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Effects of the humic acid extracted from vermicompost on the germination and initial growth of *Brachiaria brizantha* cv. MG5

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The biological effects of humic substances on vegetables depend on the source of extraction and the concentration used, on the vegetable species and on the age of the plant. This study aimed to evaluate the effect of different humic acid (HA) doses extracted from vermicompost on the germination and initial growth of *Brachiaria brizantha* cv. MG5. To that end, germination tests were conducted in germination agents, as well as emergence test in greenhouse and seedlings growth test for those agents. For all these tests, five doses of HA [0.0; 1.0; 2.0; 4.0 e 8.0 mM C.L^{-1}] were used, with and without reapplying HA. The following characteristics were analyzed: percentage of germination, percentage of normal seedlings in the first counting of the germination test, percentage of abnormal seedlings, percentage of non-germinated seedlings, percentage of emergence, emergence speed rates, fresh and dry matter of the upper and roots part, length of the upper and roots part and number of lateral roots. Positive effect of HA reapplication in the percentage of abnormal seedlings was observed. With increasing doses of HA, there was a reduction in the percentage of germination and normal seedlings in the first count. The application of HA promoted increase in shoot length and number of lateral root. There was no significant difference in the length of the root. Humus acid affects positively the initial growth of *B. brizantha* cv. MG5, with the best stimulation being observed when the 2.0 mM C.L^{-1} dose was applied.

Key words: Forage, *Urochloa* sp., vigor.

INTRODUCTION

Organic matter for soils, waters and sediments have humic substances as their main component. They influence chemical, physical and biological properties and directly affect the growth and metabolism of plants, especially the root system (Nardi et al., 2002). Vermicomposting is the post-thermophilic biodegradation of organic material through the interaction between earthworms and microorganisms (Edwards et al., 2010). The mature vermicompost is significantly enriched in humic acids, which have a well- acknowledged capability to induce plant development, especially for root systems (Canellas et al., 2010; Muscolo et al., 2013). The most studied physiological effects of the humic substances regard the promotion of root growth (Façanha et al., 2002; Rodda et al., 2006a; Zandonadi et al., 2007). The great majority of the biostimulating effects of humus acid (HA) has been credited to its similar activities to the auxins (Chen and Aviad, 1990; Canellas et al., 2002; Façanha et al., 2002), considering that these effects on the vegetable growth depend on the type of source from where the humic substances are isolated, on the type and age of the plants and on the concentration used for the essays (Kononova, 1982; Santos and Camargo, 1999; Muscolo et al., 2013; Martinez-Balmori et al., 2014).

Brachiaria seeds have difficulty to germinate in the laboratory and in the field, and the main factor contributing to this is the occurrence of natural or innate dormancy, presenting among other factors, heterogeneity of maturation (Lago and Martins, 1998). In forage species, the formation of a dense stand at sowing is fundamental to the pasture productivity (Sulc, 1998). For this to happen, there needs to be effected quickly and evenly seedling emergence, allowing the formation of a closed canopy early in the growing season, suppressing weeds and maximized light interception. A suggested hypothesis is that the positive biological effects of HS in the plant can be due to an auxin activity (Nardi et al., 2002; Piccolo et al., 1992). To this effect, auxin promotes acid growth of plants in which the cell elongation and the consequent increase in length of the plants is a function of cell turgor. This increased growth promoted by humic acid can be of great importance in the early growth of brachiaria seedlings, promoting rapid establishment of forage resulting in higher quality pastures. Given in the above, testing the hypothesis that humic acids affect the growth of *Brachiaria brizantha* cv. MG5 plants, related to the used dose and to the reapplication of the solution, we aimed to evaluate the effect of different doses of humus acid extracted from vermicompost on the germination and initial growth of *B. brizantha* cv. MG5.

