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BIOPRIMING OF CORN SEEDS BASED ON *Bacillus subtilis* UNDER DIFFERENT STORAGE PERIODS

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ABSTRACT

Corn crop (*Zea mays* L.) is one of the pillars of Brazilian agriculture, with a wide cultivation area and high productivity; Brazil is the world's second largest exporter of this cereal. For this reason, the aim of the present study was to evaluate the viability of biopriming of corn seeds with a product based on *Bacillus subtilis*, at different concentrations, up to 120 days of storage. The research was carried out in the seed technology laboratory of the State University of Londrina (UEL), using the commercial corn seeds Balu 366. The experimental design was completely randomized, with four replications, in a 7 × 5 factorial scheme, with seven concentrations of the commercial product based on *Bacillus subtilis* (Serenade) for seed biopriming (0.0, 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 %) and five storage periods in a cold chamber (0, 30, 60, 90, and 120 days). The evaluation of the physiological quality of the seeds occurred through germination, cold and seedling length tests. The variables analyzed were first germination count, germination percentage, vigor from the cold test, shoot length, length of the longest root, shoot dry mass, and root dry mass. For the statistical analysis, adjustments of regression models with the best fit were made. The biopriming of seeds proved to be an advantageous practice to enhance the physiological quality of the seeds under storage; it should be carried out at a concentration of 2.5 %, due to its economic viability, considering its efficiency equivalent to the highest concentrations. The biopriming of seeds proved to be a viable practice for seed storage for up to 60 days.
Additional keywords: Germination, seed conditioning, vigor, *Zea mays*

RESUMEN

Bioacondicionamiento de semillas de maíz con *Bacillus subtilis* durante diferentes periodos de almacenamiento

El maíz (*Zea mays* L.) es uno de los pilares de la agricultura brasileña, con gran superficie de cultivo y alta productividad; siendo Brasil el segundo mayor exportador de este producto básico en el mundo. Por tal motivo, el objetivo del presente trabajo fue evaluar la viabilidad del bioacondicionamiento de semillas de maíz, con un producto comercial a base de *Bacillus subtilis*, en diferentes concentraciones, hasta por 120 días de almacenamiento. El trabajo se realizó en el laboratorio de producción y tecnología de semillas de la Universidad Estadual de Londrina (UEL), utilizando semillas comerciales de maíz Balu 366. El diseño experimental fue completamente al azar, con cuatro repeticiones, en un esquema factorial 7 × 5, con siete concentraciones del producto comercial *Bacilus subtilis* (Serenade) para bioacondicionamiento de semillas (0,0; 2,5; 5,0; 7,5; 10,0; 12,5 y 15,0 %) y cinco periodos de almacenamiento en cámara frigorífica (0, 30, 60, 90 y 120 días). La evaluación de la calidad fisiológica de las semillas se produjo mediante pruebas de germinación, frío y longitud de plántula. Las variables analizadas fueron: primer conteo de germinación, germinación, vigor de la prueba de frío, longitud de vástago, longitud de la raíz más larga, masa seca de vástago y masa seca de raíz. Para el análisis estadístico se realizaron ajustes a modelos de regresión con mejor ajuste. El bioacondicionamiento de semillas de maíz resultó ser una práctica ventajosa para mejorar la calidad fisiológica de las semillas almacenadas; y debe realizarse a una concentración del 2,5 %, debido a su viabilidad económica, teniendo en cuenta su eficiencia equivalente a concentraciones superiores. El bioacondicionamiento del maíz demostró ser una práctica viable para almacenar semillas por hasta 60 días.
Palabras clave adicionales: Cebado de semillas, germinación, vigor de la semilla, *Zea mays*

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INTRODUCCTION

Corn (*Zea mays* L.) crop is one of the pillars of Brazilian agriculture, with an approximate cultivation area of 22 thousand hectares and production of 128,000 t. Domestic consumption is approximately 80,000 t·year^{-1} and the rest for exportation (Conab, 2023).

For success in a crop, the use of high quality seeds is a primordial factor. However it is

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necessary to point out that throughout the whole productive chain of seeds, several factors are able to influence their quality, positively or negatively, either in the production field, harvesting, processing and/or storage (Marcos, 2015).

