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Humic acid and growth promoting microorganisms in the germination and initial development of white sesame plants

Ácido humico e microorganismos promotores de crescimento na germinação e no desenvolvimento inicial das plantas de gergelim branco

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Abstract
The objective of this work was to verify the effect of humic acid application in association with the growth-promoting bacteria *Azospirilum brasilense* and *Bacillus subtilis* on seed germination and on the initial development of white sesame seedlings. The research was carried out in the Laboratory of Seed Technology and Matology (LaSeM), at the University of the State of Mato Grosso – Campus de Alta Floresta. The treatments were composed by the combination of doses of 0, 100, 200, 300, 400 and 500 mL 100 kg^{-1} of commercial product containing humic acid, composed of 18% of humic acid, in addition to treatment with the bacteria *Azospirillum brasilense* and *Bacillus subtilis*, in the same doses as humic acid. The variables analyzed were germination percentage, aerial and root length, fresh and dry mass of the roots. Humic acid showed promise in the cultivation of white sesame.
Keywords: *Sesamum indicum*; Diazotrophic bacteria; Growth promoters.

Resumo

O objetivo deste trabalho foi verificar o efeito da aplicação de ácido húmico em associação com as bactérias promotoras de crescimento *Azospirilum brasilense* e *Bacillus subtilis* na germinação das sementes e no desenvolvimento inicial de plântulas de gergelim branco. A pesquisa foi realizada Laboratório de Tecnologia de Sementes e Matologia (LaSeM), na Universidade do Estado de Mato Grosso – Campus de Alta Floresta. Os tratamentos foram compostos pela combinação das doses de 0, 100, 200, 300, 400 e 500 mL 100 kg^{-1} de produto comercial contendo ácido húmico, composto de 18% de ácido húmico, além de tratamento com as bactérias *Azospirillum brasilense* e *Bacillus subtilis*, nas mesmas doses do ácido húmico. As variáveis analisadas foram porcentagem de germinação, comprimento de parte aérea e de raiz, massa fresca e seca das raízes. Ácido húmico se mostrou promissor ao cultivo do gergelim branco.

Palavras-chave: *Sesamum indicum*; Bactérias diazotróficas; Promotores de crescimento.

Resumen

El objetivo de este trabajo fue verificar el efecto de la aplicación de ácido húmico en asociación con las bacterias promotoras del crecimiento *Azospirilum brasilense* y *Bacillus subtilis* sobre la germinación de semillas y sobre el desarrollo inicial de plántulas de sésamo blanco. La investigación fue realizada en el Laboratorio de Tecnología y Matología de Semillas (LaSeM), de la Universidad del Estado de Mato Grosso – Campus de Alta Floresta. Los tratamientos estuvieron compuestos por la combinación de dosis de 0, 100, 200, 300, 400 y 500 mL 100 kg^{-1} de producto comercial a base de ácido húmico, compuesto por 18% de ácido húmico, además del tratamiento con la bacteria *Azospirillum brasilense* y *Bacillus subtilis*, en las mismas dosis que el ácido húmico. Las variables analizadas fueron porcentaje de germinación, longitud de brotes y raíces, masa fresca y seca de las raíces. El ácido húmico se mostró prometedor en el cultivo de sésamo blanco.

Palabras clave: *Sesamum indicum*; Bacterias diazotróficas; Promotores de crecimiento.

1. Introduction

Sesame (*Sesamum indicum*) is an oilseed crop belonging to the Pedaliacea family. Since the early 1990s, sesame cultivation has gained economic importance due to new methods of using grains and their derivatives (Muller et al., 2016).

The cultivation of sesame in Brazil is concentrated in the states of Mato Grosso, Goiás, São Paulo, Northeast region in Brazil and Triângulo Mineiro region. However, the country is known as a small sesame producer as it produces an average of 15,000 tons on 24,000 hectares of land (Barros et al., 2011). Despite this, given the increase in consumption of grains of this species and the management characteristics, there is a great possibility of expanding the cultivation of this oilseed by family farming. Techniques that will increase the productive capacity, viable for small producers and with respect to the environment, are important to leverage the activity, encouraging its cultivation (Avila & Graterol, 2005).

The cultivation of sesame is a reality in several states of Brazil and has growth potential in family farming, given its characteristics, such as ease of cultivation in small areas, ease of cultural treatment, productive potential, in addition to allowing its use in rotation and intercropping of cultures. In addition, it is a rustic species, undemanding in soil fertility and water and its grains can be stored without much difficulty for long periods, for later commercialization.

