

Physiological and health quality of soybean seeds with different vigor levels treated with *Bacillus amyloliquefaciens*

Qualidade fisiológica e sanitária de sementes de soja com diferentes níveis de vigor tratadas com *Bacillus amyloliquefaciens*

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ABSTRACT

The utilisation of biological organisms in the context of seed treatment has become very common. A notable example of this is the application of *Bacillus amyloliquefaciens* in soybean seeds. The objective of this study was to evaluate the physiological and health quality of soybean seeds with varying degrees of vigour treated with *B. amyloliquefaciens*. Three concentrations of *B. amyloliquefaciens* were subjected in four batches of seeds exhibiting varying degrees of vigour. Following the treatments, the physiological quality of the seeds was assessed using germination, tetrazolium, seedling emergence, cold test, seedling length and seedling dry matter mass tests. The health quality was assessed using the *Blotter test*. Soybean seeds exhibiting superior vigour had a more pronounced response to treatment with *B. amyloliquefaciens* in terms of their physiological potential, as evidenced by enhanced emergence and initial growth. Batches exhibiting reduced vigour demonstrated a positive response to the treatment, indicating the microorganism's capacity to respond to varying levels of vigour. Our results demonstrated that the use of *B. amyloliquefaciens* has been demonstrated to reduce the incidence of pathogens such as *Fusarium* sp., *Macrophomina* sp. and *Aspergillus* sp. Furthermore, the administration of doubled doses of *B. amyloliquefaciens* exhibited optimal outcomes.

Keywords: physiological potential, seed health, viability, vigor

RESUMO

A utilização de organismos biológicos no contexto de tratamento de sementes constitui um subconjunto das tecnologias em consideração. Um exemplo notável é a aplicação de *Bacillus amyloliquefaciens* em sementes de soja. O objetivo deste estudo foi avaliar a qualidade fisiológica e sanitária de sementes de soja com diferentes níveis de vigor tratadas com *B. amyloliquefaciens*. Três concentrações de *B. amyloliquefaciens* foram submetidas a quatro lotes de sementes apresentando diferentes níveis de vigor. Após os tratamentos, a qualidade fisiológica das sementes foi avaliada usando testes de germinação, tetrazólio, emergência de plântulas, teste de frio, comprimento e massa de matéria seca de plântulas. A qualidade sanitária foi avaliada usando o *Blotter test*. As sementes de soja que apresentavam vigor superior tiveram resposta mais pronunciada ao tratamento com *B. amyloliquefaciens* em termos de potencial fisiológico, conforme evidenciado pelo aumento da emergência e do crescimento inicial. Os lotes que apresentaram vigor reduzido demonstraram uma resposta positiva ao tratamento, indicando capacidade do microrganismo de responder a diferentes níveis de vigor. Os resultados demonstraram que o uso de *B. amyloliquefaciens* demonstrou reduzir a incidência de patógenos como *Fusarium* sp., *Macrophomina* sp. e *Aspergillus* sp. Além disso, a administração de doses duplas de *B. amyloliquefaciens* apresentou ótimos resultados.

Palavras-chave: potencial fisiológico, saúde das sementes, viabilidade, vigor

INTRODUCTION

Soybean represents the primary commodity produced in Brazil and has significant economic importance, given its status as one of the leading global producers and exporters of soybeans. The 2024/25 harvest is estimated to yield a production of 166,211.1 thousand tons, marking a 12.5% increase compared to the 2023/24 harvest (CONAB, 2024). This crop has been demonstrated to offer a favourable financial return to producers, thereby stimulating new investments in this sector, with the integration of novel technologies and alternative crops (Nunes, 2022).

The utilisation of soybean seed treatment constitutes a technique that has been used to ensure the efficacy of sowing and the cultivation of high-quality produce in designated fields. In addition to chemical treatment, the utilisation of biological treatment, incorporating the application of microorganisms, is an alternative.

In the context of soybeans, one of the microorganisms employed is the bacterium *Bacillus amyloliquefaciens*. This biological agent is synchronised with the management of soybean pests, and its action is faster than that of fungi and viruses. The genus of bacteria in question has been used to control caterpillars, since after ingesting the product applied to soybean leaves, there is a significant reduction in the population of these insects, with a consequent interruption in their feeding (Borges, 2021).

