

Performance of soybean seeds associated with a biostimulant based on the seaweed *Lithothamnium* sp.

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ABSTRACT. Soybeans are one of the world's main crops, and Brazil leads the production of this oilseed, which has diverse uses. Evidence shows that seed treatment with nutrients and/or association with beneficial microorganisms can aid in germination processes and confer positive aspects to seed vigor. Products like the extract of the algae *Lithothamnium* sp., known for their richness in elements such as Ca and Mg, have been used to enhance the performance of economically important crops. In this context, the objective was to evaluate the performance of soybean seeds subjected to different methods of association with *Lithothamnium* sp. extract. The experiment had four treatments: seeds without association with any biologically active ingredient (control); seeds associated with *Lithothamnium* sp. through the physiological conditioning process; seeds associated with *Lithothamnium* sp. through the coating process; and seeds associated with *Lithothamnium* sp. through the physiological conditioning process followed by the coating process. Physiological conditioning was done with moistened paper and incubation at 25° C for 20 hours. In the coating, 1 g of biostimulant was used for 1000 seeds. Germination, seedling emergence, accelerated aging, and emergence speed index tests were performed. The experimental design adopted was completely randomized. The data obtained were subjected to tests of homogeneity of variances and residual normality. Subsequently, analysis of variance was performed, and the means were compared using the student-Newman-Keuls test at a 5% significance level. Statistical analyses were performed using R software. For all items evaluated in soybean seeds, there was an effect of the seed association treatments with the *Lithothamnium* sp.-based product. Coating soybean seeds with *Lithothamnium* sp. benefited final seedling emergence, emergence speed, and germination under stress conditions. Physiological conditioning followed by coating soybean seeds with *Lithothamnium* sp. impaired germination and seedling emergence.

Key words: physiological conditioning; *Glycine max* L.; physiological quality; beneficial microorganisms; coating.

DOI: <http://doi.org/10.33837/msj.v9i1.1757>

Received: October 30th, 2025

Accepted: April 22nd, 2026

Published online: April 29th, 2026.

Associate editor: Flávio Gonçalves de Jesus

INTRODUCTION

Soybeans are one of the world's most important agricultural products, used for various purposes, from human and animal feed to biofuel production. Brazil is the world's largest producer of this oilseed, with a record harvest of 169 million tons in the 2024/2025 cycle. This volume represents about 40% of global production, consolidating the country's leadership in the sector (USDA, 2025).

The increase in the production of this species in Brazil is the result of several factors involved in agricultural cultivation. In this scenario, seeds, which

represent the beginning of the harvest, bring to the field

The full genetic potential of a material, occupying a prominent position. To further enhance performance, products are added to the seeds. According to Brzezinski et al. (2015), the use of products associated with seeds, such as fungicides and insecticides, can ensure the initial establishment of the plant population. According to Leão-Araújo and Araújo (2024), the use of biological products in seeds, as control agents or even to benefit plant development, has increased significantly in recent years.

The association of seeds with products called biostimulants aims to enhance seedling vigor,

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promoting rapid initial development and strengthening the root system (Melo et al., 2021). Biostimulants are substances or organisms that stimulate plant growth, and may include enzymes, proteins, hormones, amino acids, bacteria, fungi, other organisms, micronutrients, and inorganic compounds (Du Jardin, 2015, Chiaiese et al., 2018, Shahrajabian et al., 2021, Wadas & Dziugiel, 2020). The use of these products has been gaining prominence in agriculture, reflected in increased commercialization, especially due to the positive effects on plant development (Joshi et al., 2020, Santoyo et al., 2021).

There are reports of the use of biostimulants in all stages of agricultural production, including seed treatment, foliar sprays during plant growth, and post-harvest applications. These inputs act in the regulation and modulation of physiological processes in plants, and can promote growth, reduce the negative effects caused by biotic and abiotic stresses, and contribute to increased crop yields (Galindo et al., 2020, Moradtalab et al., 2019).

Examples of products of this nature are calcareous algae (*Maerl* and *Lithothamnium*), which are basically composed of calcium carbonate and magnesium carbonate and more than 20 trace elements, present in varying amounts, mainly Fe, Mn, B, Ni, Cu, Zn, Mo, Se and Sr (Dias, 2000). According to Cressard (1974), the use of these algae in agriculture is very old, with records dating back to the 17th century. The genus *Lithothamnium* comprises more than 70 species and precise taxonomic identification remains complex and insufficiently explored (Sissini et al., 2022).