The use of soil biological resources, such as humic substances and growth-promoting microorganisms, should be considered as low-cost alternatives for improving plant development, in addition to their environmental bias (Rampim et al., 2020).

Among these biological resources, there are bacteria that promote plant growth, such as *Azospirilum brasiliense*, which produces growth hormones (auxins and gibberellins) and fixes nitrogen for plants, stimulating their growth and acting as a biological control agent. of pathogens (Correa et al., 2008; Hungria, 2011; Lima et al., 2021). Also, *Bacillus subtilis* facilitates phosphorus solubilization and the synthesis of siderophores, in addition to protecting plants from attack by nematodes and various fungi (Paz et al., 2012; Alves et al., 2018).

These characteristics allow the producer versatility in cultivation and post-harvest, which justifies the involvement of research in practices that allow the addition of knowledge and techniques, especially those that do not harm the environment, aiming at encouraging cultivation by small producers.

Therefore, the use of growth-promoting bacteria and humic acid can contribute to the better development of the species, promoting gains in productivity and without harming the environment. Thus, the objective of this research was to verify the effect of humic acid application and growth-promoting bacteria on seed germination and initial development of young white sesame plants.

2. Methodology

The experiment was conducted at the Laboratory of Seed Technology and Matology (LaSeM), at the University of the State of Mato Grosso – Campus de Alta Floresta. The treatments were composed by the combination of doses of 0, 100, 200, 300, 400 and 500 mL of commercial product containing humic acid, composed of 18% of humic acid for each 100 kg of seeds; and the bacteria *Azospirillum brasilense* and *Bacillus subtilis*, at the same concentrations of humic acid, since both microorganisms are also available in the form of commercial products (NitroGeo® and Panta®, respectively). The experimental design used was a completely randomized design (DIC) with 4 replications in a 3 x 6 factorial scheme, with three growth promoters (humic acid, *Azospirillum brasilense* and *Bacillus subtilis*), in six doses (0, 100, 200, 300, 400 and 500 mL of commercial product for every 100 kg of seeds) (Cândido et al., 2022).

The product containing humic acid and bacteria were applied directly to white sesame seeds, inside plastic bags, using a volumetric pipette. These were shaken for complete homogenization to cover the biostimulants in the seeds.

These were submitted to a germination test, with 25 seeds being uniformly distributed per experimental unit (transparent gerbox box), on two sheets of germitest paper moistened with distilled water at a proportion of 2.5 times their dry weight, in their respective treatments, with four repetitions of each treatment (BRASIL, 2009). These boxes were kept inside a germination chamber type B.O.D, at 25 °C of constant temperature, with 12h of light. The treatments were evaluated daily, collecting information to determine the variables.

The variables analyzed were: average percentage (%) of germination, fresh and dry mass (mg) of seedlings, stem diameter (cm), length of roots and aerial part (cm) of seedlings. Data were statistically processed using analysis of variance and means comparison test (Tukey) (Ferreira, 2014).

3. Results and Discussion

It was verified that there was significance for the variables fresh mass (MF), aerial part length (CA) and root length (CR) for the interaction between promoters and doses (Table 1).

However, for germination (%F) and seedling dry mass (DM) the significance was limited to growth promoters. And for the variable stem diameter (DC), there was no difference between treatments (isolated or with interaction).

When observing the germination percentage of white sesame seeds treated with growth promoters, it is possible to verify that humic acid was superior to the other microorganisms, promoting average germination 5.3 and 7.6% higher than *B. subtilis* and *A. brasiliense*, respectively. However, this difference is within acceptable values for germination of seeds with high vigor, demonstrating that the material used, from seed producers in the region of Canarana-MT, has high physiological quality.

After germination, the initial development of white sesame seedlings was monitored to verify the ability of the promoters to differentiate their growth. Thus, the fresh mass of the seedlings, after three days of the implantation of the experiment, was measured, and the data are shown in Figure 2.

There was fluctuation in the results obtained after the statistical analysis of the data, seeking to compare the averages collected in this period, with interaction between promoters and doses. When analyzing the promoters, it was found that both