In addition to its direct application for pest management, *B. amyloliquefaciens* has been extensively used in seed treatment, thereby enhancing plant resilience in challenging abiotic environments (Melo *et al.*, 2021). It functions as a biocontrol agent, exhibiting the capacity to impede fungal and bacterial activities across a broad spectrum of pathogens (Ansary *et al.*, 2018; Daungfu, 2019). Furthermore, it has been demonstrated to promote plant growth (Luo *et al.*, 2022).

The utilisation of microorganisms with a biostimulant effect on plants has been a subject of investigation (Santos, 2021). Nascente *et al.* (2017) evaluated the effects of different beneficial microorganisms in relation to gas exchange, phytomass production and nutrient content, and *Bacillus sp.* was identified

as having the highest values in biomass and photosynthetic dry matter rates in a rice plantation.

In the work by Ferreira *et al.* (2021) the germination of the seeds treated with the *Bacillus* isolates QST-713, AP-03 and AP-210 was higher than the inoculated and non-inoculated controls. Furthermore, for accelerated ageing, they showed higher germination than the fungicide treatments. *Bacillus velezensis* AP-03 demonstrated a 72.5% increase in germination compared to the control, along with enhanced pigment production (Ferreira *et al.*, 2021).

Dameto *et al.* (2020) analysed the yield and quality of soybean seeds inoculated by *Bacillus subtilis*, concluding that this microorganism promoted productivity increases in the cultivars tested, improving seed quality due to the increase in seedling emergence percentage and seed vigour.

As dos Santos *et al.* (2016) demonstrate, the quality of seeds is determined by a combination of genetic, physical, health and physiological attributes. With regard to the physiological potential of seeds, in addition to viability, vigor assumes importance as the parameter that determines the potential of seeds to germinate quickly and uniformly under a wide variety of environmental conditions (Marcos Filho, 2015).

Presently, vigor tests are a customary component of seed quality programmes (Marcos Filho, 2015). Consequently, as this factor is part of seed quality, it has the capacity to interfere with the efficiency of the biological treatment. Accordingly, a comprehensive understanding of how seeds with varying degrees of vigor respond to treatment with *Bacillus amyloliquefaciens* bacteria is essential for decision-making and the efficacy of this practice in comparison to alternative types of seed treatments. The aim of this study was to assess the impact of soybean seed vigor on the physiological potential and sanitary quality of soybean seeds subjected to treatment with *Bacillus amyloliquefaciens*.

MATERIAL AND METHODS

The research was conducted at the Organic Agriculture Laboratory of the Agricultural Sciences Centre of the Federal University of São Carlos. Araras, São Paulo, Brazil.

Soybean seeds (*Glycine max* (L.) Merrill) from four batches exhibiting different levels of vigour and with no prior treatment were subjected to three treatments consisting of different concentrations of *Bacillus amyloliquefaciens*, based on the recommended dose, half the recommended dose and double the recommended dose, plus a control treatment, without the microorganism (Figure 1, Table 1). The doses selected for administration were diluted in 5 ml of water, ensuring a total volume of 200 seeds per dose. The treatment was conducted within a plastic bag, whereby the mass of seeds was manually agitated to ensure complete coverage of the surface.

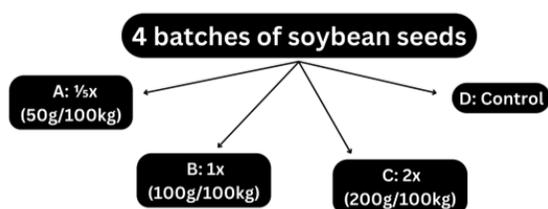


Figure 1 - Doses used for the composition of the *Bacillus amyloliquefaciens* treatments on 4 batches of soybean seeds.

A: 1/5x the recommended dose (50g/100kg), B: 1x the recommended dose (100g/100kg), C: 2x the recommended dose of *Bacillus amyloliquefaciens* (200g/100kg), diluted in 5ml of water for a total of 200 seeds and distributed in each batch.

In order to characterise the batches selected for study, the seeds were assessed for their physiological potential and health quality before and after the treatments. The following tests were used for this purpose:

Germination test: The experiment was conducted with four replicates of 50 seeds for each treatment,

in Germitest paper towel rolls. The rolls were then moistened with a quantity of water equivalent to 2.5 times the mass of the dry substrate. Subsequent to the preparation of the rolls, they were stored within a BOD germination chamber at 25 °C in conditions of darkness. The sixth and ninth day were selected for counting, according to the Brazilian Rules for Seed Analysis (Brasil, 2025).