The potential of *Lithothamnium* sp. for agricultural use has been demonstrated in the production of seedlings and in the cultivation of horticultural, fruit, oilseed, grain and forage species (Ramos et al., 2023). Some studies reveal a positive effect of *Lithothamnium* sp. in the initial seedling development of species such as *Jatropha curcas* L. (Evangelista et al., 2016), *Passiflora alata* C. (Souza et al., 2007), *Carica papaya* L. (Teixeira et al., 2009, Hafle et al., 2009) and *Coffea arabica* L. (Rodriguez et al. 2017). Other seaweed-based products from other species such as *Codium decorticatum* have been reported as biostimulants to enhance germination percentage and chlorophyll content (Vijayakumar et al., 2019).

The effects of using *Lithothamnium* sp. in agricultural crops depend, among other factors, on the dose used for each species or even genotype (Kapoor et al., 2021, Ramos et al., 2023), which suggests that the forms of association of the product in the seeds may also reveal varied behaviors.

Physiological seed conditioning is a tool used to initiate the seed hydration process and the initial phases of the germination process (Waqas et al. 2019). This activates respiration, sugar digestion, and protein

synthesis (Corbineau et al. 2023). This technique is also called priming and has been used to enhance the physiological quality of seeds (Khalaki et al. 2020). This technique can be performed with water or agents that include salts, antioxidants and bioactive molecules, beneficial microorganisms, phytohormones (hormopriming), and nanoparticles (nanopriming) (Pagano et al. 2023).

Seed coating is another way of supplying plant growth-promoting substances to seeds, which can enhance seed quality. The process consists of covering seeds with small amounts of exogenous materials, such as various species of plant growth-promoting bacteria, rhizobia, and *Trichoderma*.

MATERIALS AND METHODS

The experiment was conducted in the Seed Laboratory of the Federal Institute of Goiás - Urutaí Campus. The soybean seeds used were from a batch of the Brasmax Extrema IPRO cultivar, relative maturity group 8.1, indeterminate growth habit, and cycle ranging from 108 to 128 days.

The seed batch was separated into 4 subsamples, each subsample comprising the seeds from the four treatments described below. Treatment 1: seeds without association with any biologically active ingredient (control); Treatment 2: seeds associated with *Lithothamnium* sp. through the physiological conditioning process; Treatment 3: seeds associated with *Lithothamnium* sp. through the coating process; and Treatment 4: seeds associated with *Lithothamnium* sp. through the physiological conditioning process followed by the coating process.

The product used was LTSupra, from the company Supramar. The physical nature of the product is powder, registered with MAPA (Brazil - Ministry of Agriculture, Livestock and Supply) under number ES 0016172-1 as a mixed mineral fertilizer. In the record, the composition is 97% *Lithothamnium* seaweed and 3% calcium and magnesium silicate. The minimum guarantees are total calcium (Ca) = 29%; total magnesium (Mg) = 1.8% and total silicon (Si) = 3%.

In treatment 1, the seeds were subjected to physiological quality tests without any prior procedure. For treatment 2, the product containing *Lithothamnium* sp. in solution was used in the proportion of 30 g for every 4 liters of water, applied directly to the seeds during the conditioning process. For the conditioning process, the soybean seeds were arranged in a single layer on germination paper previously moistened with a biostimulant solution based on *Lithothamnium* sp., in the proportion of 2.5 times the dry weight of the paper. Rolls were made and kept in a Mangelsdorf-type germinator at a

constant temperature of 25 ± 0.5 °C in a dark environment. The conditioning period was 20 hours, and then the seeds were dried in an oven with air circulation and renewal at 30 °C.

For the seed coating treatment, identified as treatment 3, 1 g of the product was used for every 1,000 seeds in a Gerbox-type acrylic box. The seeds were previously and superficially moistened with distilled water using a sprayer. After superficial moistening, the seeds were placed in the acrylic box with the powdered product. The seeds were moved until the product adhered completely to the surface of the seeds, in a homogeneous manner.

The last treatment consisted of a combination of the two methodologies mentioned above; the seeds initially underwent the physiological conditioning process and then the coating process.

After the treatments were completed, the seeds were subjected to the following tests:

Germination test (G): 200 seeds were used, divided into four replicates of 50 seeds. The seeds were placed between sheets of germination paper. The papers were moistened with water in a volume corresponding to 2.5 times the weight of the dry paper. Rolls were made and kept in a Mangelsdorf-type germinator at 25 ± 2 °C. Evaluations took place on the fifth day, when the first count (PC) data were obtained, and the eighth day after sowing, when the germination data (G) were obtained (Brazil, 2009). The results were expressed as the average percentage of normal seedlings.

