Phosphorus supply for corn plants with fertilizer containing humic substances

Carlos Juliano Brant Albuquerque1*, Bruno Teixeira Ribeiro2, César Henrique Souza Zandonadi2, Rafael José de Oliveira3, Tales Graciano Coelho3, Weslei Geraldo Martins4, Matheus Gonçalves Borges4 and Erika Alice Nascimento Resende4

1Minas Gerais State Agricultural Research Corporation, Brazil.
2Federal University of Uberlândia, Institute of Agricultural Science, Minas Gerais, Brazil.
3Heringer Fertilizers, Brazil.
4Minas Gerais State Research Support Foundation, Brazil.

Received 14 August, 2015; Accepted 12 November, 2015

The introduction of new technology components increases the stability of the existing production systems in addition to maximize their efficiency, reducing costs and improving productivity. Thus, the objective was to evaluate the influence of phosphorus fertilization on the agronomic and physiological characteristics of two corn hybrids using mineral fertilizer and humic substance-rich fertilizer the benefits of association of organic compounds (humic substances) mixed to mineral fertilization. The experiment was conducted in a typical Dystrophic Latosol (clay loam Hapludox), in Uberlândia, Minas Gerais State, Brazil, in a randomized block design in a factorial scheme 2 × 7 (2 corn cultivars and 7 fertilizers) with three replications. It was used two cultivars of transgenic corn (HL1315 PRO2 and AG8088 PRO 2). It was tested doses of two fertilizers: (i) formulated organomineral (NPK): 08-28-16; (ii) formulated mineral (NPK): 08-28-16). The doses of each fertilizer were equal to 0 (control), 60, 120 and 180 kg ha⁻¹ of P₂O₅. The characteristics evaluated were bedridden plants, plant height, ear insertion height, grain yield, incidence of rot grain, 100-grain weight, chlorophyll a, chlorophyll b and total chlorophyll. Humic substance-rich fertilizer did not contribute to corn growth. However, corn plants treated with humic substances showed higher chlorophyll concentration in the leaves.

Key words: Biofertilizer, chlorophyll content, organic compounds, fertilizers, Zea mays L.

INTRODUCTION

The corn cultivation has been fairly studied in Brazil, researches involving as the obtainment and the recommendation of cultivars as the cultural management and the effect of the soil and climatic conditions in the expression of the genetic potential of the seed has received attention in the scientific community. In the last years, the culture passes through important technological changes, resulting in significant increases of the productivity and the production. The whole and rational utilization of all the resources available in the rural

*Corresponding author. E-mail: carlosjuliano@epamig.br.

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system (Harger et al., 2007), with the introduction of new technological compounds, increase the stability of the production systems existing, as well as maximize their efficiency, reducing costs and improving the productivity.

The use of chemical fertilizers in the corn cultures fertilization is broadly broadcasted (Farinelli and Lemos, 2010), however, one of the problems faced by the agriculturist is the high cost of the feedstock. Between the feedstock, the phosphorus is one of the most important nutrients because of its scarcity in Brazilian soil; its lack can cause drastic reduction in the cultures productivity. According to Kobiyama et al. (2001), the main causes of this degradation have been the deforestation, the inadequate management of the agriculture, super grazing etc. Associated to the low natural fertility of the Savana ground (Cerrado), in special the deficiency of phosphorus resulted from the low availability and this nutrient high capacity of fixation (Azevedo et al., 2004).

For Perez et al. (2005) the problem solution could be the search for the production with sustainable alternatives like, the organomineral fertilizer utilization taking detritus, from the integrated systems of production, from the nitrogen fixation etc. According to Tiritan et al. (2010) the organomineral fertilization possibilities the reduction of phosphorus dose and/or protect the phosphorus, inhibiting the reactions of the element fixation with the iron's oxide and the aluminum from the ground (Santos et al., 2005).

These improvement happens due to the gradual liberation of nutrients, that reduce process as leaching, fixation and volatilization, although depends essentially of the decomposition rate, controlled by the temperature, humidity, texture and mineralogy of the ground, beyond the chemical composition of the organic material used (Ribeiro et al., 2011). Organic fertilizers complemented with soluble mineral sources, also should be used aiming to improve the physical and biological properties of the ground (Rodrigues and Casali, 1999).