Tetrazolium test: For the control treatment, four replicates of 50 untreated seeds were used for each batch. These seeds were soaked in paper towels that had been previously moistened with a quantity of water equivalent to 2.5 times their mass. Following a 16-hour soaking period at 25 °C, the seeds were transferred to plastic cups and completely submerged in a 0.075% 2,3,5-triphenyltetrazolium chloride solution. The cups were then placed in an oven at 40 °C for a 2-hour period. After that, the seeds were extracted from the oven, thoroughly rinsed in tap water, and maintained submerged until the evaluation stage. The procedure adopted for the assessment and interpretation of vigor levels comprised the longitudinal sectioning of each seed with a razor blade, followed by the allocation of a score in accordance with the criteria established in Brasil (2025).

Seedling emergence in sand: 50 seeds were used in four replicates for each batch, which were distributed in plastic trays containing sand as a substrate, receiving an amount of water corresponding to 60% of the holding capacity. The boxes were then kept at room temperature in the laboratory and evaluated on the fourteenth day, considering the percentage of emerged seedlings for each treatment (Marcos Filho *et al.*, 2009).

Cold test: This was carried out in four replicates with 50 seeds on paper towels moistened with 2.5 times their mass of distilled water and covered with 60g of soil. The rolls were rolled up, placed

Table 1 - Dose of *Bacillus amyloliquefaciens* used on soybean seeds for each treatment. The doses are different for each batch, as the seed masses are different between the batches studied. A: 1/5 × the recommended dose, B: 1 × the recommended dose, C: 2 × the recommended dose of *Bacillus amyloliquefaciens*, diluted in 5ml of water for a total of 200 seeds.

Treatments	Batch 1	Batch 2	Batch 3	Batch 4
A	0.014g	0.023g	0.019g	0.019g
B	0.028g	0.046g	0.038g	0.038g
C	0.056g	0.092g	0.076g	0.076g

vertically and kept in a cold room at 10 °C for 7 days, then kept at 25 °C in a germination chamber for a further seven days. Normal seedlings were assessed in a single count on the seventh day after transferring the rolls to the germination chamber (Vieira *et al.*, 2010).

Seedling length: This test was carried out in a similar way to the germination test, using a millimeter ruler. Four replicates with 20 seeds were used, which were sown in a line in the upper third of the paper. The papers were previously moistened with water corresponding to 2.5 times the dry weight of the paper. The seeds were placed with the micropyle facing downwards and then the rolls were positioned vertically in a germination chamber at a temperature of 25 °C for seven days. At the end of the seven days, the normal seedlings (primary root and hypocotyl) were measured using a millimeter ruler (Vanzolini *et al.*, 2007).

Dry matter mass of seedlings: The normal seedlings from the seedling length test were separated into root and aerial part and placed in an oven with air circulation at 60 °C until the mass stabilized. The results were expressed in grams for each of the lots studied.

Blotter test: This was carried out in petri dishes disinfected with 5% sodium hypochlorite, containing three sheets of 80 filter paper as a substrate. Sterilized water was then added at a rate of 2.5 times the mass of the paper sowed, selected seeds were placed on the paper. The seeds were placed in the germination chamber for 24 hours, 24 hours in the freezer and 7 days in the BOD at 25 °C (Henneberg *et al.*, 2012).

Following a thorough evaluation of the batches, they were then ranked according to their level of vigour. This assessment was conducted by taking into account the data from the control treatment, which involved the absence of the *Bacillus amyloliquefaciens*. It is important to note that the tetrazolium test was only conducted on the control treatment, with the purpose of providing information on the ranking of the batches studied.

The experiment was set up in a completely randomised design in a 4 x 4 factorial scheme (four levels of vigour and three concentrations of

B. amyloliquefaciens + control). The results were evaluated using the analysis of variance test, and where a significant effect was identified, the Tukey test at 5% probability was used for further analysis.

RESULTS AND DISCUSSION

The batches were then ranked based on the results obtained in the control treatment in all the tests carried out without the interference of *Bacillus amyloliquefaciens* (Figure 2, Tables 2 to 4). The results of the study indicated that batch 3 exhibited the highest level of vigour, as determined by the collective evaluation of all the tests conducted (Table 2). Batch 1 exhibited the lowest level of vigour, while batches 2 and 4 demonstrated intermediate levels.

