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Humic substances: effects on potato growth and yield

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ABSTRACT

The results from humic substances (HS) application in varied crops and conditions are controversial, and the experiments with the potato crop in Brazil are scarce. The objective of this study was to evaluate the effects of HS doses on the growth and yield of two potato cultivars. Four doses of HS were tested: 0, 5.05, 10.10, and 15.15 L ha^{-1}, applied in the planting furrows of cvs. Agata and BRS F63 Camila, in Guarapuava-PR, in the 2015 and 2016 crop seasons, between October and February. The experiment was carried out using a randomized complete block design, in a factorial scheme (crop season x dose x cultivar), with four replications. Plant samplings were performed at tuber initiation, flowering, tuber bulking, and plant maturation growth stages. After shoot senescence, the total and commercial tuber yields were evaluated. Cultivars responded similarly to HS application, with no significant interaction between HS and cultivars, for most assessed variables. At tuber initiation, there was a negative linear effect of HS doses on leaf area index, number of formed tubers, and tuber and total plant dry weight. In the other evaluations, the effect of HS application was not observed regarding the assessed variables. Likewise, no effects were detected on the number and fresh weight of tubers in total and commercial yields. We concluded that HS application affected both cultivars similarly, hampering initial plant growth and not increasing potato yield.

Keywords: *Solanum tuberosum*, biostimulant, leaf area index, tuber yield.

RESUMO

Substâncias húmicas: efeito no crescimento e na produtividade de plantas de batata

Os resultados da aplicação de substâncias húmicas (SH) em variadas culturas e condições são controversos e os experimentos com essas substâncias na cultura da batata no Brasil são escassos. O objetivo do trabalho foi testar o efeito de doses de SH no crescimento e na produtividade de duas cultivares de batata. Testou-se quatro doses de SH: 0, 5,05, 10,10 e 15,15 L ha^{-1}, aplicadas nos sulcos de plantio das cultivares Agata e BRS F63 Camila, em Guarapuava-PR, nas safras 2015 e 2016, entre outubro e fevereiro. Foi utilizado o delineamento de blocos casualizados em esquema fatorial (safra x dose x cultivar) com quatro repetições. Foram realizadas avaliações fitotécnicas nos estádios de iniciação de tubérculos, florescimento, enchimento de tubérculos e na maturação de plantas. Após a senescência da parte aérea, quantificou-se a produtividade total e comercial. As cultivares responderam de forma semelhante à aplicação de SH, não se observando interação significativa entre SH e cultivar na maioria das variáveis analisadas. Na iniciação de tubérculos, observou-se efeito linear negativo das doses de SH no índice de área foliar, no número de tubérculos formados, na massa seca de tubérculos, bem como na massa seca total. Nas demais avaliações não se constatou efeito da aplicação de SH nas variáveis analisadas. Igualmente, não houve efeito das SH no número ou na massa fresca de tubérculos nas avaliações da produtividade total e comercial. Concluiu-se que a aplicação de SH afetou de forma semelhante as cultivares; afetou inicialmente de forma negativa o crescimento das plantas e não aumentou a produtividade final de batata.

Palavras-chave: *Solanum tuberosum*, bioestimulante, índice de área foliar, rendimento de tubérculos.

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Humic substances (HS) fall into the group of materials that promote plant growth, the so-called biostimulants, which increase nutritional efficiency, water stress tolerance, and the quality of agricultural products (Jardin, 2015). In recent decades, sustainable alternatives to the indiscriminate use of synthetic agrochemicals, such as fertilizers and pesticides, have been proposed (Rouphael & Colla, 2020). The prospect of increasing the use of biostimulants in agriculture worldwide is on the order of 12% per year. It has been projected to reach revenues above US$2.2 billion in 2018, demonstrating the importance of studies on the effects of biostimulants (Calvo *et al*., 2014).

Humus represents decomposed organic matter (OM) added to compounds from microbial resynthesis that are resistant to biological degradation due to the presence of lignin and other phenolic constituents (Flaig *et al*., 1975). The HS present in humus include fulvic acids, humic acids, humines, and himatomelanic acids, according to their solubility in alkaline or acidic media and ethanol (Schnitzer & Khan, 1975). Small, heterogeneous molecules associate randomly to form the hydrophobic and hydrophilic fractions of HS, combining contiguously or embedded within each other to form complex chemical structures (Piccolo, 2001). The primary sources of HS used for the industrial production of commercial products are brown coal, leonardite, peat, lake-bottom sediments, and organic waste, which differ in the OM origin and humification conditions (Yakimenko *et al*., 2018).