MATERIALS AND METHODS

Two experiments were separately performed in germination chamber Biochemical Oxygen Demand (BOD) type and in a greenhouse to evaluate the effect of HA on the initial growth of branquiaria seedlings. All tests were performed with commercial seeds of *B. brizantha* cv. MG5 ventilated with a DeLeo blower, with a 4.8 cm of aperture aiming to remove impurities and empty seeds, obtaining the pure seed fraction to conduct the tests. The humus acid used was extracted from a vermicompost obtained through the personal collection existent in the Laboratory of Environmental Microbiology and Biotechnology of the University of Vila Velha, Brazil. A vermicompost was obtained from mixture of plant residues from *Panicum maximun* Jacq., and cattle manure 5:1 (v/v). The organic residues were mixed and earthworms were added at a ratio of 5 kg earthworms (*Eisenia foetida*) per m^3 of organic residue. A bed of worms and organic residues was first prepared in a container and additional layers of organic residues were periodically placed over the pile as a function of temperature until the pile reached 50 cm. At the end of the transformation process (three months after addition of the last organic residues), worms were removed into a pile of fresh organic residue (plant + cattle manure) placed in a corner of the container. The organic matter composition of the resulting vermicompost was: pH 7.8, 46.5 g kg^{-1} total organic carbon and 17.3 g kg^{-1} HA carbon. HA were isolated from vermicompost and purified as reported elsewhere (Canellas et al., 2002). The HA were suspended in distilled water and titrated to pH 7.0 by automatic titrator (VIT 909 Videotitrator, Copenhagen) with a 0.1 KOH solution under N_2. The resulting potassium-humates were then passed through a 0.45 μm Millipore filter and freeze-dried (Canellas et al., 2010). For the usage in the experiments, lyophilized HA was solubilized with a 0.1 mM NaOH solution and it was diluted using a 2 mM $CaCl_2$ solution. The pH solution was set to be between 5.8 and 6.0 and so the dilution of the stock solution with 2 mM $CaCl_2$ solution was proceeded to obtain doses of 0.0, 1.0, 2.0, 4.0 and 8.0 mM C.L^{-1} of HA based on Canellas et al. (2010), which were used in all experiments.

On the first experiment, tests of germination in BOD and emergence tests in greenhouse were conducted. The HA doses were directly applied on the substrate, with and without reapplication of HA. In the treatment with reapplication of HA, the substrate was dampened again on the seventh and fourteenth days with HA, and on the other days it was dampened with water for another time when it was necessary. Regarding the treatment without a reapplication of HA, the substrate was dampened again with water only during all the test conduction.

Germination test

This test was conducted according to the rules for the analysis of seeds (Brasil, 2009), following a totally randomized outlining with four repetitions of 50 seeds each, by treatment. The seeds were uniformly distributed in gerbox for germination; measuring 11 × 11 × 3.5 cm, over two germitest sheets of paper dampened with 5 ml of AH solution. The gerbox containing the seeds were taken to BOD-type germination agents with a photoperiod of 16/8 h (E/L) and using an alternate temperature of 20 and 35°C, considering 16 h for the lowest temperature and 8 h for the highest one. The treatment with reapplication of HA was dampened once again with 1.0 ml of HA solution in the seventh and fourteenth days, using the same concentrations applied initially. The counting was carried out in the seventh and twenty-first day after the beginning of the test to evaluate normal, abnormal and non-germinated seedlings.

Emergence test in greenhouse

The emergence test of seedlings in greenhouse followed the outlining in randomized blocks, using four blocks, and it was established in plastic trays perforated at the bottom, using as a

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Abbreviation: HA, Humic acid.