In the viability and vigor tests, a high germination rate and adequate seedling development were observed for lots 2 and 3. However, these results were slightly lower than those recorded for lot 3 with regard to germination speed, emergence and growth. This categorisation is indicative of the mean performance of the batches, as determined by the control tests conducted (Figure 2).

The lower vigor exhibited by lot 1 can be attributed to various factors that compromise seed quality, including inadequate handling practices following maturity (Marcos Filho, 2021), natural deterioration, suboptimal storage conditions, or mechanical damage.

The superior performance of a crop is directly associated with seed quality, which can be defined by viability. In this context, seed vigour is a fundamental attribute, integrating genetic and environmental aspects to comprehensively estimate the potential performance of growing conditions (Reed, 2022).

The present study examined the relationship between the treatments with *B. amyloliquefaciens* and the batches with different levels of vigour. The results demonstrated that there was no distinction between the seeds treated with *B. amyloliquefaciens* and those with only the control treatment for germination (Table 2).

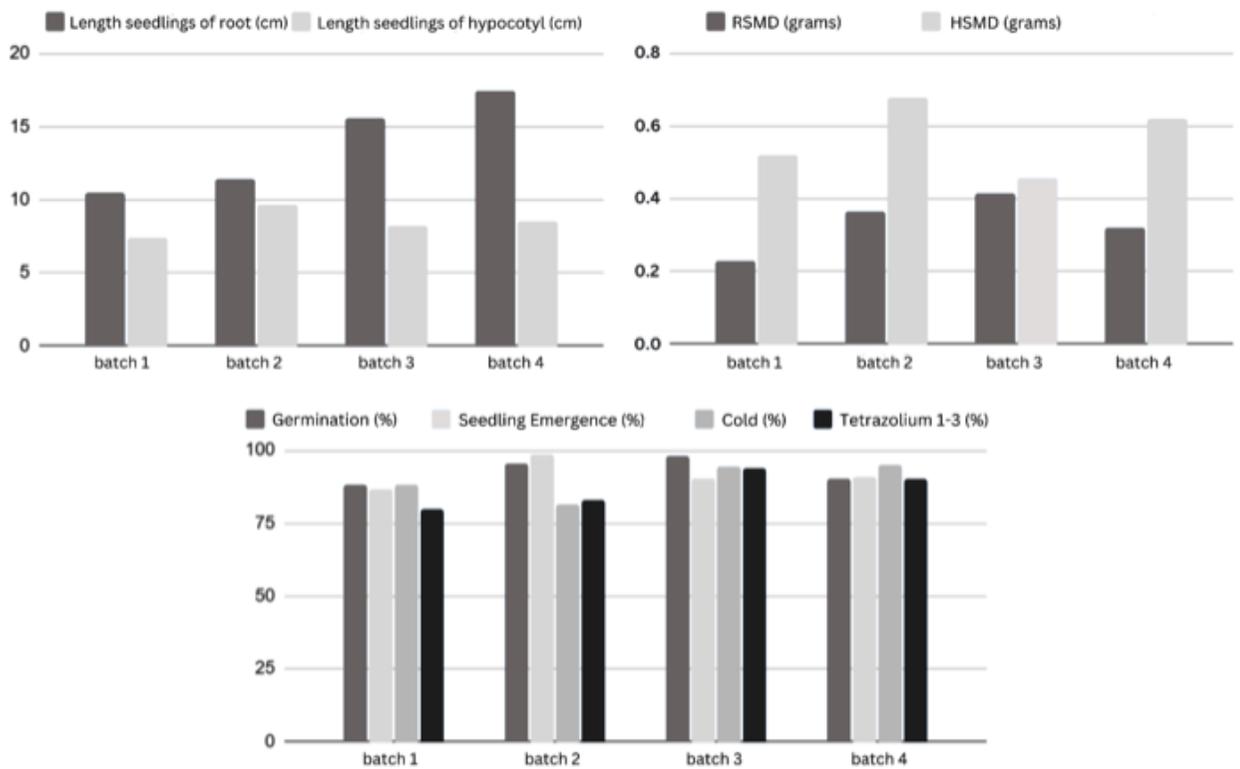


Figure 2 - Germination, seedling emergence, cold test and tetrazolium test, in percent, length of seedlings, root and hypocotyl, in cm, and mass of dry matter of root and mass of dry matter of hypocotyl, in grams (g), of four batches of soybean seeds.