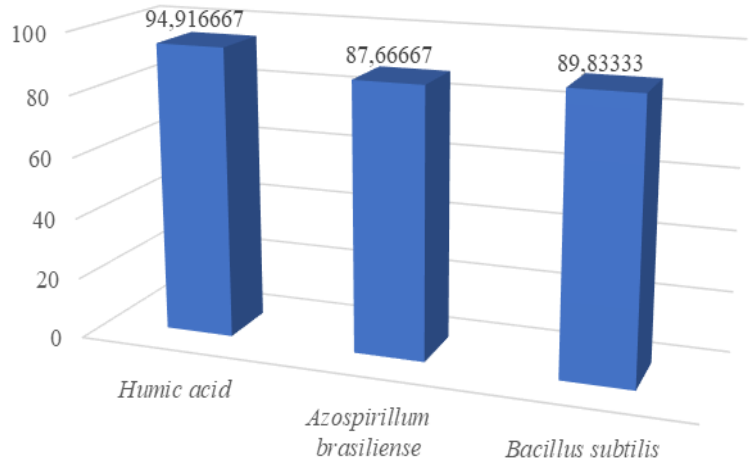
humic acid and *B.* subtilis did not influence the fresh mass of seedlings, within the doses. The treatment of seeds with *A. brasiliense*, promoted higher averages for the following doses: 0, 100, 300 and 500 mL/100 kg of seeds. Although not very consistent, it was found that, at least for this variable, this microorganism negatively affected the seedlings.

Table 1. Analysis of variance table with mean square values for seed germination percentage (%G), aerial part length (CPA), root length (CR), aerial part fresh mass (MSPA), fresh mass of root (MFR), aerial part dry mass (MSPA) and root dry mass (MSR) of white sesame seedlings whose seeds were treated with increasing doses of humic acid, *Azospirillum brasiliense* and *Bacillus subtilis*.

FV	%G	MF	MS	CA	CR	DC
P	332,3888*	0,0117*	0,0239*	0,0722*	3,9017*	161,2338ns
D	37,1222ns	0,0066*	0,0010ns	0,0758*	3,9165*	170,2230ns
P * D	21,5888ns	0,0032*	0,0014ns	0,0539*	1,7372*	171,8288ns
CV	5,91	17,86	8,55	14,09	16,55	49,24

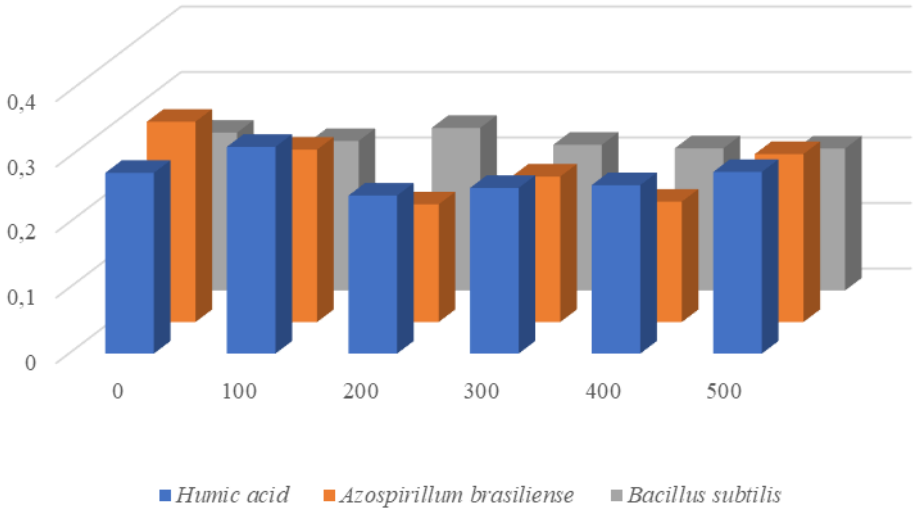
P = growth promoter; D = doses; %G = germination percentage; MF = fresh mass; MS = dry mass; CA = aerial part length; CR = root length; DA = stem diameter. *Significant at 5% probability by F test. ns not significant. Source: Authors.

Figure 1. Germination of white sesame seedlings submitted to seed treatment with increasing doses of humic acid, *Azospirillum brasiliense* and *Bacillus subtilis*.



Source: Authors.

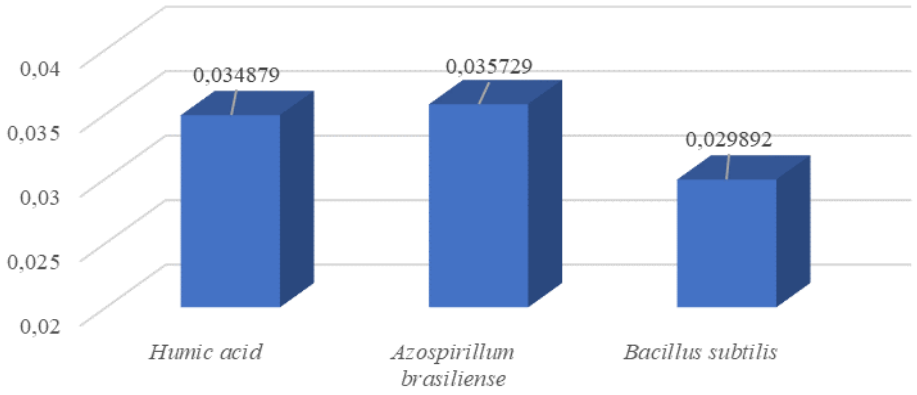
Figure 2. Fresh mass of green mung bean seedlings subjected to seed treatment with increasing doses of humic acid, *Azospirillum brasiliense* and *Bacillus subtilis*.