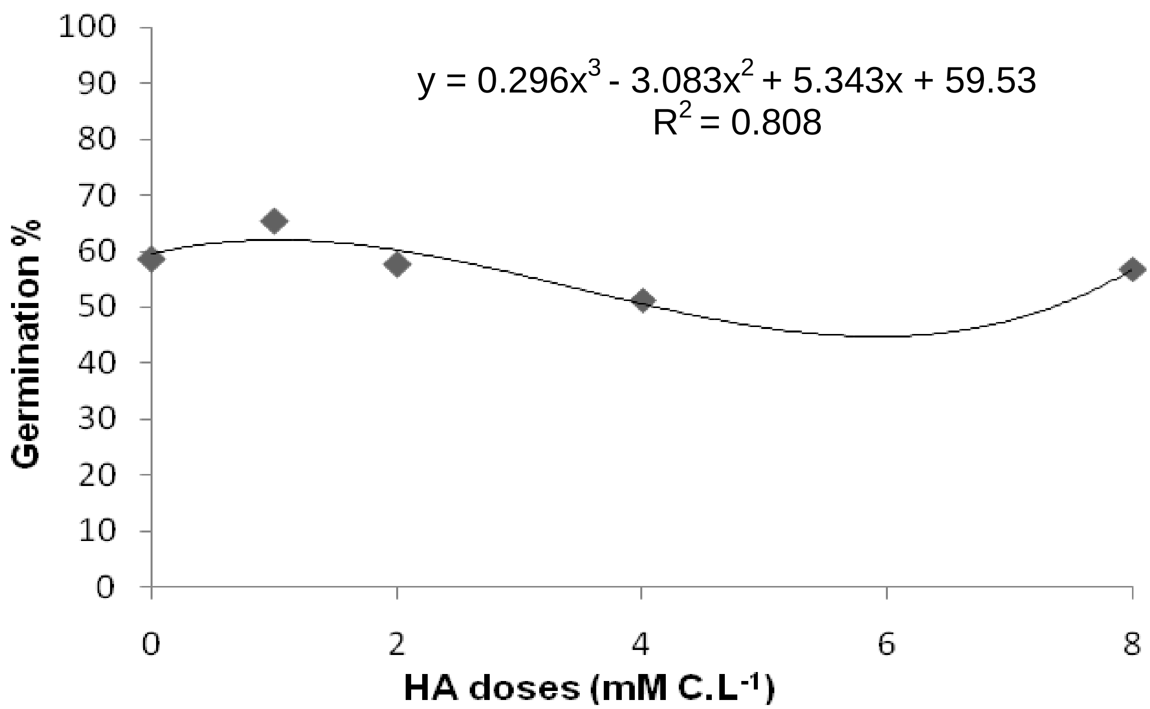

Figure 1. Germination percentage of braquiaria seedlings treated with different HA doses.

substrate a soil classified as yellow dystrophic Latosol, mixed with sand in the proportion of 2 x 1 (2 parts of soil for 1 part of sand). The substrate was dampened with 500 ml of HA solution, a volume which is enough to reach the field capacity. Therefore, 50 seeds were seeded by tray with a depth of 0.5 cm. The treatment with reapplied HA was dampened again with 100 ml of HA solution in the seventh day, using the same concentrations applied initially. Daily countings were performed regarding the number of plants emerged to calculate the emergence speed rate. The evaluation of the total of seedlings emerged was performed in the 14th day after the sowing, when the plants were carefully taken from the trays and cut in a way that the upper part of the roots could be separated from the rest. They both were packed in paper bags and weighted in a precision balance to determinate the fresh matter, followed by the determination of the dry matter according to the methodology described by Silva and Queiroz (2006).

The second experiment conducted in a germination agent aimed to evaluate the length of the upper part and the root, as well as the emission of secondary roots of braquiaria seedlings treated with different HA doses directly applied in the substrate in a single time before the sowing, with a solution volume equivalent to three times the weight of the paper being used. The substrate used was a paper roll with three germitest paper sheets, where 20 seeds of *B. brizantha* cv. MG5 were planted with four repetitions by treatment. The paper rolls were packed in plastic bags, which were arranged in plastic trays. They were taken to BOD, where they were put in a way that the inclination was set to 45° to guarantee the action of gravitropism in the growth of the roots. The test was conducted with a photoperiod of 16/8 h (E/L) and with an alternate temperature of 20 and 35°C, considering 16 h to the lowest temperature and 8 h to the highest one. After the germination, only 10 seedlings by repetition were maintained. The evaluations were performed in the 14th day after the sowing. Measures of the upper part and of the roots of all plants were taken. Then, with the help of a loupe, the number of secondary roots by plant was counted. The results obtained were submitted to the analysis of variance (ANOVA) and to regression analysis with the ASSISTAT software.