Humic substances comprise more than 80% of the OM present in the soil and have shown positive effects in increasing volume, branching, and hair of roots (Canellas & Olivares, 2014). The stability, durability, and composition of complex chemical structures will define the effects of their activity on plant development by improving soil fertility (Schnitzer & Khan, 1975); increasing cation exchange capacity; chelating mechanisms that favor the availability of micronutrients to plants; and buffering power, thus avoiding sudden changes in soil pH (Burns & Martin, 1986). Humic substances also improve the soil physical attributes (Flaig *et al*., 1975) and participate in soil remediation, reducing toxins (Martin & Focht, 1977). Furthermore, they enable the biological balance of the soil, which can reduce root diseases, besides playing a physiological role in plant growth (Flaig *et al*., 1975; Martin & Focht, 1977; Burns & Martin, 1986), through a hormone-like action, improving the intermediary metabolism, respiration, and photosynthesis (Nardi *et al*., 2002).

Despite all the above-mentioned benefits, contradictory results regarding yield increase with HS doses have been reported in various crops and conditions. Seyedbagheri *et al.* (2012) state that the contradictions obtained with HS application are mainly due to HS's complex nature, including their unknown total carbon content and clay mineral types and how they interact with HS in the tested soils, besides the application of inadequate HS doses. Responses to HS application can also vary depending on the genetic material used. For instance, Sanli *et al.* (2013) observed significant interactions between potato cultivar and HS dose for commercial yield and tuber protein content. It is therefore critical to use more than one cultivar when assessing the effects of HS application. Other studies found a positive effect of HS application on potato yield (Martins *et al*., 2020; Wadas & Dziugieł, 2020; Caradonia *et al*., 2021), but the effect depended on several factors, highlighting the importance of studies in specific environments. In light of all that has been presented, this study aimed to evaluate how HS application affects the growth and yield of potato cultivars in southern Brazil.

MATERIAL AND METHODS

Two experiments were conducted in Guarapuava-PR, Brazil. The first was installed in October 2015 on the Midwestern Paraná State University, Unicentro-Cedeteg campus (25°23'06"S, 51°29'39"W, 1,029 m altitude). The second experiment was performed in October 2016 in Rio das Pedras (25°20'32"S, 51°22'04"W, 1,055 m altitude). The soil of both experimental sites is classified as Clayey Oxisol (Michalovicz *et al*., 2014). The chemical and physical attributes of the soil in the Cedeteg Campus and Rio das Pedras, respectively, were: pH ($CaCl_2$): 5.3 and 5.5; OM: 28.2 and 43.1 g dm^{-3}; P (Mehlich 1): 5.7 and 5.7 mg dm^{-3}; K^+: 0.47 and 0.30 $cmol_c$ dm^{-3}; Ca^{2+}: 2.1 and 4.3 $cmol_c$ dm^{-3}; Mg^{2+}: 3.7 and 1.5 $cmol_c$ dm^{-3}; Al^{3+}: 0.0 and 0.0 $cmol_c$ dm^{-3}; H+Al: 3.67 and 4.26 $cmol_c$ dm^{-3}; sand: 200 and 180 g kg^{-1}; silt: 270 and 290 g kg^{-1}, and clay: 530 and 530 g kg^{-1}.

A randomized block design was used in a 2 x 2 x 4 factorial scheme: 2 crop seasons, 2 cultivars (Agata and BRS F63 Camila), and 4 doses of HS (0, 5.05, 10.10, and 15.15 L ha^{-1}) with 4 repetitions. In both crop seasons, the planting spacing was 0.8 m between rows and 0.3 m between plants. The experimental unit had 7 rows with 16 plants, totaling 4.8 m^2. Sprouted seed potatoes with 30 to 50 mm diameter were planted using 3.5 t ha^{-1} of the chemical formulation 04-14-08. The HS were applied in the planting furrow, with a product containing 20.2% (w/w) of HS obtained from diluting a liquid product composed of 25.2% HS, extracted from peat, and total carbon content of 14%.