Among the factors able to influence the seed quality, abiotic factors, such as temperature and humidity, and biotic factors, such as attack by pathogens and insects, stand out. Thus, seed treatment has been a technique commonly applied to seed protection, mainly through the use of fungicides and insecticides (Machado et al., 2013). However, in order to increase the sustainability of this technique and minimize the negative influence on the environment, several seed treatment techniques have been the target of scientific studies, with seed priming being a relevant practice for optimizing their quality, mainly biopriming, based on biological products (Lizárraga et al., 2015).

The use of *Bacillus* spp. in seed treatment or soil application has shown positive results, promoting the growth of corn plants or other crops (Chalivendra et al., 2019; Rojas et al., 2022), in addition to controlling some phytopathogens such as *Fusarium verticillioides* (Nguyen et al., 2017).

During storage, seed treatment has been a tool that aims to protect them during this period, in order to preserve the physiological, physical and health quality of seeds for a longer period. In the field, this practice has generally contributed to controlling and/or preventing attacks by pathogens and insects, and the lack of this protection can have a direct impact on yield (Silva et al., 2020).

Throughout storage, the seeds must be kept in a suitable place to avoid their degradation, but the time that these seeds would be kept under these conditions also needs to be analyzed, because factors such as relative humidity and air temperature affect the process germinative (Saeed et al., 2020). Properly conducted storage is a determining factor for maintaining seed quality for longer periods, reducing the chances of accelerating the seed deterioration process. The seeds, under storage, do not have an improvement in their physiological quality, but maintaining their quality for a long period is the main objective (Silva et al., 2022).

Due to the changes that occur in the quality of the seeds, after storage they need to be subjected to tests in order to verify their quality before being taken to the field (Silva et al., 2022). To certify the physiological quality of seeds, vigor tests have been applied in order to detect differences in the physiological potential of seed lots, which contributes to decision-making (Sena et al., 2015).

It is known that the seed production chain is extremely important for world agriculture. For this reason, studies aimed at the development of beneficial technologies for seed production, whether in the field or in post-harvest, are necessary, in order to enhance the quality of the seeds produced, in addition to minimizing their losses, contributing to the increase its viability time.

However, for the validation of technological practices to be made available to the seed production chain, it is extremely important to submit the seeds under different tests, in order to evaluate the germination and vigor of seed lots under various parameters, which will contribute for reliable results.

For this reason, the aim of the present study was to evaluate the viability of biopriming of corn seeds with a product based on *Bacillus subtilis*, at different concentrations, up to 120 days of storage, the maximum storing time generally available for corn producers between successive production cycles.

MATERIAL AND METHODS

The research was carried out in the seed technology laboratory of the State University of Londrina (UEL), using Balu 366 corn seeds. For seed biopriming, the experimental design was completely randomized, with four replications, in a 7×5 factorial scheme (concentrations and storage periods). Used seven concentrations (0.0, 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 %) of the product Serenade (aqueous solution), based on *Bacillus subtilis* [13.68 $g.L^{-1}$ (1.37 % m/v)], and five storage periods (0, 30, 60, 90, and 120 days), in a cold chamber (0 °C).

For the treatment (biopriming), the corn seeds were placed on two sheets of germitest paper, covered by a third sheet, moistened in the proportion of 2.5 times the mass of the dry substrate with the doses of Serenade, following the concentration of each treatment. The seeds remained in contact with the moistened substrate for 62 h, in a germinator at 25 °C. The imbibition

period was defined by means of pre-tests, when 50 % of the seeds showed radicle emission. Seeds with root emission were discarded, remaining only the seeds that reached the second phase of the germination process (without radicle emission).

After imbibition of the seeds, they were arranged in a thin layer, in iron trays with a protection of germitest paper to reduce the friction caused between the tray and the seed, and then placed in an oven at 40 °C for 72 hours. Every 24 hours, the trays were randomly changed positions inside the oven, in addition to being shaken to change the position of each seed, in order to standardize the drying process of the seeds, without causing thermal damage.