RESULTS AND DISCUSSION

No effect of the reapplication of HA to the variable percentage of germination was verified, where it was observed as a reduction in the germination percentage with the increase in the doses, with the greatest reduction happening when a 4.0 mM C.L^{-1} dose was applied (Figure 1). However, in the highest dose, there was a positive stimulus of HA again in the germination percentage. This behavior may have happened in function of the bioactivity of the HA in brachiaria seeds having been affected by the degree of seeds ripeness, since they have an uneven ripeness inside the panicle (Martins and Silva, 2001). They have, a same lot, seeds with different degrees of maturity, which influences the permeability of the tegument and possibly the sensibility of these seeds to distinct HA doses. The results of this study confirm those by Ayuso et al. (1996), that also observed a reduction in the germination percentage in dampened paper substrate with an HA solution, considering that this effect was mainly observed in seeds with a greater sensibility to negative external factors.

According to Azam and Malik (1983), the best effects of the dampened materials in the germination of seeds happen when they are immersed in the solution before the germination itself and when they are germinated in

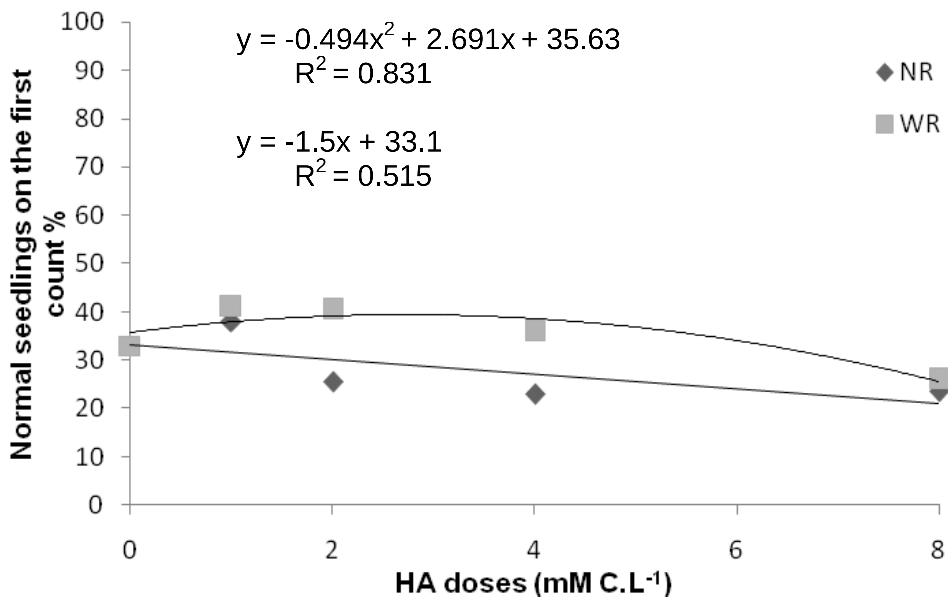

Figure 2. Percentage of normal seedlings on the first counting of the test on germination of braquiaria seedlings treated with different HA doses with no reapplication (NR) and with reapplication (WR) of HA.