The experimental areas were not irrigated. The management of weeds, pests, and diseases was done manually and following the regional standard procedures. Desiccation of plants was performed when 70% of leaves turned yellow, thus determining the end of the growth cycle of the two cultivars.

The leaf area index (LAI) of each plot was evaluated by quantifying the leaf area of 35 to 45 fully developed leaves, using a leaf area integrator (LI-3100, Licor, USA), and the respective dry weight (DW) of the sampled leaves. From the relationship between leaf DW and leaf area, the specific leaf area of the sample was obtained. Using the total leaf DW of the plot and the planting density, the LAI of each plot was estimated. The number of initiated (diameter less than 1 cm) and formed (diameter greater than 1 cm) tubers was recorded. The total DW and the DW of the formed tubers, obtained after oven-drying the samples at 65°C until constant weight, were also quantified. Samplings were performed at the phenological stages corresponding to the initiation of tubers, flowering, tuber bulking, and plant maturity, at approximately 15, 31, 47, and 63 days after emergence (DAE), respectively. Samples were taken from 4 plants per plot, one in each of the central rows. At harvest, total and commercial (tubers with transverse diameter ≥42 mm) yields were estimated by manually sampling 12 plants per plot, 3 in each of the central rows. The values of fresh weight and number of tubers were also recorded.

Average temperature data and monthly accumulated precipitation during the two crop seasons were obtained from the SIMEPAR weather station located about 50 m from the experiment site of the first crop season and 15 km from the second crop season site. The historical average data (30 years, 1986-2015) were obtained from Clima Tempo (2021). The average monthly temperature ranged from 20.1 to 22.7°C, with an average of 21.4°C in the first crop season, while the second crop season had 18.2-22.8°C, with an average of 20.7°C. The historical average for the municipality brings variation from

17.5 to 21.0°C, with a general average of 19.8°C. The total rainfall observed in the first crop season (1,042 mm) was 20% above the historical average (865 mm), with October (191 mm) being the least rainy month and February (246 mm) the month with the most rainfall. In the second crop season, November (151 mm) had the least rainfall, while December (208 mm) had the most rainfall. The total volume observed in the second crop season, 866 mm, was similar to the historical average.

The homogeneity of variance of the data was tested using Cochran's test, and normality was tested using Shapiro-Wilk's test, with the consequent transformation of non-normal data utilizing potentiation, square root, and logarithm in base ten. Next, analysis of variance and linear and polynomial (2nd order) regression analysis were performed. Finally, the significant regression with the highest determination coefficient (R^2) was adopted.

RESULTS AND DISCUSSION

Climatic conditions

The average monthly temperatures in the two experiments met the potato crop requirements for high yields under Brazilian conditions, ranging from 10 to 25°C (Lopes *et al*., 2011). Furthermore, the total rainfall during the two crop seasons also satisfactorily met the crop requirement, with 300-800 mm (King *et al*., 2020).

Statistical analyses

All obtained variances were considered homogeneous, but there were data with non-normal distribution. These data were transformed. No triple interactions were observed among crop seasons, cultivars, and HS doses. The interactions of HS doses and crop seasons were observed only for DW of formed tubers at the tuber initiation stage, wherein values were higher in the second crop season for all HS doses. The interaction between HS and cultivars was only observed for number of tubers initiated at the tuber bulking stage, with cv. Agata presenting higher values than cv. BRS-Camila at the doses 0 and 10.10 L ha^{-1}. Therefore, the data presented are the average of the two crop seasons and the two cultivars.

Effects of HS application

At the tuber initiation stage, negative linear regressions were fitted to LAI (Table 1 and Figure 1A), number of formed tubers (Table 1 and Figure 1B), tuber DW (Table 1 and Figure 1C), and total DW data (Table 1 and Figure 1D). In addition, quadratic regressions better-fitted LAI according to HS doses at the tuber bulking stage (Table 1 and Figure 1E).

The decrease in LAI with increasing HS doses seems to have impacted the number and DW of formed tubers and total DW accumulation at the tuber initiation stage. Oliveira (2000) observed that greater shoot growth and development influenced tuber fresh weight and total and commercial yield when comparing nitrogen doses between 40 kg ha^{-1} and 200 kg ha^{-1}.