Seedling emergence in the field (E): carried out in seedbeds with four replicates of 50 seeds. Irrigation was carried out regularly, and seedling counts were performed eight days after sowing, considering only those with at least 2 cm of shoot (Nakagawa et al., 2018). Data was obtained 15 days after sowing. Results were expressed as an average percentage.

Emergence speed index (ESI): was calculated by summing the number of seeds counted in the previous test daily, divided by the number of days elapsed between sowing and evaluation, according to the formula defined by Maguire (1962):

$$ESI = \frac{E1}{N1} + \frac{E2}{N2} + \dots + \frac{En}{Nn}$$

Where:

E1, E2, En = number of seedlings counted in the emergence tests in the first, second, and up to the last count.

N1, N2, Nn = number of days from the first, second, and up to the last count performed, where emergence stabilized.

Accelerated aging (AA): 200 seeds were used, placed in Gerbox-type acrylic boxes on stainless steel screens, containing 40 mL of distilled water at the bottom. The boxes remained in an oven regulated at 41 ± 0.2 °C for 24 hours (Dutra & Vieira, 2004). After this period, the seeds were subjected to a germination test, with four replicates of 50 seeds. Evaluations were performed on the fifth day after sowing on the paper.

The experimental design adopted was completely randomized. The data obtained were subjected to tests of homogeneity of variances (Levene) and normality (Shapiro-Wilk). Subsequently, analysis of variance (ANOVA) was performed, and the means were compared using the Student-Newman-Keuls (SNK) test at a 5% significance level. Statistical analyses were performed using the R software (R Core Team, 2022).

RESULTS AND DISCUSSION

For all evaluated items, there was an effect of the seed association treatments with the *Lithothamnium* sp.-based product (Table 1). That is, there is a difference in the behavior of the germination process (final percentage, speed, stress tolerance) and emergence (final percentage and speed) of soybean seeds according to the presence or absence of the association and type of association with *Lithothamnium* sp.

In terms of seedling emergence in the field, treatment 4 showed inferior performance (Figure 1A). The other treatments, however, did not show a statistically significant difference in final seedling emergence in the field.

Table 1. Summary of the analysis of variance (ANOVA) for the physiological quality of soybean seeds associated with *Lithothamnium* sp.-based biostimulant.

	First germination count	Germination	Accelerated aging	Seedling emergence in the field	Emergence speed index
P-value (ANOVA)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
CV (%)	4.47	5.17	6.65	8.28	12.83
Residual normality	0.85	0.87	0.85	0.70	0.27
Homoscedasticity	0.73	0.36	0.25	0.66	0.07

Figure 1. Seedling emergence (A) and emergence speed index (B) of soybean seeds subjected to different forms of association with a biostimulant based on *Lithothamnium* sp. and seeds without any association (control).

