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Effect of humic acids extracted from vermicompost on the germination of brachiaria seeds: doses and methods of application

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ABSTRACT

The aim of this study was to determine the best dose and method of application of the humic acid extracted from vermicompost for the velocity and percentage of germination of Brachiaria brizantha cv. MG5 seeds. This study conducted three different methods of application for different doses of humic acid for seed treatment: vacuum (V), immersion (I), and direct application on substrate (S), with one humic-acid re-application (CR) or with no re-application (SR), and different HA doses: [0.0; 0.5; 1.0; 2.0; 4.0 and 8.0 mM C.L-1]. The application of the 1.0 mM C.L-1 dose in vacuum, without HA re-application, led to the best results regarding the percentage of germination and the non-germinated seeds. As for the velocity of germination (first count) and average time of germination, the best results were observed with the application of 0.5 mM C.L-1 with the immersion method and without HA re-applications.

KEYWORDS forage, humic substances, vigor.

Introduction

The growing use of forage grass has stimulated the development of the seed industry in Brazil, making it the biggest producer, consumer and exporter of forage grass seeds in the world (Silva Filho, 2009). Amongst the used species, *Brachiaria brizantha* cv. MG-5 has been receiving increasing attention worldwide. In forage species, the formation of a thick stand is of crucial relevance to the productivity of the pasture (Sulc, 1998). In order for it to happen, it is necessary to have quality seeds, with a fast and uniform emergence of seedlings.

According to Vaughan and Malcom (1985), the humic fractions influence biochemical and physiological processes during germination. In general, these processes include the reduction of the time before germination, a greater percentage of germination, and a greater length during the first stages of plant growth (Petrovic *et al.*, 1982; Vaughan & Malcom, 1985; Dell'Amico *et al.*, 1994).

The presence of humic substances is important in every stage during the development of the plant, but it is of particular relevance in the first stages. It is for that reason that the pre-planting treatment of the seeds is of great important. Even before the germination starts, the vital forces are awakened, and the immune system is stimulated (Levinsky, 2013). Studies on the effects of humic acids in corn have shown that it accelerates the development cycles, *i.e.*, the germination, the flowering, and the beginning of fructification (Eyheraguibel, 2008).

In order to test the hypothesis that the humic acids affect the germination of seeds according to its dose and method of application, the aim of this study was to determine the best dose and method of application of humic acids for the velocity and percentage of germination of *Brachiaria brizantha* cv. MG5 seeds.

Material & Methods

This study was conducted as part of the research developed in the Seed Technology Sector of the Plant Science Laboratory at State University of North Fluminense Darcy Ribeiro (UENF).

Materials

Commercial *Brachiaria brizantha* cv. MG5 seeds were winnowed with a De Leo blower, with an opening of 4.8 cm, to remove impurities and empty seeds, obtaining only a pure seed fraction for the conduction of tests.

The humic acid extracted from vermicompost was obtained from a private collection of the Microbiology and Environmental Biotechnology Laboratory 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 (3 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 treatment of the seeds, the lyophilized humic acid was solubilized in a 0.1 mM NaOH solution and diluted in 2.0 mM $CaCl_2$ to obtain the following doses: 0.0; 0.5; 1.0; 2.0; 4.0 and 8.0 mM $C.L^{-1}$, according to the percentage of carbon in their elemental characterization.

Methods of Application

This study conducted three different methods of humic acid application for seed treatment: vacuum (V), immersion (I), and direct application on substrate (S). Every method was submitted to one humic-acid re-application (CR) or with no re-application (SR).

For the vacuum application, the seeds were placed in beakers containing 5 ml HA solutions in different concentrations. The beakers were taken to the desiccator attached to a vacuum pump; the vacuum was applied intermittently as follows: 5 minutes in the vacuum + 5 minutes without vacuum + 5 minutes in the vacuum, adapted from Vieira and Barros (1994). The immersion treatment consisted on the immersion of seeds in humic acid in the different concentrations for 24 hours. In the treatment with direct application on the substrate, 5.0 ml of humic solutions were utilized to humidify the substrate for germination.