In the context of the seedling emergence test, batch 3, which exhibited the highest levels of vigour, demonstrated no significant outcomes. In batches exhibiting reduced vigour (1), emergence was observed to be higher in the lower doses (A) or the recommended dose (B).

In the work of Henning *et al.* (2010), the author posits that accelerated emergence can confer an initial advantage upon plants from vigorous seeds, enabling them to optimise their utilisation of water, light and nutrients. Consequently, the absence of a discernible effect in the most vigorous batch (batch 3) can be attributed to the heightened capacity of highly vigorous seeds for germination and seedling development, irrespective of the prevailing test conditions or applied doses.

A comparison of the results obtained from the various treatments reveals that the seeds from the most vigorous and intermediate batches (2, 3 and 4) exhibited superior performance in response to

the elevated doses of the treatment (Treatment C) when compared to the seeds from batch 1 (Table 2).

The assessment of seedling growth can be facilitated through the measurement of length and dry matter mass, as these parameters are both objective and measurable (size and mass, respectively). In Meneguzzo (2021) study, soybean seeds exhibiting superior vigour were observed to yield seedlings of greater length. This finding corroborates other studies which also indicate a positive relationship between seed vigor and seedling growth, directly reflecting the quality of emerging plants and initial development.

With regard to root length (Table 3), it can be seen that the batches exhibiting the lowest levels of vigour (1 and 2) demonstrated inferior root development, irrespective of the treatment applied, while the batches with the highest vigour (3 and 4) exhibited greater root length. To the length of the seedlings in relation to the hypocotyl, no significant

Table 2 - Germination (G), First Germination Count, Seedling Emergence in sand (SE) and First Seedling Emergence Count in sand, in soybean seeds treated with different concentrations of *Bacillus amyloliquefaciens*. A: $\frac{1}{5}$ × the recommended dose, B: 1× the recommended dose, C: 2× the recommended dose of *Bacillus amyloliquefaciens*, diluted in 5ml of water for a total of 200 seeds. D: Control treatment with seeds treated only with water. Averages followed by the same capital letter in the rows for each test and lower case in the columns do not differ by the Tukey test at 5% probability

Batch	Germination (%)				First Germination Count (%)			
	A	B	C	D	A	B	C	D
1	85.0Ab	90.5Aa	86.0Ab	88.0Ac	74.5Ab	79Ab	77.5Ab	75Ab
2	93.5Aa	91.5Aa	92.0Aab	95.5Aab	93.5Aa	86,5Ba	88,5Ba	94.5Aa
3	96.5Aa	92.0Aa	94.5Aa	98.0Aa	92.5Aa	91,5Aa	93,5Aa	96.5Aa
4	94.0Aa	91.0Aa	93.0Aab	90.0Abc	94Aa	89Ba	89Ba	89.5Ba

Batch	Seedling Emergence (%)				First Emergence Count (%)			
	A	B	C	D	A	B	C	D
1	92.5Aab	94.5Aab	86.0Bb	86.5Ba	71.5Aa	74.5Aa	70.5Aa	66.5Ba
2	96.5Aa	82.0Cc	90.5Bab	98.5Ba	70.5Aa	62Bb	65Bb	60Ba
3	93.0Aab	89.0Ab	90.5Aab	90.0Aa	70.5Aa	67.5Ab	64,5Bb	67.5Aa
4	89.5Bb	96,5Aa	93.5Aba	90.5Ba	63Bb	71.5Ab	60.5Bb	64Ba

increase was observed for any of the *B. amyloliquefaciens* treatments applied in comparison to the control treatment, but a decrease was noted. More vigorous seeds exhibit longer primary roots and greater total seedling lengths.

Thus, a comprehensive evaluation of the data concerning seedling length reveals that, while substantial variations were observed among the various batches with respect to the length of the seedling or its components, only the length of the root exhibited a correlation with the data pertaining to seedling emergence. Therefore, it is feasible to substantiate the findings of researchers who have indicated that root length exhibits heightened sensitivity in differentiating between batches of soybeans (Nakagawa, 1999), including in contexts pertaining to the consequences observed in response to the biological treatment of soybean seeds.