After biopriming, except for the seeds that were used to evaluate the 0 (zero) storage time, they were stored in a cold chamber without the presence of light, at 0 °C. These seeds were stored, under these conditions, for 30, 60, 90, and 120 days. The latter was the maximum evaluated storing time because the experiment was carried out be applied by producers whose seed production occurs from one harvest to next one. From the biopriming of corn seeds, the analyzed variables were:

Germination test: 50 seeds were used per repetition, totaling 200 seeds evaluated per treatment. The seeds were arranged on sheets of germitest paper, as explained before. Then, the paper rolls were placed in a germinator at 25 °C. The evaluations were carried out four and seven days after the installation of the test, according to the Rules for Seed Analysis (Brasil, 2009), and the data were expressed in percentage of germinated seeds. From this test, the following variables were evaluated: first germination count (seeds germinated on the fourth day after installing the test) and germination (sum of seeds germinated on the fourth and seventh day after installing the test), expressed as a percentage of the total.

Cold test: 50 seeds were used per repetition, totaling 200 seeds evaluated per treatment. Arranged in rolls of germitest paper as before. Then, the rolls were placed in a transparent plastic bag, sealed with adhesive tape, and then placed in an incubator at 10 °C for 7 days (Conceição, 2008). After this period, each experimental unit was removed from the plastic bag and placed in a germinator at 25 ºC; performing the count of normal seedlings after 7 days (Brasil, 2009). The germination percentage represents the vigor from the cold test.

Seedling length test: 20 seeds were used per repetition, totaling 80 seeds evaluated per treatment, which were placed in the upper third of the germitest paper rolls, moistened with distilled water in a proportion of 2.5 times their dry mass and placed in a germinator at 25 °C. After 7 days, 10 normal seedlings from each repetition were randomly selected to measure shoot length and length of the longest root, from the collar of the seedling to the end of the coleoptile, using the methodology adapted from Meneguzzo et al. (2020).

After measuring the shoot and root lengths of the seedlings of each treatment, the shoots and roots were sectioned and placed separately in paper bags. Then, each experimental unit was placed in an oven with forced air circulation at a temperature of 80 °C for 24 hours. After cooling in a desiccator, weighing was performed on an analytical balance, with a precision of 0.001 g, calculating the average.

For the statistical analyses of the factorial arrangement of the product concentration and storing period, adjustments were made to regression models with the best fit for the percentage of first germination count, germination, vigor from the cold test, shoot and root length, and shoot and root dry mass; and F test was used to establish significant differences using the program SISVAR (Universidade Federal de Lavras, MG, Brazil). When no interactions existed, the individual effect of each factor is presented.

RESULTS AND DISCUSSION

The interaction between the factors concentrations and storage periods showed significant differences for the variables first germination count, germination, vigor from the cold test, root length and root dry mass (Table 1). The variables shoot length and shoot dry mass showed significant differences for the factors of concentrations and storage periods, individually (Table 1).

When analyzing the variables first germination count and germination, it is possible to observe a quadratic tendency of increase of the variables as the concentrations increase, for the evaluated

storage periods (Figure 1). Storage period of 60 days provided a higher percentage of both first germination count and germination, for most of the evaluated concentrations (Figure 1).

Table 1. F test for the variables first germination count (%), germination (%), vigor from the cold test (%), shoot length (cm), root length (cm), shoot dry mass (g) and root dry mass (g) of corn seeds submitted to different concentrations of a product based on *Bacillus subtilis* for biopriming, under storage

First germination count	p-value	**Germination**	p-value
Concentration (C)	0.0000*	Concentration (C)	0.0000*
Storage period (SP)	0.0000*	Storage period (SP)	0.0000*
C × SP	0.0017*	C × SP	0.0153*
CV %	6.91	CV %	6.54
Vigor from the cold test	p-value	**Shoot length**	p-value
Concentration (C)	0.0000*	Concentration (C)	0.0000*
Storage period (SP)	0.0000*	Storage period (SP)	0.0000*
C × SP	0.0000*	C × SP	0.7543 ns
CV %	9.91	CV %	11.12
Root length	p-value	**Shoot dry mass**	p-value
Concentration (C)	0.0000*	Concentration (C)	0.0079*
Storage period (SP)	0.0000*	Storage period (SP)	0.0000*
C × SP	0.0097*	C × SP	0.8728 ns
CV %	14.63	CV %	30.99
Root dry mass	p-value		
Concentration (C)	0.1129 ns		
Storage period (SP)	0.0393*		
C × SP	0.0097*		
CV %	41.40		