Source: Authors.

For seedling dry mass, higher averages were found for humic acid and *A. brasiliense*, indicating that these promoters were able to promote, regardless of the dose used for seed treatment, formation and accumulation of dry plant mass in young sesame plants (Figure 3). This result may indicate a favorable element for its use in the treatment of seeds, since one of the advantages of the promoters is the reduced value for the acquisition of the products, with little impact on the cost of final production, in situations of large-scale production.

Figure 3. Dry mass of white sesame seedlings submitted to seed treatment with increasing doses of humic acid, *Azospirillum brasiliense* and *Bacillus subtilis*.



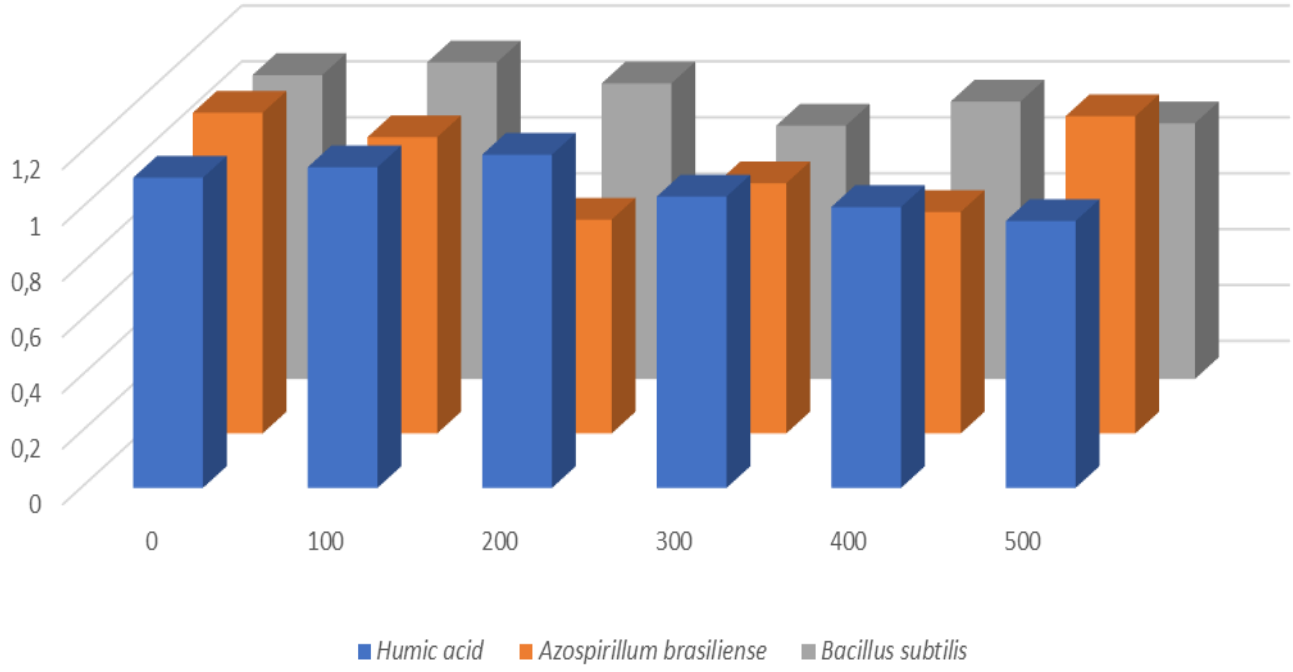
Source: Authors.

The effect of *A. brasilense* inoculation was also reported by Cândido et al (2020), Gonçalves et al. (2020) and Ribeiro

et al (2020) in forage, soybean and corn crops. Studies carried out by Sabundjian (2016) demonstrate that the effects provided by *A. brasilense* are derived from morphological and physiological changes in the roots of inoculated plants, resulting in an increase in the absorption of water and nutrients.

