coelho, Faria, Silva, and Donato (2012). One analysis of variance involved the root length data in a split-split plot design, considering the factors HS dose, presence/absence of plant extract and soil depth. The randomized block design was used for two analyses: one considering root length data as a mean of five distances from the plant (0.25, 0.50, 0.75, 1.0, and 1.25 m) and another evaluating the effect of HS doses on root effective depth (EDepth) and effective distance (EDist). EDepth and EDist, where 80% of the roots are found in terms of depth and horizontal distance from the plant, respectively (Coelho, Simões, Carvalho, & Coelho Filho, 2008), were determined according to Santana et al. (2012).

Soil physical attributes were evaluated 12 months after planting only in the sub-plots with the application of HS alone in the layer 0-0.20 m. The attributes total porosity, macroporosity, microporosity, soil density, and water content at the tension of 10 kPa were evaluated at the distance of 0.30 m from the plant, using an Uhland sampler, according to Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA, 2011).

Based on the analysis of variance, using the statistical program Sisvar (Ferreira, 2011), a regression analysis was performed for the quantitative sources of variation, and tests of comparison of means were performed for the qualitative variables.

Results and discussion

The analysis of variance detected the effect of HS doses and plant extract on the mean root length (RL) in the evaluated profile. Soil depth also affected RL, as well as the interactions between HS doses x Plant extract (PE) and between HS doses x Soil depth.

According to the regression analysis for the dependent variable root length (RL) as a function of HS doses combined with PE, there was no fitting of the RL data, thus, there were no differences observed for this variable between doses. However, except for the HS doses of 0 and 28.28 L ha^{-1}, the application of the other HS doses with the plant extract resulted in higher mean root length than with only HS application (Table 2).

Table 2. Comparison of mean root length (RL) with and without the application of plant extract (PE) and doses of humic substance (HS).

	Doses of humic substance (L ha^{-1})			
PE	0	21.21	28.28	42.42
With	372.5 a	473.1 a	385.2 a	347.3 a
Without	367.2 a	307.6 b	375.2 a	294.6 b

Means followed by the same letters in columns do not differ by the Tukey test at 5%.

The use of plant extract combined with HS contributed to root system growth. This contribution may be due to the biocidal aspects of triterpenoid saponins, the main component of the plant extract, which may have favoured the sanitation of the root environment, possibly acting on nematodes, which damage the root system (Ritzinger, Borges, Ledo, & Caldas, 2007), resulting in better root development than with only HS application. The action of these natural pesticides based on triterpenoid saponins, essential oils, is related to the repellent, intoxicating and/or antagonistic activity of the substances present in the plants.

Considering HS application alone, root length (RL) was influenced by HS dose ($p < 0.05$), showing second-degree polynomial behaviour as a function of HS dose (Figure 1): 75% of RL variation can be explained by the variation in HS doses. This result is different from the combination of HS and PE, for which there was no fitting of RL data as a function of HS dose.

These results corroborate the influence of plant extract, modifying the effect of HS when applied together, as the saponin-based plant extract improves soil conditions for root development and overshadows the effects of HS. These results are also consistent with Piccolo, Conte, and Cozzolino (1999), who related humic substances to root growth, as well as other authors (Zandonadi, & Busato, 2012; Zandonadi, Santos, Busato, Peres, & Façanha, 2013; Nikbakht et al., 2008), who claim that one of the main effects observed with the use of humic substances is root development.

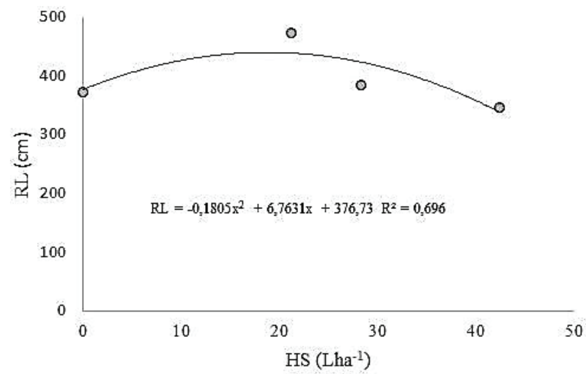

Figure 1. Banana root length (RL) as a function of the isolated application of humic substances (HS).

The HS dose that promoted the highest RL value (Figure 1) was 18.73 L ha^{-1}, a value considered high for commercial purposes, as the recommendation for use is 7.07 L ha^{-1} per banana cycle (35 L ha^{-1} of the commercial product). The RL variation rate was positive until the dose of 18.73 L ha^{-1}, equivalent to the maximum dose, followed by a negative rate from this dose onward. The RL variation rate with respect to HS dose ranged from 6.76 cm per unit of L ha^{-1}, 0 HS dose application, to 0 cm per unit of L ha^{-1} for the dose of 18.73 L ha^{-1} HS. The increase in RL per L ha^{-1} in the condition of no HS application (0 dose) up to the dose of 7.07 L ha^{-1} varied from 6.76 cm to 4.21 cm per L ha^{-1} of HS.