ns: not significant, * :significant

Ibanhes et al. (2021) noted in their studies that the doses of *Bacillus subtilis* applied, together with physiological conditioning or pelliculation (a technology that allows the addition of agrochemicals to seeds, without changing their size or shape) can be used for treatment of bean seeds without harming seed lot viability. Tavanti et al. (2020) observed that the inoculation of *Bacillus subtilis* promotes yield increments in both soybean cultivars tested, besides improving seed quality due to the increase in seedling emergence percentage and seed vigor.

When analyzing the variable vigor from the cold test, an increase of the variable is verified as the concentrations are increased up to 5.0 %, with a tendency of stabilization in higher concentrations, for the storage periods of 0, 90, and 120 days. For storage periods of 30 and 60 days, the tendency for the variable to stabilize is achieved at a concentration of 2.5 % (Figure 2).

In addition, it is possible to observe that when storing the corn seeds subjected to biopriming for up to 60 days, it allowed the seed lot to express greater vigor compared to the other evaluated periods. This practice proved to be viable for seeds stored for up to 120 days (Figure 2). According to Oliveira and Gomes (2010), osmopriming of sorghum seeds did not influence germination, but promoted benefits in their vigor, as observed in the present study from the cold test, perhaps because bacteria could help minimize the deterioration process in seeds, especially during storage.