One of the possible hypotheses that could explain the lower initial accumulation of DW in plants that received HS would be its negative effect due to the high iron content of the experimental soils. In soils with high levels of aluminum and iron, HS is inactivated and form stable cement, which reduces soil permeability (Rowberry & Collin, 1977). Another hypothesis would be the physical blocking of pores in the cell wall of root cells from the epidermal surface, promoted by the accumulation of HS absorbed along with water, adversely impacting root hydraulic conductivity, leaf growth, transpiration, and plant tolerance to drought (Asli & Neumann, 2010).

Despite finding an effect of HS application on the LAI at the tuber bulking stage (Table 1 and Figure 1E), the highest dose applied (15.15 L ha^{-1}) resulted in similar values of LAI without HS application (0 L ha^{-1}), therefore, bringing no advantage.

In the other samplings, no effect of HS application was detected for the variables analyzed. LAI values were 3.84 and 0.75 in flowering and plant

Table 1. Leaf area index (LAI), number of initiated and formed tubers, tuber dry weight, and total plant dry weight at tuber initiation (15 days after plant emergence, DAE), flowering (31 DAE), tuber bulking (47 DAE), and plant maturation (63 DAE) stages of cvs. Agata and BRS-Camila subjected to four doses of humic substances. Guarapuava, Unicentro, 2016-2017.

Variable	Growth stages			
	Tuber initiation	Flowering	Tuber bulking	Plant maturation
LAI	1.06	3.85	3.62	0.75
Regression[1]	L**	ns[3]	Q*	ns
CV (%)[2]	6.27	23.1	19.4	63.0
N. initiated tub. (nº $plant^{-1}$)	3.37	4.82	2.88	1.63
Regression	ns	ns	ns	ns
CV (%)	32.6	16.1	43.4	36.1
N. formed tub. (nº $plant^{-1}$)	2.93	9.83	10.3	9.94
Regression	L**	ns	ns	ns
CV (%)	54.9	17.0	17.0	19.5
Tuber dry weight (g m^{-2})	5.84	213.6	585.8	667.9
Regression	L**	ns	ns	ns
CV (%)	82.0	27.2	21.9	24.9
Total dry weight (g m^{-2})	74.9	415.8	822.8	760.9
Regression	L**	ns	ns	ns
CV (%)	2.67	20.6	19.1	24.2

Average of two crop seasons, two cultivars, and four doses of humic substances; [1]L: linear equation; Q: quadratic equation; [2]CV (%): coefficient of variation; [3]ns: statistical difference not significant (p>0.05), * and ** statistical difference at 5% (p<0,05) and 1% (p<0.01), respectively.

maturity stages, respectively (Table 1). The number of tubers initiated per plant was 3.37, 4.82, 2.87, and 1.63 at the tuber initiation, flowering, tuber bulking, and plant maturity stages, respectively. The number of tubers formed per plant was 9.83, 10.3, and 9.94 for flowering, tuber bulking, and plant maturity stages, respectively. Mean tuber DW was 214, 586, and 668 g m^{-2}, and mean total DW was 416, 823, and 761 g m^{-2} at flowering, tuber bulking, and plant maturity stages, respectively.

There was no effect of HS application on the number of total and commercial tubers; the average of the treatments showed 9.3 and 5.2 total and commercial tubers plant^{-1}, respectively (Figure 2A). Additionally, for total yield, which averaged 46.88 t ha^{-1}, and commercial yield, with 39.37 t ha^{-1} on average, evaluated at approximately 68 DAE, there was no effect of HS application (Figure 2B).