The evaluation of RL means (Figure 2) in the profile of 0-0.40 m, considering all the applied doses, revealed that HS, whether alone or combined with plant extract, resulted in an increasing behaviour in the profile or in part of it. The HS applied with PE positively contributed to the alteration of these variables in the soil (Table 2), and both the observed values and the ones estimated using second-degree polynomial models were higher than the means obtained for isolated HS application, which indicates a positive effect of HS on root development through the interaction with PE. The superiority of RL means for the combined application of HS and PE occurred until the depth of 0.35 m, which is the limit for the influence of HS and PE in the profile.

Figure 3 shows the interaction of HS dose x Soil depth and RL means at four depths as a function of the isolated application of HS. The variations in RL at the depths of 0.30 and 0.40 m corresponded to the variation of HS dose through quadratic models; more than 95% of RL variation is explained by the variations in soil depth. At the depths of 0.10 and 0.20 m, there was a linear reduction in root length with increasing HS doses. These results indicate, for the depths of 0.10 and 0.20 m, that root length has a reduction rate of 1.34 and 2.09 cm per L ha^{-1} of HS, respectively.

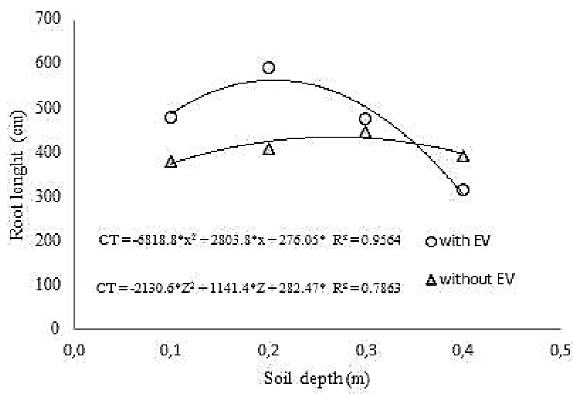

Figure 2. Banana root length (RL) as a function of the application of humic substances (HS) with and without PE.

The reduction of roots at the depths of 0.10 and 0.20 m with increasing HS dose was not expected, as there was no linear reduction of RL with increasing HS dose applied with plant extract (Table 2) and, in general, there was a quadratic polynomial behaviour of RL with increasing HS doses (Figure 1). It should be noted that, at the depths of 0.10 and 0.20 m, RL is naturally higher than at greater depths in the soil profile for the banana crop, according to Santana et al. (2012).

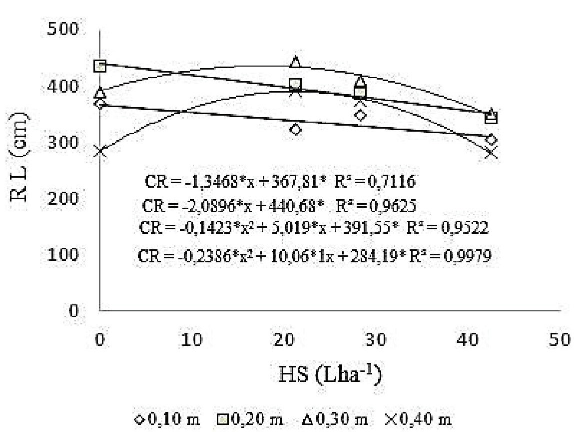

Figure 3. Root length (RL) as a function of doses of humic substances (HS) applied through fertigation: 0 L ha^{-1} (control), 21.21 L ha^{-1} (1.5 Reference dose-RD), 28.28 L ha^{-1} (2 RD) and 42.42 L ha^{-1} (3 RD).

The increase in the variation rate of RL for the depths of 0.30 and 0.40 m with increasing HS dose occurred until 17.63 and 21.09 L ha^{-1}, respectively, where RL reached its maximum values, which may be due to the vertical movement of the HS dissolved in the soil solution. Higher doses caused more vertical movement of the soil solution, which moved downward in the profile and reached depths of 0.30 and 0.40 m; at these depths, doses higher than 17.63 and 21.09 L ha^{-1}, respectively, do not maintain the HS solution enough to cause a positive effect on RL.