It was observed that the different doses of *Bacillus amyloliquefaciens* tested had a significant impact on the dry matter mass of the roots and hypocotyls of the soybean seedlings (Table 3), varying according to the batch. The treatments with the lowest doses (A) or the recommended dose (B) exhibited higher Root Dry Matter Mass (RSMD) values, particularly in batches of intermediate and higher vigour. Treatments involving higher doses (C) have been observed to yield higher Hypocotyl Dry Matter

Mass (HSMD) values. Furthermore, it is notable that batch 3, which exhibited the highest levels of vigour, also possessed the lowest root dry matter mass. However, this batch was distinguished by its significantly higher hypocotyl dry matter mass in comparison to the control treatment (D). This phenomenon may be associated with a differential allocation of energy and metabolic resources during the initial development of the seedlings. It has been demonstrated that seeds exhibiting greater vigor tend to prioritise the growth of the aerial parts, such as the hypocotyl, over that of the root system, with the objective of facilitating faster and more efficient emergence (Rossi, 2017).

The investigation revealed no statistically differences in the germination percentage among the four batches examined in the cold test, nor was there any discernible effect in relation to the *Bacillus amyloliquefaciens* treatments (Table 4). The impact of the control treatment was the only observable phenomenon, and this was pertinent to the general characterisation of the batches with regard to vigour levels. In his study, Binsfeld (2014) reported that the cold test was not significantly relevant in the interaction with the treatments of biostimulant, bioactivator, and nutrients applied. The author posits that this outcome can be ascribed to the inherent correlation between the effect of the product and the physiological quality of the seeds.

Table 3 - Root Seedling Length (RSL), Hypocotyl Seedling Length (HSL), Root Dry Matter Mass (RSMD) and Hypocotyl Dry Matter Mass (HSMD) in soybean seeds treated with different concentrations of *Bacillus amyloliquefaciens*. A: $\frac{1}{5}$ × the recommended dose, B: 1× the recommended dose, C: 2× the recommended dose of *Bacillus amyloliquefaciens*, diluted in 5ml of water for a total of 200 seeds. D: Control treatment with seeds treated only with water. Averages followed by the same capital letter in the rows for each test and lower case in the columns do not differ by the Tukey test at 5% probability

Batch	Root length (cm)				Hypocotyl length (cm)			
	A	B	C	D	A	B	C	D
1	12.3Abc	9.1Ab	10,2Ab	10.5Ac	7.2Ab	6.8Ab	6.8Ab	7.3Aa
2	10.7Ac	11.4Ab	10.7Ab	11.4Abc	11.4Aa	9.3Aab	11.5Aa	9.6Aa
3	18.3Aa	16.8Aba	13.8Bab	15.5ABab	7.9Ab	8.0Ab	7.2Ab	8.2Aa
4	15.8Aab	13.3Aab	16.0Aa	17.4Aa	8.2Bb	11.2Aa	8.2Bb	8,5Ba

Batch	Root Dry Matter Mass (grams)				Hypocotyl Dry Matter Mass (grams)			
	A	B	C	D	A	B	C	D
1	0,231Aa	0,166Ab	0,204Ac	0,229Ac	0.456ABb	0,433Bb	0.552Ab	0.518ABbc
2	0.307Ba	0.366ABa	0.400Aa	0.366ABab	0,504Bb	0,524Bb	0.701Aa	0.675Aa
3	0,303Ba	0,287Ba	0.307Bb	0.415Aa	0.634Aa	0.662Aa	0.659Aa	0.455Bc
4	0.271Aa	0,345Aa	0.346Aab	0.320Ab	0.619Aa	0.665Aa	0.668Aa	0.618Aab

The response of the seeds to the treatment is contingent on their pre-existing vigour and physiological capacity. It is important to note that the cold test, by evaluating only the response under adverse temperature conditions, cannot fully reflect the influence of the internal quality of the seeds on the performance of the treatments. It can also be posited that seeds with lower vigour exhibit a marginally lower germination rate following the cold test, due to their reduced physiological capacity. This is due to the fact that they possess reduced energy reserves and less efficient cell structures, which engenders difficulties in the process of germination under adverse conditions. The greater the amount of reserves in the seeds, the greater the vigour of the seedlings that originate from them.

This finding suggests that the treatments did not interfere with the viability of the seeds. In the work of Coppo *et al.* (2017), no substantial disparities were observed in the vigour of soybean seeds when employing a treatment comprising plant and fungal extracts. The author posits that this phenomenon can be attributed to the rapid germination and emergence of the seeds, a consequence of their robust physiological potential. Consequently, the utilisation of treatment was deemed to be either unnecessary or irrelevant. The author also noted that seeds exhibiting low vigour demonstrated no response to the treatment, remaining susceptible to fungal infection present in the coat, exhibiting no substantial changes.