This way, the goal of the work was: Evaluate the influence of phosphorus supply on the agronomic and physiological characteristics for two hybrids of corn using conventional sources and sources containing humic substances in the NPK's formulation; show the benefits of the association of organic compounds (humic substances) to the mineral fertilization.

**MATERIALS AND METHODS**

**Location and description of the experimental area**

The experiment was conducted in a dystrophic Latosol (clay loam Hapludox) under pasture (*Brachiaria decumbens*), located in Uberlândia Minas Gerais State, Brazil, inas, 18°57' S and 48°16' W and 866 m of altitude. The local weather is Aw (Köppen's classification). The period of ten days precipitation in the experiment period is showed in Figure 1. Some attributes of the ground fertility of interest, before the implantation of the
The herbicide Gezaprim® 500 (atrazine), in the dosage of 4 L ha⁻¹ of incorporation with plow in a depth of 20 cm.

The application of limestone was made by haul followed by the 14th December 2013, in the grooves, taking to bases the double of plowed again and furrowed. The sowing was made manually on evaluation of each parcel was considering the two central lines. Four lines of 5 m (spaced in 50 cm) constituted each parcel. For the needed plants for the obtainment of the intended plants density, 60,000 plants ha⁻¹. After the thinning, was realized the coverage developed, was realized the thinning to reach the population of 48,980 plants ha⁻¹.

By the occasion of the experiment installation, the area was plowed again and furrowed. The sowing was made manually on 14 December 2013, in the grooves, taking to bases the double of needed plants for the obtainment of the intended plants density. Four lines of 5 m (spaced in 50 cm) constituted each parcel. For the evaluation of each parcel was considering the two central lines.

After the sowing, when the plants show five leaves completely developed, was realized the thinning to reach the population of 60,000 plants ha⁻¹. After the thinning, was realized the coverage fertilization applying the equivalent to 250 kg ha⁻¹ of urea and 200 kg ha⁻¹ of KCl.

It was used two cultivars of transgenic corn (HL1315 PRO2 e AG8088 PRO 2). The choice of these cultivars was due to its wide recommendation for the cultivation in the region of Triângulo Mineiro, MG.

It was tested doses of two fertilizers: (i) organomineral formulated (NPK): 08-28-16; (ii) mineral formulated (NPK): 08-28-16). The doses of each fertilizer were determined by the supply of 0 (control), 60, 120 and 180 kg ha⁻¹ of P₂O₅. It was used urea and potassium chloride to balanced quantities of nitrogen and potassium between the treatments in function of the highest dose of fertilizer. The experiment was conducted following a design in randomized blocks, in factorial scheme 2x7 (2 cultivars, 7 fertilization) with three repetitions.

For the control of weed plants was used, in the post-emergency, the herbicide Gezaprim® 500 (atrazine), in the dosage of 4 L ha⁻¹ of the commercial product.

**Deployment and conduction of the experiment**

In January of 2013 was used 4 L ha⁻¹ of glyphosate for the pasture desiccation. Then, was applied 6 Kg ha⁻¹ of dolomitic limestone (PRNT of 90%) aiming to elevate the saturation by bases to 80%. The application of limestone was made by haul followed by the incorporation with plow in a deep of 20 cm.

The obtained data were initially subjected to a variance analysis and after to Scott-Knott (1974) test, to 5% of probability and to 2 cm from one of the leaf's border.

### Plants height (cm) and ear's insertion high

The height of the plants was taken from the insertion point of the flag leaf until the ground, measuring in meters, four plants by useful area of the portion, after the physiological maturity of the grain. The insertion height of the obtained ear, measuring in meters the superior era until the ground of four plants by useful area of the parcel, after the physiological maturity of the grain.