The analysis of variance performed to verify whether HS dose influenced the effective depth or the effective distance of the root system did not indicate the effect of the doses on these variables ($p > 0.05$). The values of mean effective depth (0.38 m) and mean effective distance (0.78 m) for the HS doses (Table 3) show that, despite the differences in these variables for the doses, there was no fitting that could adequately explain their variation. The mean values of effective depth and effective distance for the HS doses were close to the values reported by Coelho et al. (2008) and lower than the mean values obtained by Santana et al. (2012), namely 0.74 and 0.63 m, respectively.

Table 3. Mean values of Effective Depth (EDepth) and Effective Distance (EDist) of the root system of 'BRS Princesa' banana as a function of the dose of humic substances (HS).

HS dose (L ha^{-1})	EDepth (m)	EDist (m)
0	0.38	0.77
21.21	0.38	0.78
28.28	0.38	0.78
42.42	0.38	0.78

In this study, the analysis of variance did not show a significant effect of HS dose on the variables total porosity, macroporosity, microporosity, soil density, water retention at 10 kPa and mean weighted diameter of the aggregate (Table 4) at the distances of 0.25 and 0.50 m and in the layer 0-0.20 m, evaluated after 12 months of application. Thus, HS application did not cause any significant change in the evaluated attributes for any of the applied doses, which disagrees with Santos, Silva, Canellas, and Camargo (2008), who claim that root development is related to improvements in soil structure, aggregation and water infiltration and retention, which are intensified by HS, and Canellas et al. (2002), for whom improved soil structure reduces its mechanical resistance, favouring root development.

Table 4. Means of soil physical attributes (total porosity, macroporosity, microporosity, bulk density, water retention at 10 kPa, mean weighted diameter of the aggregate) for the layer 0-0.20 m in the plots under application of humic substances (HS) alone.

HS doses (L ha^{-1})	Total Por. (%)	Macrop. (%)	Microp. (%)	Bulk Dens. (kg dm^{-3})	Water content at 10 kPa (%)	MWD (mm)
0	33.99	12.06	19.97	1.69	18.60	2.55
21.21	31.93	11.66	20.37	1.67	19.07	2.52
28.28	29.23	10.68	20.12	1.69	17.93	2.95
42.42	31.19	9.86	20.64	1.71	18.65	2.62

The doses of HS with or without PE did not affect the growth variables (pseudostem diameter and leaf area) and yield variables (number of fruits/hand (NFH); number hands/bunch (NHB); hand yield (HY, t ha^{-1}); bunch yield (BY, t ha^{-1}); length (LCF, cm) and diameter (DCF, cm) of the central fruit of the second hand; however, the means of HY, BY, LCF and DCF with the application of HS and PE were statistically superior to the ones with HS application alone (Table 5).

Table 5. Means of variables: number of fruits/hand (NFH); number hands/bunch (NHB); hand yield (HY, t ha^{-1}); bunch yield (BY, t ha^{-1}); length (LCF, cm) and diameter (DCF, cm) of the central fruit of the second hand; treatments with HS with and without ad PE application.

Plant extracts	NFH (Unt.)	NHB (Unt.)	HY (t ha^{-1})	BY (t ha^{-1})	LCF (cm)	DCF (mm)
HS + PE	101.10a	6.42a	27.56a	31.34a	17.89a	36.79a
HS	100.70a	6.36a	25.87b	29.35a	16.88b	35.45b
CV (%)	3.63	7.53	5.33	4.82	8.21	5.59

Means followed by the same letters in a column do not differ by F test at 0.05 probability.

The fact that HS applied without PE did not affect the growth variables is in agreement with Yona and Aviad (1990) who claim that HS stimulation of root growth is generally more apparent than stimulation of shoot growth. In addition, the response to the application of HS may depend on other factors, such as the composition of the substances (Hooks, Wang, Ploeg, & Mcsorley, 2010), in addition to the fact that the nature of these organic compounds is relatively complex and there is unconsolidated knowledge on the action of the humic fraction on plants due to its composition and chemical structure, which have not been elucidated and are variable (Baldotto, & Baldotto, 2014). The effect of the application of PE together with HS might be due to its biocide action on harmful organisms, such as nematodes, as reported by Hooks et al. (2010), which may have promoted the preservation of the root system and stimulated root growth.

Conclusion

Root length did not decrease or increase for the application of 0 to 42.42 L ha^{-1} of humic substances with plant extract in the soil profile of 0-0.40 m for the 'BRS Princesa' banana cultivar.

Root length showed a positive variation rate until the dose of 18.73 L ha^{-1} in the soil profile of 0-0.40 m with only humic substances.

Root length in the soil profile of 0-0.40 m with the application of humic substances and plant extract was higher than with the application of humic substances alone.

The application of 0 to 42.42 L ha^{-1} of humic substances without plant extract did not reduce or increase the effective depth and effective distance of the root system of the 'BRS Princesa' banana cultivar.

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