When the aerial part length of white sesame seedlings was determined, results were similar to those reported for fresh mass, since, in the interaction of factors, both humic acid and *B. subtilis* did not cause any difference within the doses. Only with the treatment at doses of *A. brasiliense*, the averages were higher for 0, 100, 300 and 500 mL per 100 kg of seeds (Figure 4). And, within the doses, only with the treatment of 200 mL per 100 kg of seeds, there was a difference between the promoters, and higher averages for this variable were verified for *B. subtilis* and humic acid.

Figure 4. Length of aerial part of white sesame seedlings submitted to seed treatment with increasing doses of humic acid, *Azospirillum brasiliense* and *Bacillus subtilis*.



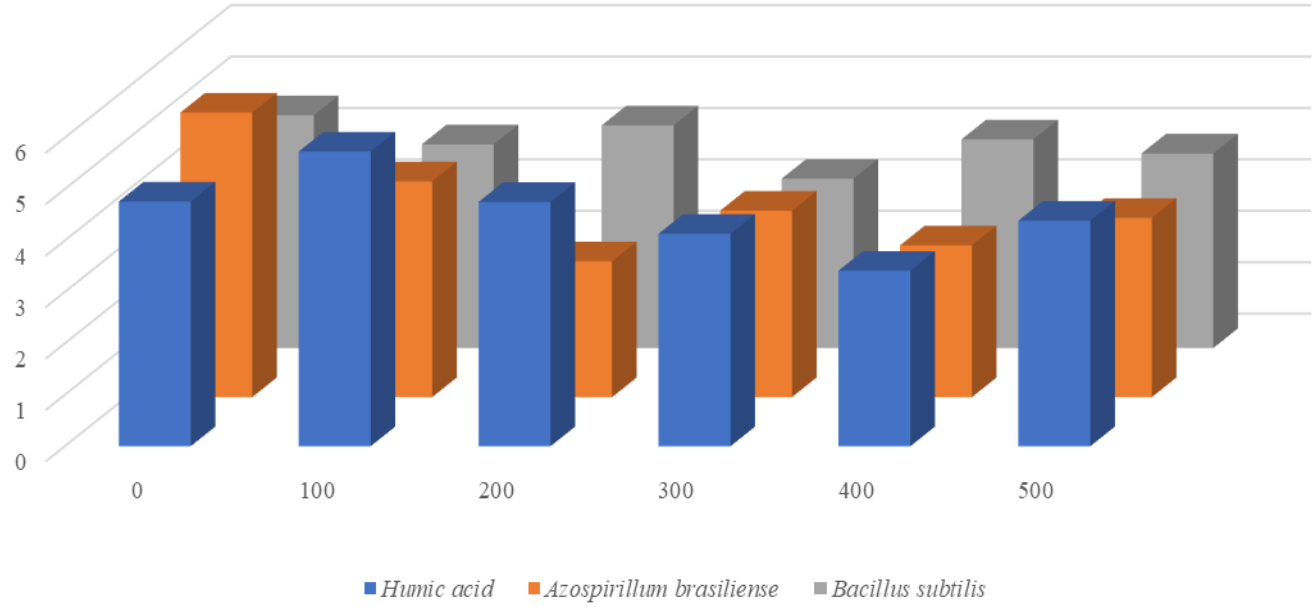
Source: Authors.

On the other side, when evaluating the length of the root part of white sesame seedlings submitted to seed treatment, it was found that there was an interaction between the factors, with a difference only in the increasing doses of humic acid and *A. brasiliense*. As for *B. subtilis*, there was no statistical variation in the means obtained (Figure 5).

Beneficial results from the application of *B. subtilis* in seed treatment or applied to the soil during sowing have been reported in other species, positively influencing seed germinability, nutrient solubilization, plant development and productivity, in addition to causing antagonism to pathogens (Miranda et al., 2020). The growth promotion mechanisms by the action of bacteria such as *Bacillus* are based on the production of phytohormones that help balance and allow plants to develop intensely (Araújo et al., 1999; Araújo, 2008; Araújo et al., 2010; Lima, 2010).

For this same variable, when analyzed, within the doses, the means were significantly different only when treated with 100 and 200 mL per 100 kg of seeds, and in both, the humic acid was higher.

Figure 5. Length of root part of white sesame seedlings submitted to seed treatment with increasing doses of humic acid, *Azospirillum brasiliense* and *Bacillus subtilis*.



Source: Authors.

4. Conclusion

Treatment with growth promoters is an important practice that can promote gains for the producer, with reduced environmental contamination.

Under the conditions of the experiment, despite some inconsistency in the results, humic acid at a dose close to 100 mL per 100 kg of seeds is the promoter that best responds to the formation of white sesame seedlings.

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