**Grain yield (kg ha⁻¹)**

The relative data to the productivity of the grains in the parcel, after the threshing, were corrected by the humidity degree of 13% and turned to t ha⁻¹ using the following expression: \( P_{13\%} = \frac{P}{1 - U} / 0.87 \) where: \( P_{13\%} \): grains productivity (t ha⁻¹) corrected by the pattern humidity degree of 13%; \( P \): productivity of grains without the correction; \( U \): degree of the grains humidity observed in the harvest moment.

**Incidence of sour grains**

The incidence of sour grains was determined according to procedure proposed in the ordinance n° 11, of 12/04/96 (BRASIL, 2009). The method consists in the visual separation and the percentage determination of grains with discoloration symptom in one quarter of a total surface, from one sample of 250 g of grains by parcel. The weight of 100 grains was obtained by the weighing of four samples of 100 grains collected randomly by the grains gathered in the useful area of each parcel and corrected by 13% of humidity.

**Physiological evaluations (chlorophyll content)**

It was accomplished physiological evaluation of the chlorophyll a, chlorophyll b and total chlorophyll content by the way of the chlorophyll meter ClorofiLOG, CFL 1030 model, operated according to the producer specifications (Falker, 2008). The determinations were accomplished in the estates of 6 to 7 and 10 to 11 leaves completely developed and in the silking, using five leaves per parcel. In the vegetative stages, the lecture with chlorophyll meter were accomplished in the 6th and 10th leaves totally expanded, corresponding, respectively, to the estates of six to seven leaves and 10 to 11 expanded leaves. In the silking estate, the lectures were accomplished in the index leave (first bellow the era). The lectures in the chlorophyll meter (two by leaf) were made in points situated in the half to two third of the sampled leaf length, from the base, and to 2 cm from one of the leaf's border.

**Statistical analysis**

The obtained data were initially subjected to a variance analysis and after to Scott-Knott (1974) test, to 5% of probability and
analysis of regression to the doses. The values referring to the percentage of the sour grains, bedridden and broken plants were changed \((\sqrt{x+1})\). All the analysis were accomplished using the statistical program SISVAR® (Ferreira, 2000).

**RESULTS AND DISCUSSION**

In the Table 2 found out the resume of the analysis for the bedridden plants features, height of the plants, height of the ears, grains productivity, sour grains and one hundred grain’s weight. It was not detected significant difference for the percentage of bedridden plants and the percentage of sour grains. However, for the other agronomic features it is possible to visualize significant effect.

The general average to bedridden plants showed values almost nulls demonstrating a genetic resistance from the hybrids to this independent feature of the used fertilization. Related to the sour grains the values are considered normal for the internal market with an average of 3.58% (Table 2). The fungus act in a deleterious way in the grains quality and are acceptable maximum values of 2% the sour grains to the exportation and the 6% to the commercialization in the internal market (Mendes et al., 2012).

Related to the grains' productivity occurred differences \((p<0.01)\) to the cultivars and fertilization effects (Table 2). The grains’ productivity was considered low with average value of 1.55 t ha\(^{-1}\). It is important to highlight that the rainfall precipitation occurred during the conduction of the experiment was 380 mm. This value is considered less than that necessary to obtain the good corn productivity, furthermore, the distribution of rain was not uniform occurring a large period without rain during the summer, between 50 and 70 days after the sowing (Figure 1). The reduction of water level disposable in the ground in the anthesis possibly reduced the fertilization capacity and, consequently, the grains’ production. It is important to highlight that the average temperatures were always over 20°C and under 25°C, values considered great for the plant's good development. Therefore, it is possible to assure that the low precipitation during the conduction of the experiment was the restrictive weather factor to the good development of the culture. The corn culture demand a minimum of 400 to 600 mm of pluvial precipitation to manifest the productivity potential, without the necessity to use the irrigation practice, being that it is consumptive, frequently, oscillates between 4 and 6 mm/day. However, the understood period between the booting/bolting phase and the milky grains, figure as the most sensitive to the hydrous stress, resulting in significant lost and irreversible production (Fancelli and Dourado, 2005).