In experiments with HS doses in soil with low fertility, pH between 8.0 and 8.2, and OM of 0.9% to 1.0%, a positive effect on yield was observed with HS application (Seyedbagheri *et al.*, 2012). Seyedbagheri *et al.* (2012) reported tuber yield of 37.6 t ha^{-1} in the untreated plots and 43.1 t ha^{-1} in the plots that received 37 L ha^{-1} of the commercial product containing 6.0% (w/w) of HS, a little less than half of the lowest dose used in the present study. Probably, the very different soil characteristics from the present study, especially concerning pH and OM content, influenced this result. Recently, a study with three potato cultivars in bags with 88.3% sand and fertilizer and HS foliar applications showed positive effects of treatments on several variables evaluated, including tuber yield (Al-Zubaidi, 2018). Moreover, HS application associated with fertilizer led to potato tuber yield 9.3% higher compared to fertilizer treatment without HS, in soil with pH 7.2 (Selladurai & Purakayastha, 2016). In another experiment with fertigated potato and application of HS and nutrients in sandy soil with pH 8.4, greater tuber yield and contents of nutrient, starch, and total soluble solids were observed. This result was attributed to less leaching of macro and micronutrients provided by HS application, without, however, neutralizing the effect of the N, P, and K application (Selim *et al.*, 2009). Without considering the probable effects of N, P, and K application associated with HS, in an experiment in loamy soil with pH 8.2 and 1.3% of OM, the effects of 0, 200, 400, and 600 kg ha^{-1} of leonardite containing 50.5% HS, in addition to N, P, and K, on four potato cultivars were evaluated (Sanli *et al.*, 2013). In this study, a significant interaction was observed between HS doses and cultivars regarding commercial tuber yield and protein content. An increase in the number of tubers per plant, commercial yield, and total yield was observed at higher HS doses (Sanli *et al.*, 2013). Further, in an experiment with different irrigation regimes and with the application of 1.5 g L^{-1} of HS to potato planted in sandy soil, pH 8.1 and 0.16% OM, positive effects were found on shoot growth and tuber fresh weight, as well as on tuber yield (Alenazi *et al.*, 2016).

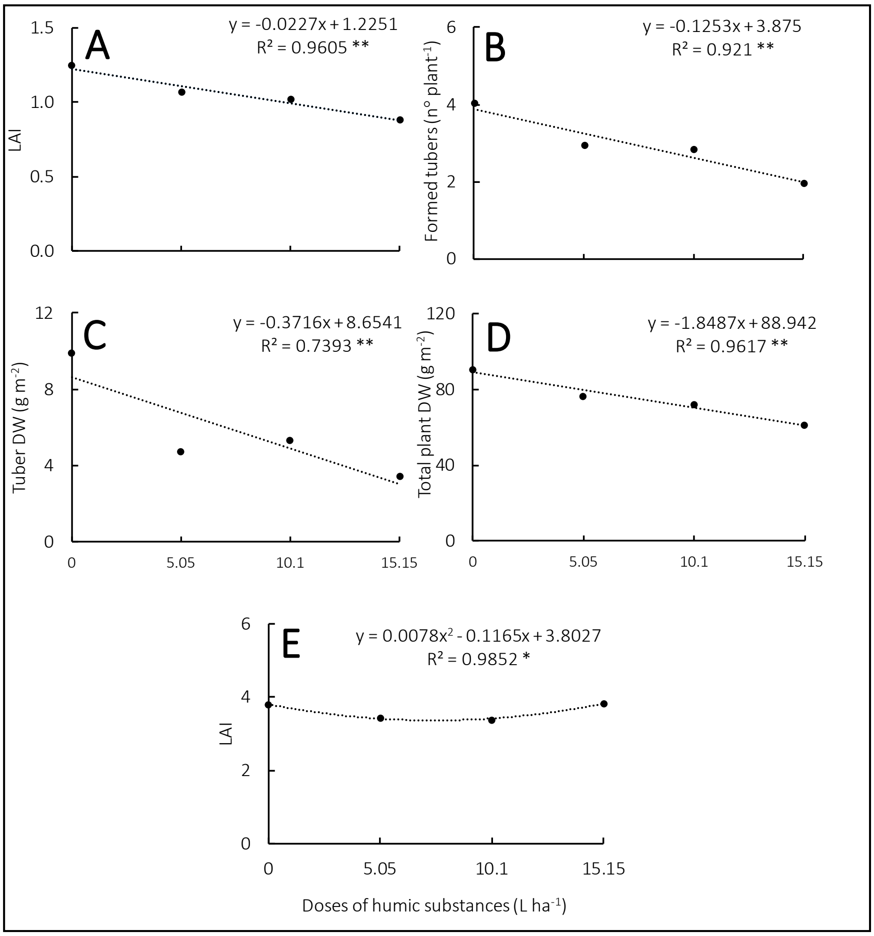

Figure 1. Linear regressions of leaf area index (LAI) (A), number of formed tubers (B), tuber dry weight (DW)(C), and total plant DW (D) at the tuber initiation stage, and quadratic regression of LAI at tuber bulking stage in plants subjected to doses of humic substances (HS) (L ha^{-1}). Average of two crop seasons and two cultivars. * and **: regression equation significant at 5% ($p<0.05$) and 1% ($p<0.01$), respectively. Guarapuava, Unicentro, 2015-2016 and 2016-2017.