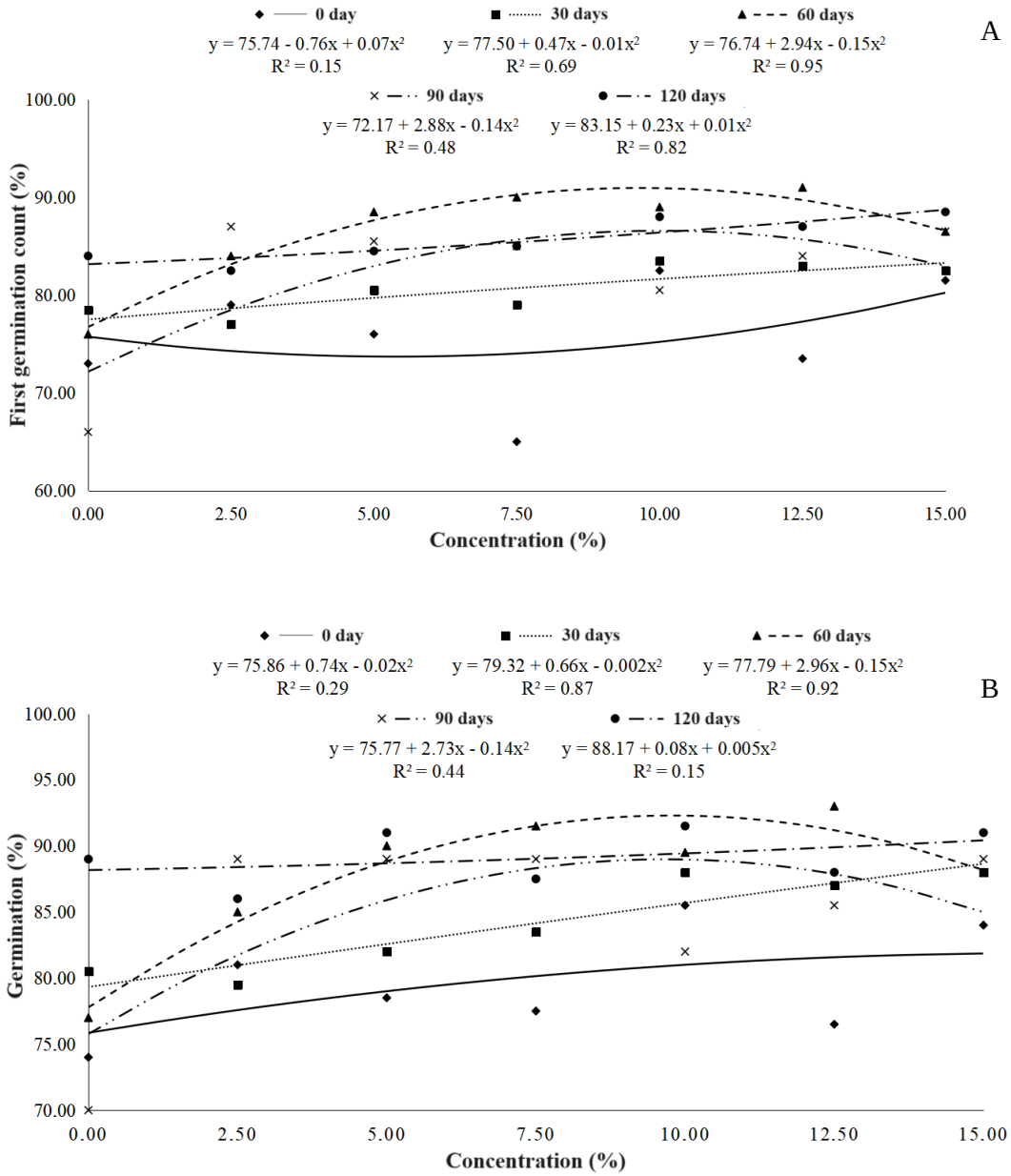

Figure 1. A- First germination count, and B- Germination of corn seeds subjected to biopriming under different concentrations of a commercial product based on *Bacillus subtilis*, under storage

For shoot length, it was observed that the factors were individually significant. Thus, in figure 3A it is possible to notice that the increase in the concentration of the product based on *Bacillus subtilis*, used in the biopriming of corn seeds, favors the increase in shoot length. Significance was also found for the storage period factor, again showing a quadratic behavior, with a storage period of 60 days favoring an increase in shoot length, with a tendency for the variable to decrease over longer storage periods (Figure 3B).

For root length, it is observed that intermediate concentrations (5.0, 7.5, and 10.0 %) tended to reduce the variable for storage periods of 0, 30, and 120 days, with an upward trend in the highest concentrations (12.0 and 15.0 %). In the storage period of 60 days, the observed trend differs from the others, considering that in this condition there

is an increase in root length as the concentration increases from 0.0 to 2.5 %, with a subsequent tendency to stabilize (Figure 4).

For shoot dry mass, it is observed that the factors were individually significant as well as verified for the variable shoot length. Thus, in figure 5A, it is possible to notice that the increase in the concentration of the product based on *Bacillus subtilis*, used in the bioconditioning of Balu 366 corn seeds, contributes to the increase in the shoot dry mass. The storage period factor was also significant with quadratic behavior. From figure 5B, it is possible to observe that in a storage period of 60 days there is a tendency to increase the shoot dry mass as the concentration increases to 7.5 %, with a subsequent tendency of reduction of the variable in longer periods.

For root dry mass, it can be observed that, in general, in the storage periods evaluated, there was an increase in that variable with the increase in Serenade concentrations up to 7.5 %; with the exception in the storage period of 120 days, when a reduction in root dry mass was observed at intermediate concentrations (7.5 and 10.0 %). The storage period of 60 days was what allowed to obtain the highest indices of root dry mass, starting from the concentration of 2.5 %; only at the concentration of 15.0 % it was observed that the highest root dry mass was obtained when the seeds were stored for 120 days (Figure 6). Therefore, when the objective is to store the seeds for a long period (120 days), it would be necessary to use a concentration from 12.5 %. However, for storage periods of less than 120 days, the 2.5 % concentration proved to be the highest recommended given the economic viability.

From the variables analyzed in the present study, it appears that the biopriming of Balu 366 corn seeds, with a product based on *Bacillus subtilis*, contributes to the expression of vigor and viability of the seed lot tested, proving to be a simple and viable practice to be carried out by producers who intend to preserve the seeds for up to 60 days.

The promotion of the bacteria on seedlings and initial plant growth may be attributed to possible biochemical changes associated to chlorophylls, carotenoids, and different enzymes as shown by Obrzut et al. (2021) in tomato crop.