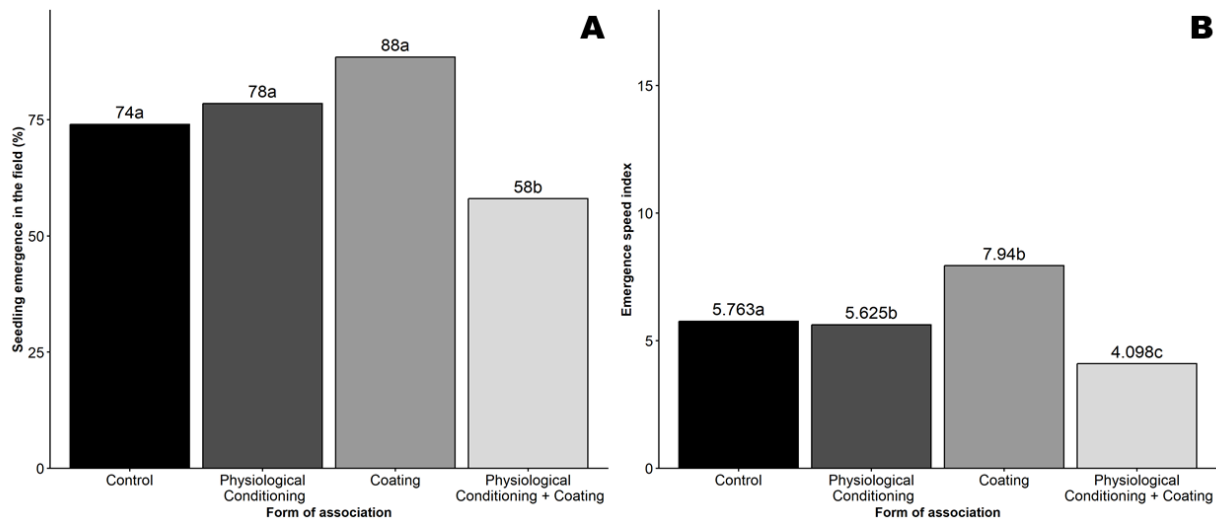
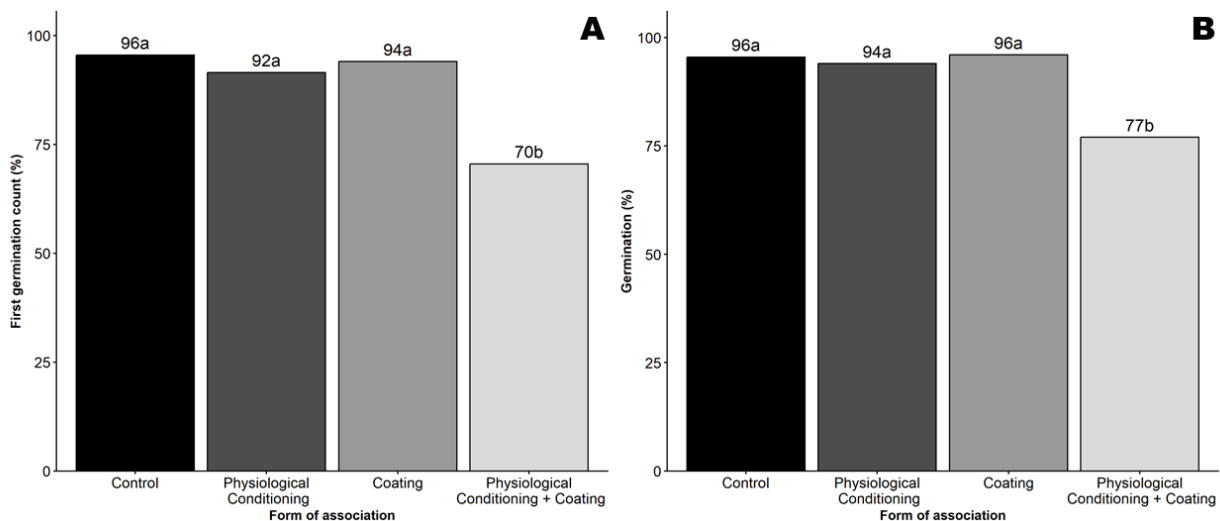


Figure 2. First germination count (A) and final germination count (B) of soybean seeds subjected to different forms of association with a biostimulant based on *Lithothamnium* sp. and seeds without any association (control).



In the work of Evangelista et al. (2016), the effect on the emergence of *Jatropha curcas* seedlings was influenced by the dose of *Lithothamnium* sp. applied to the soil. These authors revealed a linear effect on the seedling emergence response with the application of the biostimulant, with a greater response when higher levels were applied. The authors reported an improvement in microporosity, consequently better water retention capacity, to justify the beneficial effect. Possibly, this same effect was not identified in our study because the application via seed may not have been sufficient to alter the physical characteristics of the soil.

For the evaluation of the speed of the emergence process, measured by the emergence speed index, treatment 3 showed the highest speed, revealing superior behavior to the other treatments (Figure 1B). The seeds that did not receive any treatment (control) and the seeds from treatment 2 showed intermediate behavior regarding the speed of seedling emergence. The seeds from treatment 4 showed the worst emergence speed index, agreeing with the other evaluations. This beneficial result of using the biostimulant in the coating (T3) can be explained by the fact that biostimulants such as seaweed extracts can be a source of important phytohormones, including gibberellin, auxins, and cytokinins (Stirk et al., 2020).

Gibberellin is especially involved in metabolic recovery in seeds during germination and the synthesis of enzymes that hydrolyze stored reserves (Bewley, 1997), which are directed to the embryonic axis that originates the seedling. A reduction in germination time with exogenous application of gibberellin has already been reported in the literature (Ali and Elozeiri, 2017). Jesus et al. (2020) stated that conditioning greenish soybean seeds with brassinolide, a natural analogue of brassinosteroids, showed better performance in variables related to vigor. Brassinosteroids are plant hormones that signal molecular, physiological, and biochemical responses (Taiz and Zeiger et al. 2017).