For the treatments with the reapplications, the substrate was humidified daily with 1 ml of the different solutions. The treatments without re-application were humidified with the same amount of distilled water.

Germination Test

The test was conducted according to the Rules for Seed Analysis (Brasil, 2009), with four repetitions of fifty seeds each for each treatment. The seeds were uniformly distributed in 11 cm x 11 cm x 3.5 cm germination boxes (gerboxes) on two sheets of germitest paper humidified with 5 ml humic acid. The gerboxes containing the seeds were then taken to the germinator with a photoperiod of 16/8 h (D/L) and altering temperature between 20°C and 35°C (16 h for 20 °C and 8 h for 35°C, under white light). The counts were conducted on the seventh and on the twenty-first days after the beginning of the test to evaluate normal seedlings, abnormal seedlings, and non-germinated seeds. Throughout the germination tests, daily counts were conducted to calculate the average germination time by using the formula proposed by Edmond and Drapala (1958).

Statistical Analysis

All the data were submitted to the analysis of variance (ANOVA), and the significant averages were compared by using Duncan's Multi-Directional Reach Test with the Assistat software.

Results & Discussion

The results regarding the effect of the HA doses and the methods of application on the percentage of germination are in Table 1. The method of application affected the percentage of germination only with the 1.0 mM $C.L^{-1}$ HA dose, when the vacuum utilization without HA re-application as a method of application led to the greatest percentage of germination (85%), a value also observed when the 4.0 mM $C.L^{-1}$ dose was applied directly to the substrate, with the re-application of HA. However, the increase in relation to the control group was higher in the 1.0 mM $C.L^{-1}$ dose, with vacuum and without HA re-application (8,28%) when compared to 4.0 mM $C.L^{-1}$ dose applied directly on the substrate, with HA re-application (1.19%). These results are probably due to the fact that the application of vacuum forces the seeds to absorb the solution, allowing for the smaller HA doses to the effective.

Table 1 – Effect of humic acids on the percentage of germination.

Germination (%)												
Doses of HA (mM $C.L^{-1}$)	Vacuum				Immersion				Substrate			
	SR		CR		SR		CR		SR		CR	
	Averages	PV	Averages	PV	Averages	PV	Averages	PV	Averages	PV	Averages	PV
0.0	78.5 abAB	0	79.3 abAB	0	72.5 abB	0	64.5 bC	0	78.5 aAB	0	84 aA	0
0.5	76.5 bA	-2.55	74.5 abA	-6.05	77.5 abA	6.9	75.5 abA	17.05	80.5 aA	2.55	76.5 aA	-8.93
1.0	85 aA	8.28	71 bB	-10.47	69.5 bB	-4.14	73 abB	13.18	74.5 aB	-5.09	75 aB	-10.71
2.0	76.4 bA	-2.7	79 abA	-0.38	74.5 abA	2.76	73.5 abA	13.95	74 aA	-5.73	78 aA	-7.14
4.0	81 abA	3.18	80.5 aA	1.51	80 aA	10.34	74.5 abA	15.5	78.5 aA	0	85 aA	1.19
8.0	80 abA	1.91	78 abA	1.64	77.3 abA	6.6	79.5 aA	23.25	71.5 aA	-8.92	82.5 aA	-1.78

Averages followed by the same lower case letter in the columns and capital letter in the rows have no difference amongst them, according to Duncan's test ($p<0.05$).

SR = without HA re-application; CR = with HA re-application; PV = percentage of variation when compared to the control.