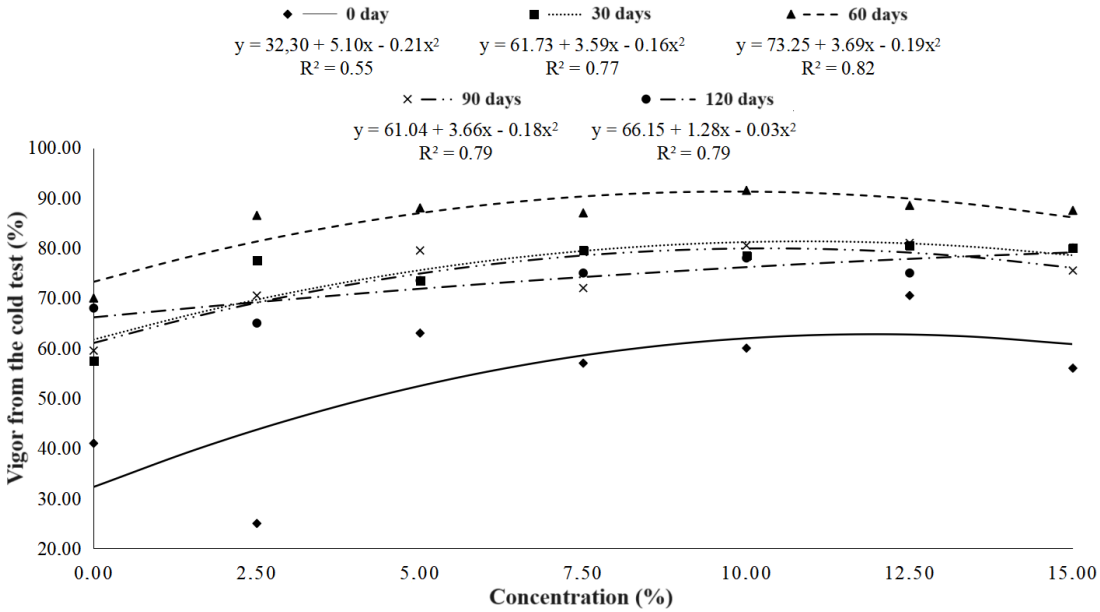

Figure 2. Vigor from the cold test of corn seeds subjected to biopriming under different concentrations of a commercial product based on *Bacillus subtilis*, under storage