In research conducted on beans, with applications of fertilizers containing HS in 35 experimental fields, no beneficial effects of the products used were detected (Mahoney *et al.*, 2016), corroborating the present study. Another work assessed five commercial formulations of HS, with carbon contents ranging from 240 to 410 g kg^{-1} of dry matter, 4 to 21 g kg^{-1} of N, 0.1 to 77 g kg^{-1} of P, and 92 to 177 g kg^{-1} of K, applied to lettuce and tomato crops in four soil types (Hartz & Bottoms,

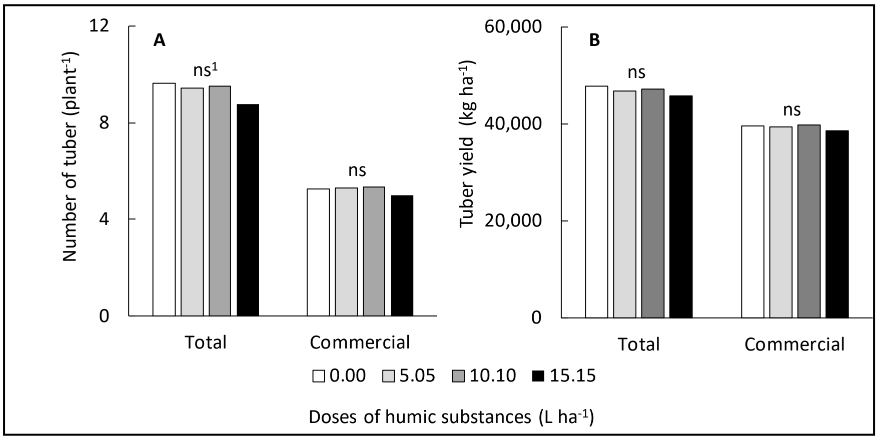

Figure 2. Number (A) and fresh weight (B) of tubers from total and commercial yield of potato plants subjected to doses of humic substances (HS) (L ha^{-1}). Average of two crop seasons and two cultivars. [1]ns: statistical difference not significant ($p>0.05$). Guarapuava, Unicentro, 2015-2016 and 2016-2017.

2010). In this study, no positive effect was observed on lettuce emergence or P uptake. In tomato, no positive effect was found on P uptake, total or commercial yield, initial growth, or concentration of any nutrient (Hartz & Bottoms, 2010). Therefore, the researchers concluded that HS application is inefficient (Hartz & Bottoms, 2010), corroborating the results obtained in the present study.

In research that evaluated the effect of three foliar applications and soil applications of HS at doses much higher (40 and 80 g m^{-2}) than the ones used here, no differences were identified in the number of tubers, total yield, and chemical composition of tubers in plants treated with foliar applications (Suh *et al.*, 2014). However, Suh's group found an increase in the weight of extra-large tubers, which resulted in higher incidence of hollow heart (Suh *et al.*, 2014). Soil applications of HS did not affect tuber number, total yield, and chemical composition of tubers; however, at 80 g m^{-2} (i.e., 800 kg ha^{-1}), the incidence of hollow heart was reduced (Suh *et al.*, 2014). Despite coming from much higher doses than those adopted in the present study, these results corroborate the absence of HS effects on total and commercial yields (Figure 2). Experiments conducted with potatoes under organic farming conditions and HS delivered to seed potato in pre-planting and by foliar applications concluded that the benefits of the biostimulant were limited (Osvalde *et al.*, 2016).

From these results, it is assumed that in certain soil conditions, such as high pH and low OM and clay content, the application of high volumes of HS, especially when the applied products contain nutrients, could positively affect tuber yield in potato plants. This assumption derives from the possibility of nutrient effects and positive interactions of nutrients with HS. The climatic conditions observed in other studies may also have contributed to the contradictory results since the environment was fully adequate to the crop needs in the present study.

The application of up to 15.15 L ha^{-1} of HS, extracted from peat, in furrows of potato cultivated in soils with acid pH and high levels of iron, OM, and clay, promoted an initial detrimental effect on plant growth and did not benefit tuber yield of the potato cultivars Agata and BRS-Camila.

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