water. It is better than when they are germinated in HA solutions. It probably happens because the humic substances penetrate the seeds tissues during the immersion (Ayuso et al., 1996). To the normal seedling on the 1st counting, a negative effect was observed when a higher level of HA doses was used, which suggests that there is a decrease on the vigor of the seeds when submitted to higher HA doses (Figure 2). This decrease of vigor indicates a reduction on the germination speed, which may have happened because of the increase on the time of soaking caused by a difference in the osmotic potential in the HA solution. Another factor that may have influenced the response to the HA doses would be the sensibility to external conditions presented by this species of seed, since there are other species with a greater sensibility to negative external factors, such as, the ones suggested by Zucconi et al. (1985) for seeds of watercress and by Ayuso et al. (1996) for seeds of tobacco and watercress. A significant effect on the reapplication of HA in the percentage of normal seedlings was verified (Figure 3). When the seeds were not submitted to reapplication of HA, a linear increase of the percentage of abnormal seedlings was observed with the increase of doses too. However, when HA was reapplied, a contrary effect was demonstrated, because there was a decrease on the percentage of abnormal seedlings increasing the HA doses. The reduction in the percentage of abnormal seedlings in function of the increase of the doses when HA was reapplied may be explained by the fact that the HA solution retards the hydration of the tissue and the gas exchanges, allowing a longer time to repair or reorganize the plasma membranes, also allowing the tissues to be formed in a more organized form, reducing the risks of damage to the embryonic axis (McDonald, 2000; Windauer et al., 2007). This way, the chances of generating abnormal seedlings also decrease. There was no significant difference to the following characteristics: percentage of non-germinated seeds, percentage of emergence and emergence speed rate, in which no effects of none of the doses tested in none of the treatments were observed.

In Table 1, the data of fresh and dry matter in the upper part and in the root, as well as the perceptual variation in relation to the control regarding the function of HA doses and the reapplication or non-reapplication of HA solution are presented. The greatest increments on the fresh matter, as in the upper part (19.48%) as in the root (25.58%), were observed when a 4.0 mM C.L^{-1} was applied with no reapplication of HA. In the treatment with the reapplication of HA, a positive effect of HA was verified, being the 8.0 mM C.L^{-1} the one that presented the best response, increasing the fresh matter of the upper part (5.54%) and of the root (16.56%), and dry part of the upper part (6.77%) and of the root (12.5%) when compared to the control. In general, a greater increment in the fresh matter of the upper and root parts was observed in relation to the dry matter of the upper and root parts, indicating that the stimulus stated in the growth of the plants could be hypothetically attributed to the action of humic acids on the cell elongation by vacuum turgidity (Rayle and Cleland, 1992). Any significant effect of the doses for the root length was identified. In Figure 4, the data of the length of the shoot are presented. Although, regarding the growth of the