For the vigor test that assesses the speed of occurrence of normal seedling formation, first count, the effect was negative in treatment 4 (Figure 2A). There was no benefit for the seeds associated with the algae product compared to those that did not receive treatment.

The same behavior observed in the first count test occurred in the germination test (Figure 2B). There was no difference between untreated seeds and seeds from treatments 2 and 3; only treatment 4 showed a deleterious effect.

According to Ramos et al. (2023), the impacts of algae such as *Lithothamnium* sp. on plant growth and development depend on the dosage used. The negative effect of treatment 4 may be related to the effect of the high concentration of the product, since two methodologies for associating *Lithothamnium* sp. with the seeds were used. Cruz et al. (2008) also reported a negative effect on seedling growth, such as root length, when high doses of *Lithothamnium* (Afertil®) were used.

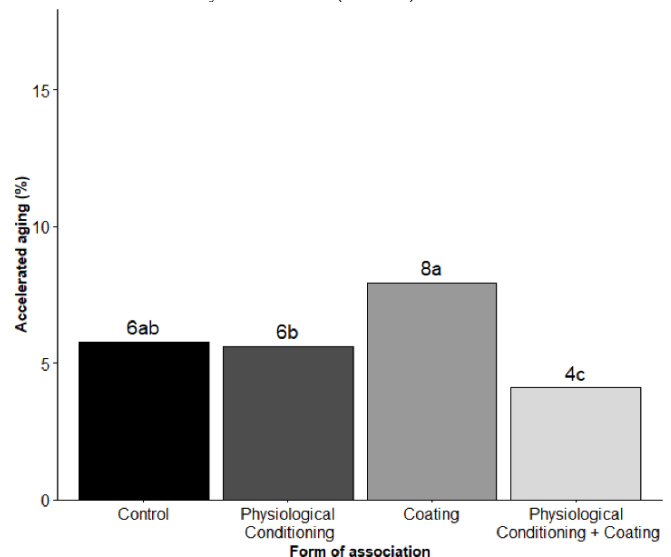
The absence of a positive effect in these cases, first count and germination tests, may be related to the ideal environmental conditions provided in the germination tests, which may overestimate seed quality (Henck et al., 2025) or at least not reflect the varied conditions that may be encountered in the field (Krzyzanowski et al., 2020). According to Marcos-Filho (2015), the germination test provides ideal conditions for the occurrence of the process.

When the tolerance of seeds to the stress provided by the accelerated aging test was evaluated, beneficial behavior was observed in treatment 3 (Figure 3). The seeds from treatment 2 showed intermediate behavior. The seeds from treatment 4, however, showed a sharp drop of 30% in the percentage of normal seedlings after stress.

These results corroborate those of Dmytryk et al. (2015), who revealed beneficial effects on the germination process of wheat seeds, such as a faster rate of occurrence of the process, when associated with biomass extracts of *Spirulina tuvoiron*, a microalga.

Tests involving simulations of field conditions (seedling emergence in the field and emergence speed index) or those that express vigor (accelerated aging) are more likely to be influenced by treatments because they assess how the germination process occurs, and are therefore more sensitive to detecting differences (Krzyzanowski et al., 2020).

Figure 3. Accelerated aging of soybean seeds subjected to different forms of association with a biostimulant based on *Lithothamnium* sp. and seeds without any association (control).



Since algae extracts can be a source of phytohormones (Stirk et al., 2020), *Lithothamnium* sp. associated with soybean seeds in our work may have favored the germination process of seeds in a more pronounced deterioration process, as simulated in the accelerated aging test. Mortelet et al. (2008) had already reported a more pronounced and effective effect of applying bioregulators based on cytokinin, auxin and gibberellin in soybean seeds exposed to stress conditions.

CONCLUSION

Coating soybean seeds with *Lithothamnium* sp. benefited final seedling emergence, emergence speed, and germination under stress conditions. Physiological conditioning followed by coating soybean seeds with *Lithothamnium* sp. impaired germination and seedling emergence.

ACKNOWLEDGMENTS

To the Goiano Federal Institute (IF Goiano), Center of Excellence in Bioinputs (CEBIO) and the Research Support Foundation of Goiás State (FAPEG).

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To cite this paper, use:

Souza, A.D.V., Xavier, J.G.M., Ribeiro, P.G.S., Lima, M.L.P., Barbosa, A.M. & Araújo, E.F.L. (2026). Performance of soybean seeds associated with a biostimulant based on the seaweed *Lithothamnium* sp. *Multi-Science Journal*, 9(1): 30-36. DOI: <http://doi.org/10.33837/msj.v9i1.1757>