When compared to the effect of the doses on the increase of the percentage of germination in relation to the control group, the 8.0 mM $C.L^{-1}$ dose using the immersion method for 24 hours, with HA re-application, promoted the significant increase of 23.25% in relation to the control group. These results agree with those found by Matysiak *et al.* (2011), who observed an increase in the percentage of germination of corn seeds immersed for 24 hours in HA solution when compared to the control group. These findings support Azam and Malik (1983), who suggest that the best stimulatory effect of the humic acid is obtained through the immersion of the seeds in humic acid for a determined time, with the germination in distilled water occurring afterwards.

The HA bioactivity in brachiaria seeds may have been affected by the level of seed maturation, since they have an uneven maturation within the panicle (Martins & Silva, 2001). Thus, within the same seed lot, there are seeds with different levels of maturity, which will influence the permeability of the skin and possibly the sensitivity of the seeds the different HA doses.

According to the data presented in Table 2, referring to the effect of the HA doses and to the methods of application on the percentage of normal seedlings on the first count of the germination test, the method of application presented a significant difference with the application of the 0.5 mM $C.L^{-1}$, considering the immersive method without the re-application of HA, which led to a greater percentage of normal seedlings on the first count of the germination test (63%) and also presented a greater increase when compared to the control group (50%).

Table 2 – Effect of humic acids on the the first count of the germination test.

Normal seedlings on the first count (%)												
Doses ofHA (mM $C.L^{-1}$)	Vacuum				Immersion				Substrate			
	SR		CR		SR		CR		SR		CR	
	Averages	PV	Averages	PV	Averages	PV	Averages	PV	Averages	PV	Averages	PV
0.0	40 aA	0	36.5 aA	0	42 aA	0	43.5 aA	0	52 aA	0	50 aA	0
0.5	35 aB	-12.5	33.5 aB	-8.22	63 aA	50	41 aB	-5.75	45 aB	-13.46	33.5 bB	-33
1.0	42 aA	5	35 aA	-4.11	44.5 aA	5.95	52.5 aA	20.69	39 aA	-25	37 abA	-26
2.0	48.5 aA	21.25	45.5 aA	24.66	55.5 aA	32.14	51 aA	17.24	43.5 aA	-16.35	45 abA	-10
4.0	49 aA	22.5	40 aA	9.59	51.5 aA	22.62	40.5 aA	-6.89	47.5 aA	-8.65	40.5 abA	-19.5
8.0	47.5 aA	18.75	40 aA	9.59	46 aA	9.52	55.5 aA	27.59	45.5 aA	-12.5	34 bA	-32

Averages followed by the same lower case letter in the columns and capital letter in the rows have no difference amongst them, according to Duncan's test ($p<0.05$).

SR = without HA re-application; CR = with HA re-application; PV = percentage of variation when compared to the control.

In the treatments with the direct application on the substrate, with or without HA re-application, a reduction on the percentage of normal seedlings can be observed in every dose of the test (Table 2). This indicates that the HA solution, when applied to the substrate, reduces the velocity of germination of the seeds of the studied species, possibly due to a difference in the osmotic potential, which would hinder imbibition and increase the necessary time for the seeds to go from Stage 1 to Stage 2 in the germination process (Carvalho & Nakagawa, 2012).

For the percentage of non-germinated seeds, there was no significant effect from the method of HA application (Table 3). The smaller percentage of non-germinated seeds (12%) was observed when the 1.0 mM $C.L^{-1}$ dose was applied in vacuum with no HA re-application. Nevertheless, the smaller reduction in the percentage of non-germinated seeds when compared to the control (-47.14%) occurred with the application of 8.0 mM $C.L^{-1}$, with a 24 hour immersion and a HA re-application.

Table 3 – Effect of humic acids on the percentage of non-germinated seeds.