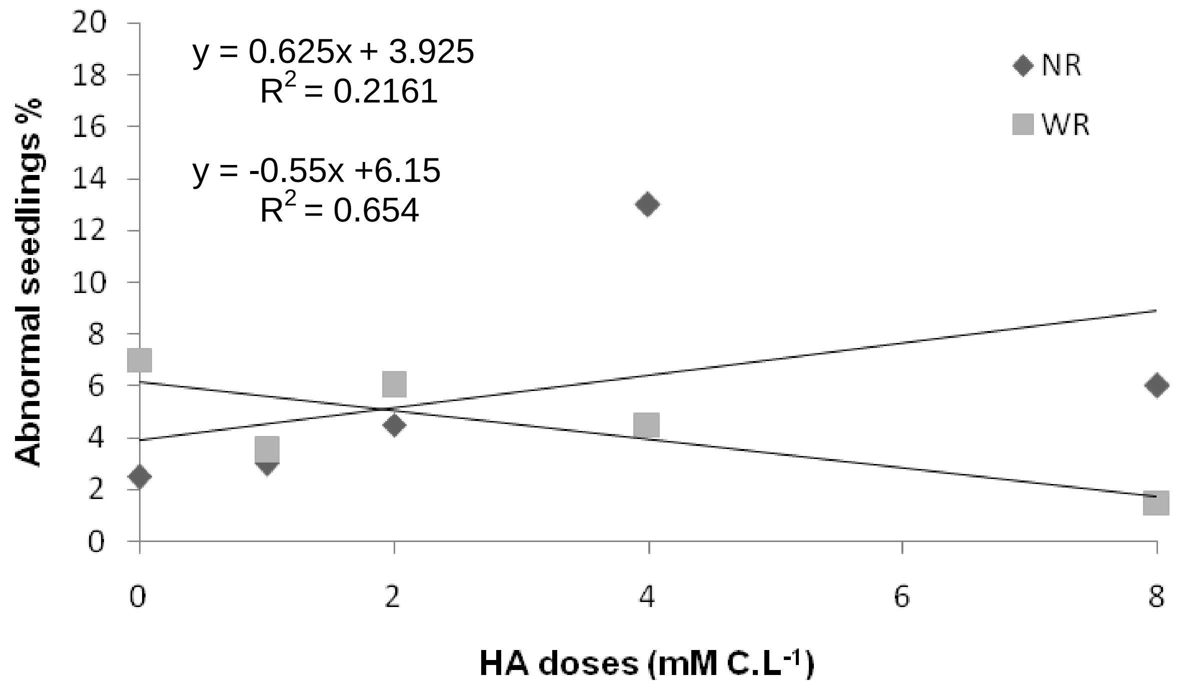

Figure 3. Percentage of abnormal seedlings on germination test of braquiaria seeds treated with different HA doses with no reapplication (NR) and with reapplication (WR) of HA.

Table 1. Fresh and dry matter in the upper and root part of braquiaria plants in response to different HA doses with or without reapplication of HA.

HA (mM C.L-1)	Dry matter of shoot (mg/pl)	Fresh matter of the root (mg/pl)	Dry matter of shoot (mg/pl)	Fresh matter of the root (mg/pl)
No reapplication of HA				
0.0	99.33	64.37	8.06	5.56
1.0	104.46 (5.16)	63.67 (-1.09)	8.54 (5.95)	5.48 (-1.44)
2.0	102.29 (2.98)	52.79 (-17.99)	8.30 (2.98)	3.56 (-35.97)
4.0	118.68 (19.48)	80.19 (24.58)	7.86 (-2.48)	4.97 (-9.17)
8.0	92.52 (-6.85)	66.07 (2.64)	7.41 (-8.06)	4.50 (-19.06)
With reapplication of HA				
0.0	104.51	62.36	8.72	4.56
1.0	99.51 (-4.78)	71.94 (15.36)	8.56 (-1.83)	5.00 (9.65)
2.0	99.86 (-4.45)	60.88 (2.37)	8.23 (-5.62)	3.90 (-14.47)
4.0	90.91 (-13.01)	53.37 (-14.42)	8.04 (-7.80)	3.19 (-30.04)
8.0	110.30 (5.54)	72.69 (16.56)	9.316.77)	5.13 (12.5)

*The values between brackets represent the percentage variation in relation to the control.

upper part, two peaks of HA bioactivity in the seedlings growth could be observed, considering that this happened when 2.0 mM C.L^{-1} and 8.0 mM C.L^{-1} doses were applied.

A dose effect on the number of lateral roots was observed, in which there was an increase in the number of lateral roots with the increase of the doses, reaching a maximum and decreasing again when the highest dose was applied (Figures 5 and 6). The dampened organic matter extracted from the worm composting presents a confirmed hormonal activity (Muscolo et al., 1999; Canellas et al., 2002). The possible presence of growth inducing substances of roots of the auxins type in the worm composting humate promotes the development of