The hybrid HL 1315 showed higher plants and higher ears (1.36 and 0.71 m) when compared to AG 1080 (1.19 and 0.54 m). Higher productivity of grains were observed for the hybrid HL 1315, in this case 1.74 t ha\(^{-1}\), already the AG 1080 reached 1.33 t ha\(^{-1}\). The height of hybrid grains HL 1315 contributed to higher productivity, cause the dry matter of 100 grains of this genotype (34.26 g) was significantly superior than AG 1080 (29.76 g).

The result of the regression equations for the plants height, ears height and grains productivity in function of the doses of P\(_2\)O\(_5\) originated from the formulated 08-28-16 organomineral and 08-28-16 mineral are showed in Figure 2.

Significant squared relation \((y = y^0 + ax + bx^2)\) for the plants height, ears height and grains’ productivity in function of the P\(_2\)O\(_5\) doses only for the formulated 08-28-16 organomineral and 08-28-16 mineral are showed in Figure 2.

**Table 2.** Variance analysis to bedridden plants (PA), plants’ height (AP), ear’s height (AE), grains’ productivity (PG), percentage of sour grains (GA) and weight of 100 grains (P100) obtained to the two cultivars in function of different fertilization.

<table>
<thead>
<tr>
<th>Variation source</th>
<th>GL</th>
<th>PA (%)</th>
<th>AP (m)</th>
<th>AE (m)</th>
<th>PG (kg ha(^{-1}))</th>
<th>GA (%)</th>
<th>P100 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>2</td>
<td>0.02</td>
<td>0.21</td>
<td>0.10</td>
<td>0.06</td>
<td>0.01</td>
<td>8.66</td>
</tr>
<tr>
<td>Cultivar (C)</td>
<td>1</td>
<td>0.02(^*)</td>
<td>0.31(^*)</td>
<td>0.31(^*)</td>
<td>1.70(^*)</td>
<td>0.06(^*)</td>
<td>224.02(^*)</td>
</tr>
<tr>
<td>Fertilization (A)</td>
<td>6</td>
<td>0.05(^*)</td>
<td>0.28(^*)</td>
<td>0.13(^*)</td>
<td>1.94(^*)</td>
<td>0.26(^*)</td>
<td>8.07(^*)</td>
</tr>
<tr>
<td>C × A</td>
<td>6</td>
<td>0.05(^*)</td>
<td>0.02(^*)</td>
<td>0.02(^*)</td>
<td>0.12(^*)</td>
<td>0.16(^*)</td>
<td>2.07(^*)</td>
</tr>
<tr>
<td>Error</td>
<td>26</td>
<td>0.02</td>
<td>0.04</td>
<td>0.01</td>
<td>0.07</td>
<td>0.11</td>
<td>6.33</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>5.70</td>
<td>14.84</td>
<td>17.76</td>
<td>17.81</td>
<td>15.65</td>
<td>7.87</td>
</tr>
<tr>
<td>General average</td>
<td></td>
<td>0.02</td>
<td>1.27</td>
<td>0.62</td>
<td>1.55</td>
<td>3.58</td>
<td>31.98</td>
</tr>
</tbody>
</table>

\(^*\)Not significant; \(^\ast\) significant at 0.05; \(^**\) significant at 0.01.
Figure 2. Plants height (A), ears height (B) and grains’ productivity (C) in function of the dose of organomineral and mineral fertilizer.

formulated generated a linear relation for the plant’s height, where for each kg ha⁻¹ of P₂O₅, were evidenced increase by 3.20 cm.

The same trends were noticed for the ear’s height and grains’ productivity. In other words, the equations of second grade adjusted better for the fertilization with mineral formulated, while the fertilization with organomineral promoted increasing gains in the ear’s height or in the grains’ productivity (Figure 2A and C).

When these results were analyzed it was possible to deduce a major stress trendy caused by mineral formulated. It is important to highlight that the experiment was conducted in condition of higher hydrous stress, in this way, Taiz and Zeiger (2013) reported that the ground dehydration increase the saline concentration in the ground solution, resulting in a reduction of the hydrous potential and possible osmotic stress as well as ionic specific effects. When connect this statement with the results obtained, we can infer that the cultivars fertilized with organomineral formulated were affected by the osmotic stress.