Non-germinated seeds (%)												
Doses of AH (mM $C.L^{-1}$)	Vacuum				Immersion				Substrate			
	SR		CR		SR		CR		SR		CR	
	Averages	PV	Averages	PV	Averages	PV	Averages	PV	Averages	PV	Averages	PV
0.0	16 abBC	0	14.5 aC	0	23.5 aB	0	35 aA	0	17.5 aBC	0	16.5aC	0
0.5	16.5 abA	3.12	19.5 aA	34.48	19.5 aA	-17.02	20.5 bA	-41.42	15 aA	-14.28	18.5aA	12.12
1.0	12 bA	-25	24 aA	65.51	22.5 aA	-4.25	24.5 abA	-30	19.5 aA	11.43	20.5aA	24.24
2.0	21.5 aA	34.37	17.5 aA	20.69	25 aA	6.38	23 bA	-34.28	21 aA	20	19.5aA	18.18
4.0	16 abA	0	17 aA	17.24	18 aA	-23.4	21 bA	-40	19.5 aA	11.43	13.5aA	-30.3
8.0	18.5 abA	15.62	16.5 aA	13.8	19.5 aA	-17.02	18.5 bA	-47.14	23 aA	31.43	15aA	-3.03

Averages followed by the same lower case letter in the columns and capital letter in the rows have no difference amongst them, according to Duncan's test ($p<0.05$).

SR = without HA re-application; CR = with HA re-application; PV = percentage of variation when compared to the control.

Brachiaria brizantha cv. MG5 may cause dormancy, which, for this species, is associated with physical causes, probably related to the restriction of the exchange of gases enforced by the seed covering, including lemma, palea, pericarp and integumentary coverings (Cardoso *et al.*, 2014). This may be the cause to the results regarding the percentage of non-germinated seeds, since the treatments that benefit imbibition were the ones with the higher reductions in the percentage of non-germinated seeds.

According to the data in Table 4, the average germination time was influenced by the method of application only with the application of the 0.5 mM $C.L^{-1}$ dose, with the immersive treatment without HA re-application, which led to the smaller average time of germination (4.18 days). However, the greatest significant reduction (-6.6%) when compared to the control group was seen with the dose of 4.0 mM $C.L^{-1}$ in vacuum and without HA re-application. The increase in the velocity of germination that does not affect the percentage of germination observed in the vacuum treatment is probable due to the fact that, when seeds undertake this type of treatment, the imbibition process occurs in a reduced period of time, reducing the first stage of germination (Carvalho & Nakagawa, 2012).

'Table 4 about here'

In the treatments with direct application on the substrate, there was an increase in the average time of germination for every dose, with the greatest significant increase (16.7%) when compared to the control group occurring with the 8.0 mM $C.L^{-1}$ dose directly applied on the substrate, with no HA re-application.

Conclusions

Humic acids positively affect the germination of *Brachiaria brizantha* cv. MG5 seeds. The best results for the percentage of seed germination and for non-germinated seeds were observed after the application of the 1.0 mM $C.L^{-1}$ dose in vacuum, without HA re-application. The best results for the velocity of germination (first count) and for the average time of germination were seen after the application of the 0.5 mM $C.L^{-1}$ with the immersive method and without the re-application of HA.

Table 4 – Effect of humic acids on the average time of germination.

Average time of germination (days)												
Doses of AH (mM $C.L^{-1}$)	Vacuum				Immersion				Substrate			
	SR		CR		SR		CR		SR		CR	
	Averages	PV	Averages	PV	Averages	PV	Averages	PV	Averages	PV	Averages	PV
0.0	4.85 aA	0	4.81 aA	0	4.54 aA	0	4.30 aA	0	4.61 bA	0	4.73 aA	0
0.5	5.09 aA	4.95	4.66 aAB	-3.12	4.18 aB	-7.93	4.58 aAB	6.51	4.86 abA	5.42	4.85 aA	2.54
1.0	4.62 abA	-4.75	5.05 aA	5	4.76 aA	4.86	4.37 aA	1.63	5.00 abA	8.46	5.02 aA	6.13
2.0	4.68 abA	-3.5	4.74 aA	-1.45	4.35 aA	-4.18	4.46 aA	3.72	5.02 abA	8.89	4.84 aA	2.32
4.0	4.53 bA	-6.6	4.66 aA	-3.12	4.60 aA	1.32	4.65 aA	8.14	5.16 abA	11.93	5.53 aA	16.91
8.0	4.67 abA	-3.71	4.70 aA	-2.29	4.89 aA	7.71	4.72 aA	9.77	5.38 aA	16.7	5.61 aA	18.6

Averages followed by the same lower case letter in the columns and capital letter in the rows have no difference amongst them, according to Duncan's test ($p<0.05$).