Table 4 - Cold test on soybean seeds treated with different concentrations of *Bacillus amyloliquefaciens*. A: $\frac{1}{5}$ × the recommended dose, B: 1× the recommended dose, C: 2× the recommended dose of *Bacillus amyloliquefaciens*, diluted in 5ml of water for a total of 200 seeds. D: Control treatment with seeds treated only with water. Averages followed by the same capital letter in the rows for each test and lower case in the columns do not differ by the Tukey test at 5% probability

Batch	Cold test (%)			
	A	B	C	D
1	87.0Aab	93.5Aa	87.0Aa	88.0Aab
2	82.0Ab	90.5Aa	92.0Aa	81.5Ab
3	93.5Aa	91.5Aa	91.5Aa	94.5Aa
4	82.5Bb	93.0Aba	86.0Aba	95.0Aa

The health of the seeds is directly related to their storage capacity and vigour. Furthermore, transmitted pathogens have the capacity to interfere with the growth habits of plants, thereby reducing their reproductive efficiency and causing significant losses in productivity (Porto, 2019; Dadlani e Yadava, 2022).

The presence of inoculum in seeds constitutes a significant phytosanitary risk, as it can facilitate the gradual dissemination of diseases within the field, thereby compromising both the yield and the commercial quality of the crop. This phenomenon can be attributed to the ability of the seed to function as a vehicle for the pathogen, thereby ensuring its survival and maintaining its viability from the harvesting stage to the subsequent sowing (Nunes, 2015; Campos *et al.*, 2020).

As demonstrated in Figure 3, the treated soybean seeds exhibited a reduced incidence of fungi, particularly for *Aspergillus*, *Macrophomina* and *Fusarium*. Rocha *et al.* (2014) observed that *Aspergillus ochraceus* significantly compromises the vigor of soybean seeds, causing total destruction of the tissues when the inoculum exceeds 50% of the seed surface. At infection levels of less than 50%, seed germination is impaired, resulting in reduced physiological performance.

It is notable that other fungi, including *Cladosporium* sp. and *Fusarium* sp., have been observed to induce latent infections in seeds during storage, despite not resulting in immediate damage. When left unchecked, these infections have been observed to trigger seed deterioration, as they act as a gateway for *Aspergillus* and *Penicillium* species. These