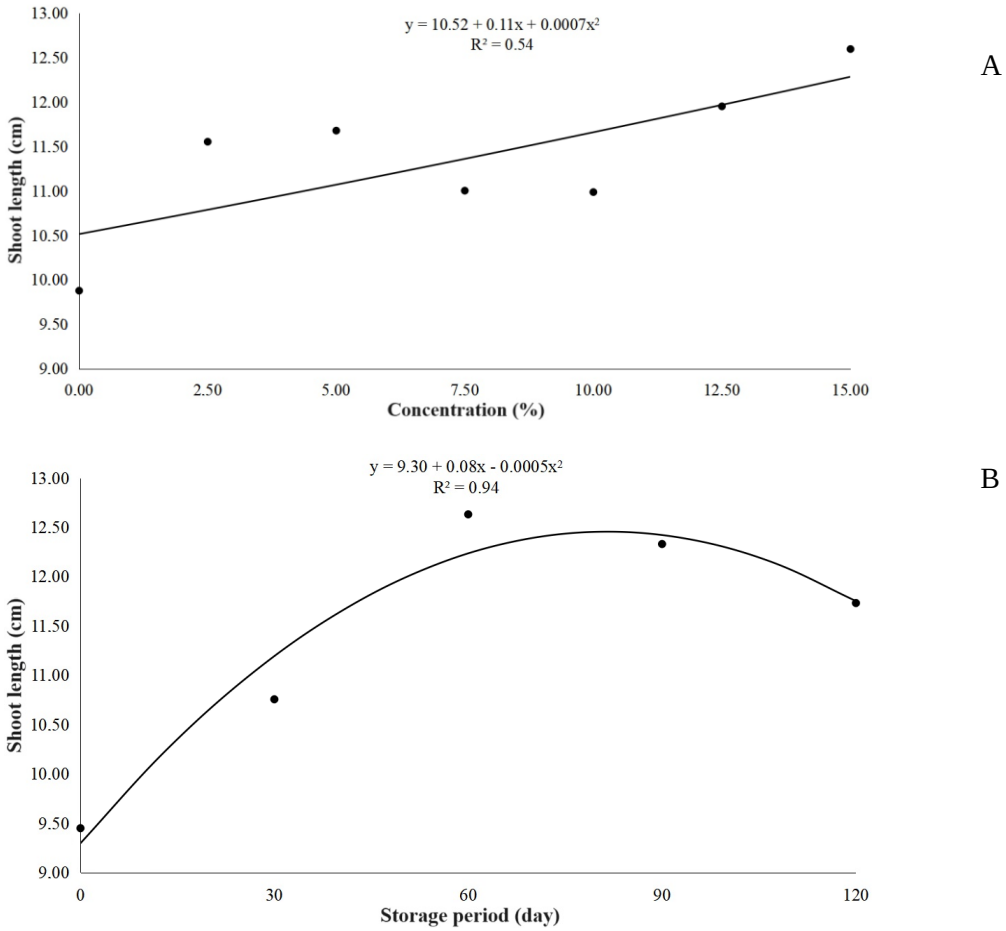

Figure 3. Shoot length of corn seeds subjected to biopriming under different concentrations of a commercial product based on *Bacillus subtilis*, under storage. A. For individual effect of concentrations. B. For individual effect of storage periods

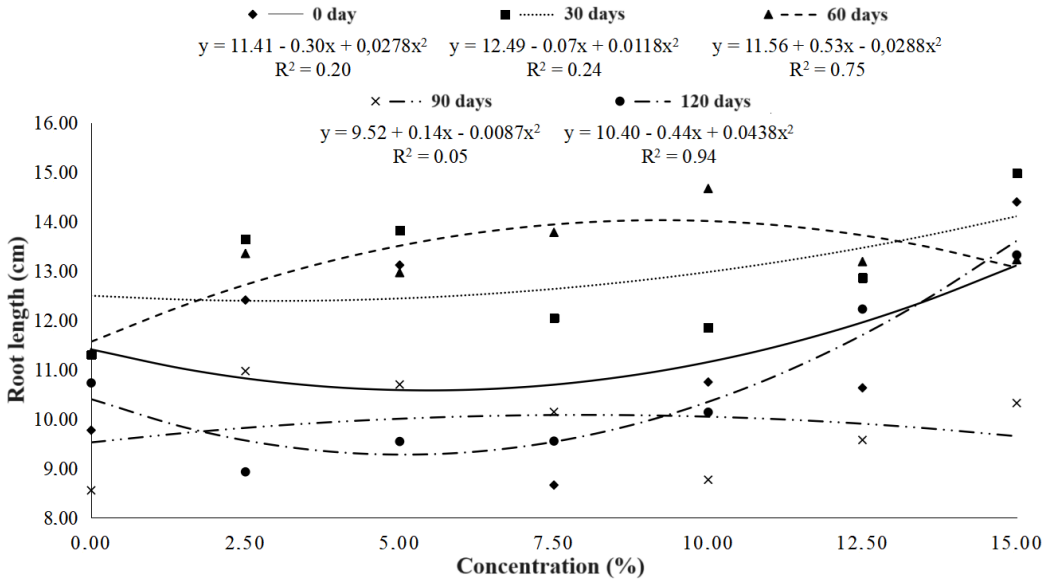

Figure 4. Root length (cm) of corn seeds subjected to biopriming under different concentrations of a commercial product based on *Bacillus subtilis*, under storage