SR = without HA re-application; CR = with HA re-application; PV = percentage of variation when compared to the control.

REFERENCES

Azam, F., & Malik, K. (1983). Effect of humic acid soaking seeds on seedling grown of wheat (Triticum aestivum L.) under different conditions. Pakistan Journal of Botany, 15, 31-38. || Brasil (2009). Ministério da Agricultura. Regras para análises de sementes. Brasília, DF: SNAD/DNDV/CLAV. || Canellas, L.P., Piccolo, A., Dobbss, L.B., Spaccini, R., Olivares, F.L., Zandonadi, D.B., Façanha, A.R. (2010). Chemical composition and bioactivity properties of size-fractions separated from a vermicompost humic acid. Chemosphere. 78, 457–466. || Cardoso, E.D., Sá, M.E., Haga, K.I., Binotti, F.F.S., Nogueira, D.C., Valério Filho, W.V. (2014). Desempenho fisiológico e superação de dormência em sementes de Brachiaria brizantha submetidas a tratamento químico e envelhecimento artificial. Semina: Ciências Agrárias, 35, 21-38. || Carvalho, N.M., & Nakagawa, J. 2012. Sementes: ciência, tecnologia e produção. 5.pub. Jaboticabal: FUNEP. || Dell'Amico, C., Masciandaro, G., Ganni, A., Ceccanti, B., Garcia, C., Hernandez, M.T., Costa, F. (1994). Effects of specific humic fractions on plant growth. In:Senesi, N., Miano, T. M. (eds.) Humic Substances in the Global Environment and Implications on Human Health, New York: Elsevier, pp. 563-566. || Edmond, J.B., & Drapala, W.J. (1958). The effects of temperature, sand and soil, and acetone on germination of okra seeds, Proceedings of American Society of Horticultural Science, 71, 428-434. || Eyheraguibel, B., Silvestre, J., & Morard P. (2008). Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize. Bioresource Technology, 99, 4206-4212. || Levinsky, B. (2013). All about humates. Focus on form. Disponível em: http://www.teravita.com/Humates/HumateIntro.htm. Accessed jan. 2014. || Martins, L., & Silva, W.R. da. (2001). Comportamento da dormência em sementes de braquiária submetidas a tratamentos térmicos e químicos. Pesquisa Agropecuária Brasileira, 36, 997-1003. || Matysiak, K., Kaczmarek, S., & Krawczyk, R. (2011). Influence of seaweed extracts and mixture of humic and fulvic acids on germination and growth of Zea mays L. Acta Scientiarum Polonorum,Agricultura, 10, 33-45. || Petrovic, P., Vitorovic, D., & Jablanovic, M. (1982). Investigations of biological effects of humic acids. Acta Biol Med Exp, 7, 21-25. || Vaughan D., & Malcolm, R.E. (1985). Influence of humic substances on growth and physiological process. In: Vaughan, D., Malcolm, R.E. (eds.), Soil Organic Matter and Biological Activity. The Netherlands: Kluwer Academic Publishers, pp. 37–75. || Vieira, H.D., & Barros, R.S. (1994). Responses of seed of Stylosanthes humilis to germination regulators. Physiologia Plantarum, 92, 17-20. || Silva Filho, J.P. (2009). Qualidade de sementes de forrageiras. Informativo ABRATES, 19, 81-82. || Sulc, R.M. (1998). Factors affecting forage stand establishment. Scientia Agricola, 55 (esp.), 110-115. |