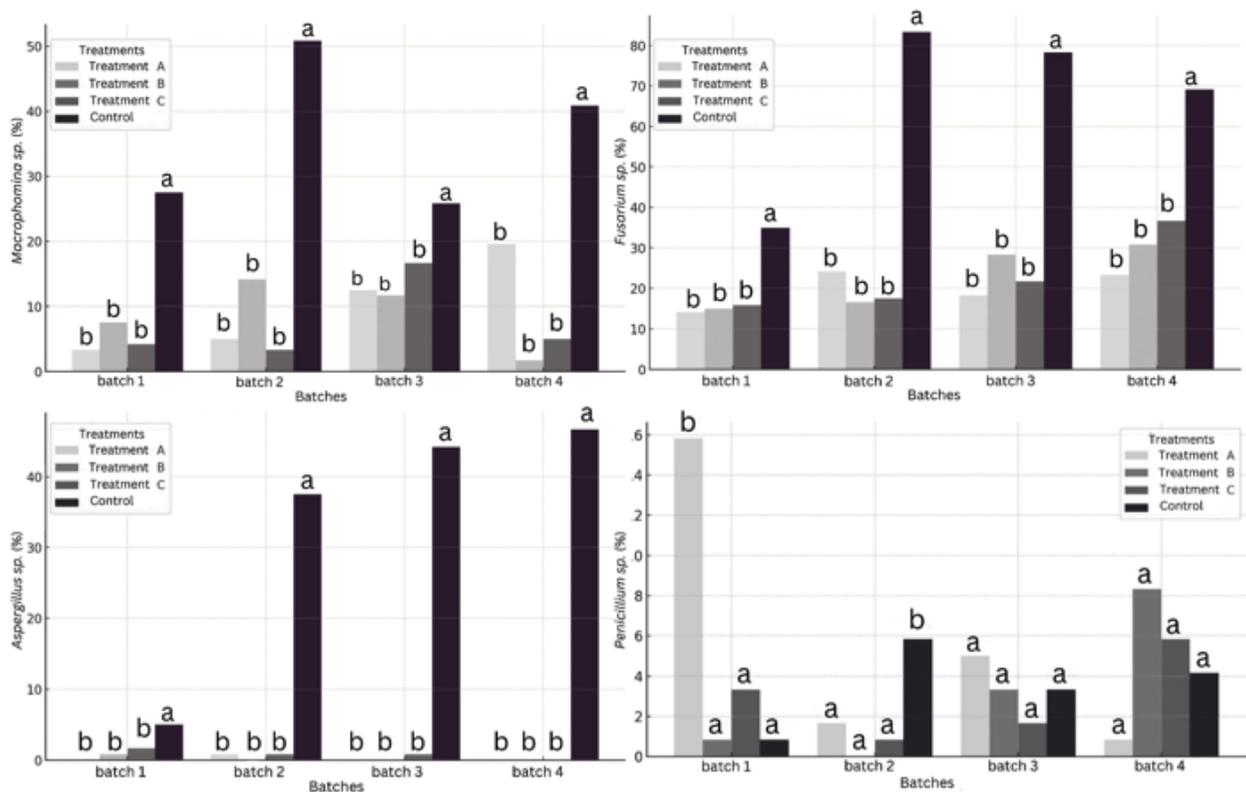


Figure 3 - Incidence of *Macrophomina* sp., *Fusarium* sp., *Aspergillus* sp. and *Penicillium* sp. in soybean treated in *Bacillus amyloliquefaciens*. A: 1/5x the recommended dose, B: 1x the recommended dose, C: 2x the recommended dose of *Bacillus amyloliquefaciens*, diluted in 5ml of water for a total of 200 seeds. D: Control treatment with seeds treated only with water. Averages followed by the same letter for each batch do not differ by the Tukey test at 5% probability.

organisms thrive in conditions of elevated temperature and relative humidity; however, they are capable of survival in environments characterised by low humidity. Their proliferation is initiated by the action of field fungi, thereby contributing to the process of seed deterioration (Wetzel, 1987).

Among the various species of *Fusarium*, the most prevalent (representing 98% or more) found on soybean seeds is *Fusarium semitectum*. This fungus is regarded as being pathogenic due to its association with germination issues in laboratory settings. *F. semitectum* has been frequently linked to seeds that have been harvested at a late stage or that have undergone deterioration as a consequence of excessive humidity in the field. During storage, this pathogen rapidly diminishes in viability under ambient conditions (Goulart, 2018).

In the work by Ishikawa *et al.* (2018), it is demonstrated that soils infested with *Macrophomina phaseolina* have a detrimental effect on the development of soybean plants, resulting in stunted growth. This pathogen is regarded as being challenging to control, but practices that enhance the physical conditions of the soil can assist in minimising the impact of rot (Almeida *et al.*, 2024).

Therefore, observing the data from the health test indicates the potential of this microorganism to reduce the incidence of diseases and improve the health quality of seeds, ensuring better plant performance in the field.

The results indicate that *Bacillus amyloliquefaciens* exerts a significant effect on seed health, with the vigour of the seed batches interfering with the effect of the treatment. It is evident that the use of the

bioproduct with vigorous seed batches, especially at higher doses, can promote faster and more uniform emergence and hypocotyl length. Conversely, seeds exhibiting reduced vigour (batch 1) demonstrated lower performance; nevertheless, they exhibited a response to the *B. amyloliquefaciens* treatment, particularly at lower doses, suggesting that the treatment can exert an effect even on seeds with low initial vigour.

The use of this microorganism can serve as an effective strategy for enhancing the performance of soybean crops, promoting healthy plant growth and reducing the incidence of pathogens. The optimal dosage for this treatment is generally considered to be double (2x), although lower doses (1/2x) have been observed to be effective in some cases.

CONCLUSION

Soybean seeds exhibiting varying degrees of vigour demonstrate divergent responses to biological treatment with *Bacillus amyloliquefaciens*, with seeds of greater vigour exhibiting enhanced physiological potential as a consequence of the treatment. However, seeds of lower vigour can also benefit, especially with adjusted doses of the treatment, suggesting that the microorganism can be successfully applied in different conditions of vigour, as long as it is properly dosed. The administration of a doubled dose (200g/100kg) of *Bacillus amyloliquefaciens* has been shown a more pronounced response in terms of physiological and health effects. Furthermore, a substantial decline in the prevalence of fungal species, including *Macrophomina* sp., *Fusarium* sp. and *Aspergillus* sp., was observed.

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