It is important to highlight that the experiment was conducted under strong hydrous stress. The shortage of water is one of the most common causes of reduction in the sorghum’s plants height due to the shorter cell expansion caused by the hydrous stress (Grima and Krieg, 1992). The cell’s expansion inhibition cause leaf expansion slowness and for consequence smaller area for perspiration, keeping the water supply on the ground for a longer period. This is the first strategy of the plants against the drought (Taiz and Zeiger, 2013). Therefore, the use of organomineral formulated promoted beneficial changes for the plant. In the practice, the two main factors that change the balance of the electric charges on the grounds with variable charges is the liming to increase the pH’s value and the addiction of the organic matter content.

Conus et al. (2009) evaluating the seeds germination and the force of corn seedlings subjected to the saline stress inducing by different salts concluding that the germination was not affected by the saline stress. However, the decrease of the osmotic potential in the solutions of KCl and NaCl, caused decrease in the length of air part and increase in the length of primary root. According to Benites and Mendonça (1998), the addiction of organic fertilizer to the ground cause dispersive or aggregating effect, according to the quantity and quality of the fertilizer.

For the corn, the production potential is early defined, in other words, by the occasion of 4ª leaf emission, could extend until the 6ª leaf, mainly in function of the protandry nature of the mainly genotype used in Brazil. The
attributed stage is denominated floral differentiation, which also coincide with the end of the leaves differentiation stage. Therefore, in this stage will be defined the potential foliar area that the plant should be showed. The same way, the confirmation of the ears' row number (ovary), will occur between the period correspondent to the 7ª and 9ª leaves emission completely expands, due to the transformations occurred in the axillary gem that will give origin to the ear.

In physiological and ecophysiological studies related to the plant's stress, Baker (2008) recommended the use of the chlorophyll meter. So, to a better understand of the agronomical results discussed previously, were evaluated the fluorescence of the chlorophyll to examine the photosynthetic performance and stress in the plant in the different fertilizations.

In Table 3 are featured the resume of the variance analysis for the contents of chlorophyll A, chlorophyll B, total chlorophyll for both cultivars in function of different fertilizations with the plants in the estate of 6ª and 7ª leaves totally expanded. Occurred differences (p<0.01) for the effect of cultivars and fertilization for chlorophyll a and chlorophyll for the total chlorophyll.

When analyze the content of chlorophyll in the estate of 10ª to 11ª leaves totally expanded noticed effect highly significant only for fertilization (Table 4).

When accomplish regression study was noticed significant squared relation to chlorophyll a, chlorophyll b and total chlorophyll using a mineral fertilizer. Nevertheless, for the organomineral fertilizer noticed simple linear relation for all the types of chlorophyll. These results reinforce what was previously discussed in the evaluation of estates 6ª and 7ª leaves totally expanded.

Santana (2012) highlighted that organomineral fertilizers promoted effects in biometric compounds as: length, volume and corn roots area. The effects in the radicular system of organomineral products noticed by these authors, evidenced improvement in the plants rhizosphere.

Moreover, these reports, can explain the better physiological activity of the plants obtained with the use of organomineral formulated in the current work (Figure 4). It is also important to highlight, that the quantity of water available for the culture, is in the dependence of the roots exploratory capacity, storage of the ground's water and the magnitude of the plants radicular system. So, the rational management of the ground and the culture, in case of great importance, for the growth and distribution of the radicular system, favoring the efficient utilization of water and nutrients. The corn should be prepared by eventual restriction of carbohydrates coincident with the phase of silking and grains filling, that could be remedied, in case that the plant feat the possibility of excedent accumulation of photo assimilates in the culm, in the stage understood between the 6ª and 10-12ª leaf.