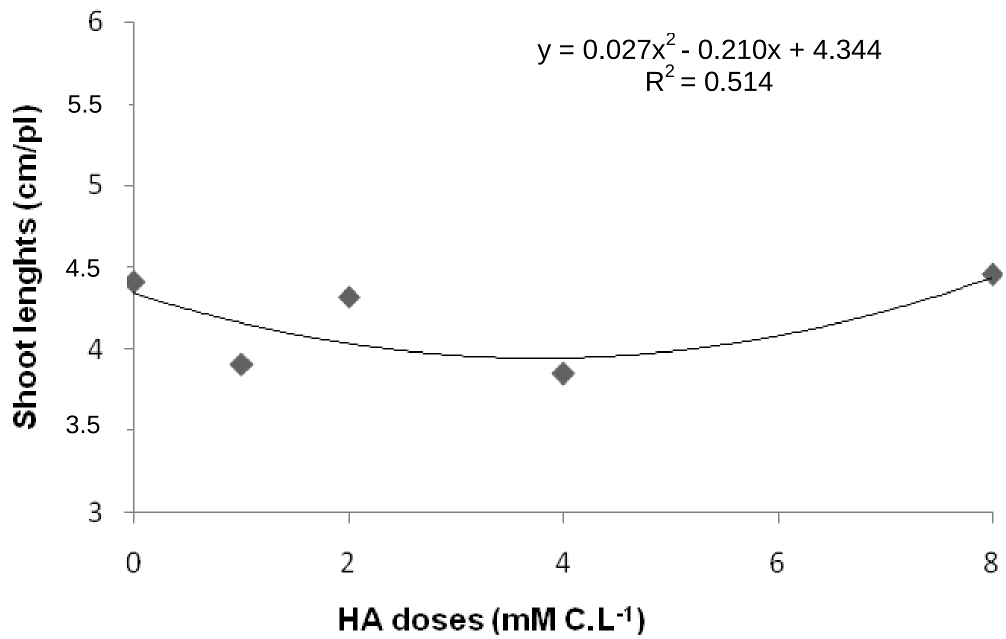

Figure 4. Shoot lengths of braquiaria seedlings in response to different HA doses.

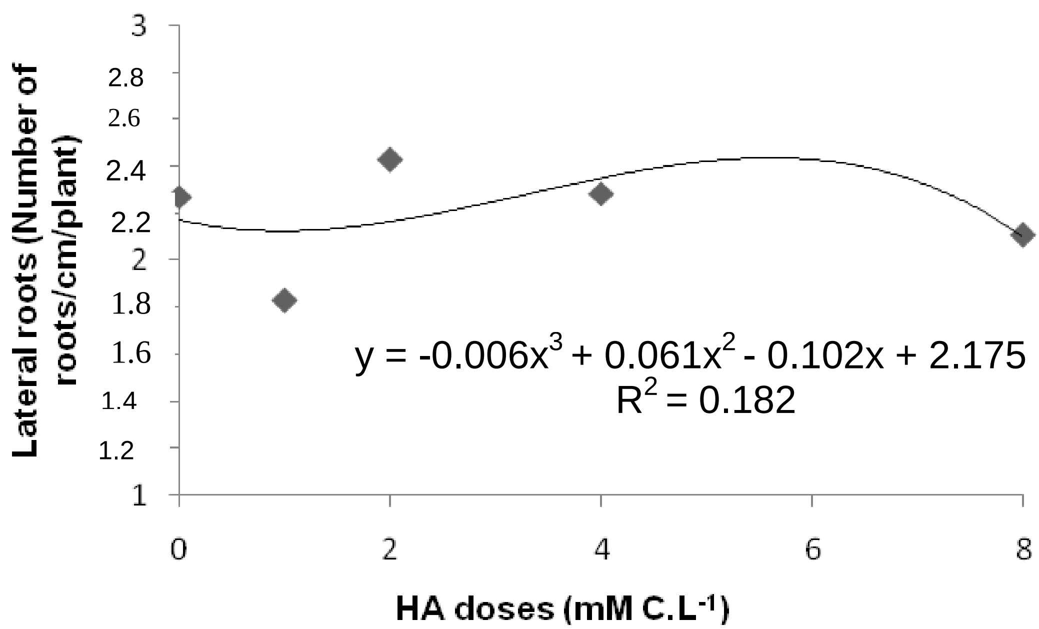

Figure 5. Number of lateral roots of braquiaria seedlings in response to different HA doses.

Lateral roots and meristematic regions (Rodda et al., 2006b).

Conclusion

Humic acids affect positively the initial growth of *B. brizantha* cv. MG5, with the best stimulus being observed when a 2.0 mM $C.L^{-1}$ dose was applied.

Conflict of interests

The authors did not declare any conflict of interest.

Figure 6. Seedling growth at 0.0 (A), 1.0 (B), 2.0 (C), 4.0 (D) and 8.0 (E) mM C.L^{-1} of HA.

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