The content of chlorophyll a, chlorophyll b and total chlorophyll evaluated in the silking were changed in function of the fertilization (Table 5). The cultivars did not affect the content of chlorophyll in the silking, only the fertilization caused significant changes for these features. The graphic representation of the joint regression equation obtained for the two cultivars considering the

<table>
<thead>
<tr>
<th>Variation source</th>
<th>Df</th>
<th>Chlorophyll a</th>
<th>Chlorophyll b</th>
<th>Total chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocks</td>
<td>2</td>
<td>12.52</td>
<td>3.40</td>
<td>28.26</td>
</tr>
<tr>
<td>Cultivar (C)</td>
<td>1</td>
<td>18.14*</td>
<td>6.25&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>45.68&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fertilizations (A)</td>
<td>6</td>
<td>9.38*</td>
<td>3.04&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>22.18&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>C X A</td>
<td>6</td>
<td>1.77&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.77&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>4.21&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Error</td>
<td>26</td>
<td>3.64</td>
<td>1.51</td>
<td>9.11</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>4.60</td>
<td>11.44</td>
<td>5.78</td>
</tr>
<tr>
<td>Geral Average</td>
<td></td>
<td>41.46</td>
<td>10.76</td>
<td>52.22</td>
</tr>
</tbody>
</table>

<sup>Not significant; *significant to 0.05; ** significant to 0.01.</sup>
contents of chlorophyll a, chlorophyll b and total chlorophyll in function of the different fertilization in the silking are featured in Figure 5.

It was noticed linear equation for the contents of chlorophyll a, chlorophyll b and total chlorophyll when used organomineral fertilizer. Nevertheless, for the mineral formulated was noticed linear equation in the contents of chlorophyll b and total chlorophyll, while for chlorophyll a, visualized a squared equation for better presentation of the data. According to Baker (2008), the use of chlorophyll a fluorescence to examine the photosynthetic performance and stress in plants is more important than other chlorophyll. Therefore, independent of the stage of the plants development, the organomineral fertilizer had beneficial effect for the plants' photosynthetic metabolism pointed by the increase of chlorophyll with the fertilization increase.

So, the results obtained in the current work point that the organic matter present in the organomineral formulated was important in the activation of the plants metabolism...
Figure 4. Chlorophyll a (A), chlorophyll b (B), and total chlorophyll (C) in function of the dose of mineral and organomineral fertilizer in the estate of 10ª and 11ª leaves totally expanded.

Table 5. Variance analysis for chlorophyll a, chlorophyll b and total chlorophyll obtained for the two cultivars in function of the different fertilizations in the silking.

<table>
<thead>
<tr>
<th>Variation source</th>
<th>Df</th>
<th>Chlorophyll a</th>
<th>Chlorophyll b</th>
<th>Total chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean square</td>
<td>Mean square</td>
<td>Mean square</td>
</tr>
<tr>
<td>Blocks</td>
<td>2</td>
<td>27.01</td>
<td>3.55</td>
<td>49.66</td>
</tr>
<tr>
<td>Cultivar (C)</td>
<td>1</td>
<td>0.75&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.56&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>4.47&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fertilizations (A)</td>
<td>6</td>
<td>48.53**</td>
<td>15.85**</td>
<td>118.77**</td>
</tr>
<tr>
<td>C X A</td>
<td>6</td>
<td>2.44&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.07&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>6.49&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Error</td>
<td>26</td>
<td>4.65</td>
<td>1.37</td>
<td>10.70</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>5.32</td>
<td>11.51</td>
<td>6.46</td>
</tr>
<tr>
<td>Geral average</td>
<td></td>
<td>40.49</td>
<td>10.19</td>
<td>50.68</td>
</tr>
</tbody>
</table>

<sup>ns</sup>Not significant; <sup>*</sup>significant to 0.05; <sup>**</sup> significant to 0.01.

improving physiological processes highlighted by the linear regression for the chlorophyll content. Furthermore, that application and evaluation follow by many years being fundamental to improve the comprehension of their effects in the improvement of chemicals, physicals and biological features of the ground, beyond the effects in corn's agronomic and physiologic compounds.

Conclusions

Humic substance-rich fertilizer did not contribute to corn
growth. However, corn plants treated with humic substances showed higher chlorophyll concentration in the leaves.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are grateful to FAPEMIG and Heringer Fertilizers.

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