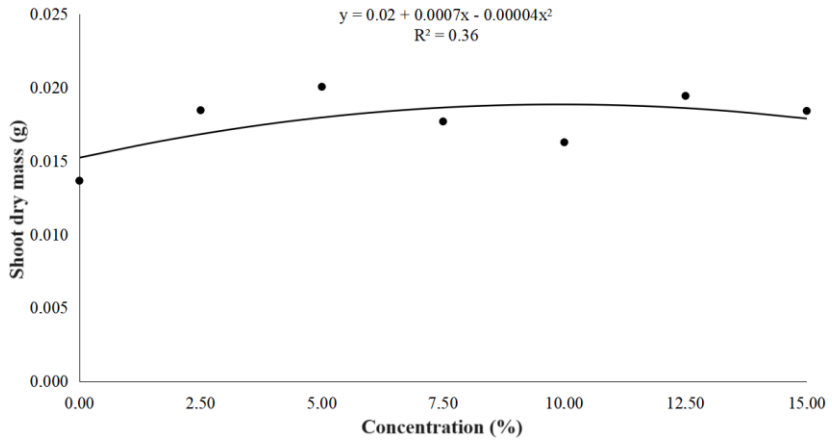

Figure 5. Shoot dry mass of corn seeds subjected to biopriming under different concentrations of a commercial product based on *Bacillus subtilis*, under storage. A- For individual effect of concentrations. B- For individual effect of storage periods

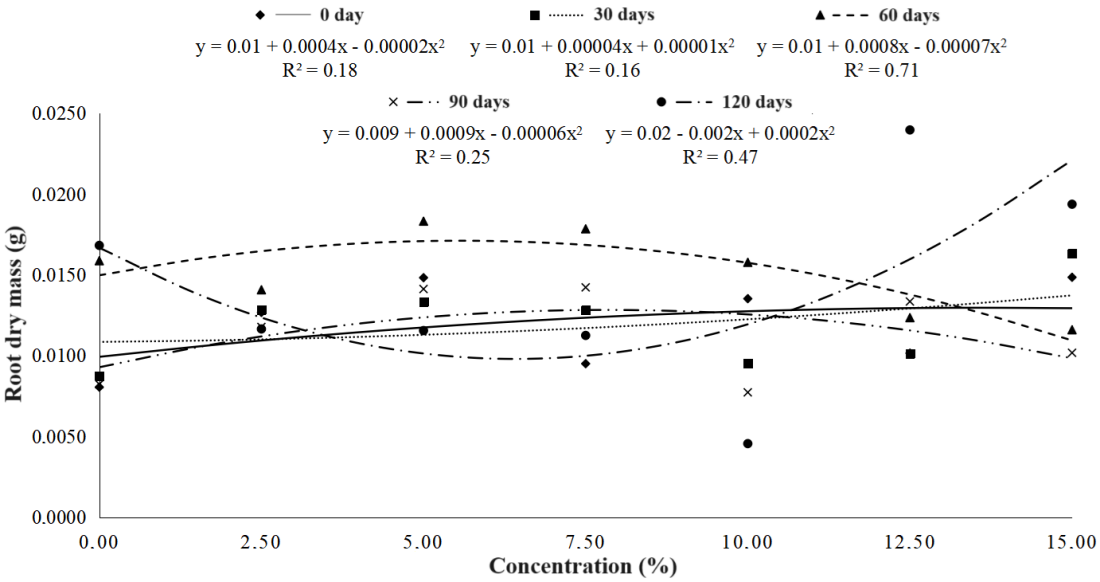

Figure 6. Root dry mass of corn seeds subjected to biopriming under different concentrations of a commercial product based on *Bacillus subtilis*, under storage

CONCLUSION

The biopriming of Balu 366 corn seeds with a product based on *Bacillus subtilis* (commercial product Serenade) proved to be an advantageous practice to enhance the physiological quality of the seeds under storage.

The biopriming should be carried out at a dose of 2.5 %, due to its economic viability, considering its efficiency equivalent to the highest concentrations.

The biopriming proved to be a viable practice for seed storage for up to 60 days.

LITERATURE CITED

1. Brasil. 2009. Regras para Análise de Sementes. Ministério da Agricultura Pecuária e Abastecimento. Brasília, DF. https://n9.cl/5aow
2. Chalivendra, S. and J.H. Ham. 2019. Bacilli in the biocontrol of mycotoxins. In: M. Islam, M. Rahman, P. Pandey, M. Boehme and G. Haesaert (eds.). Bacilli and agrobiotechnology: phytostimulation and biocontrol. Springer. Cham, Kanton Zug. pp 49-62.
3. Conab. 2023. Acompanhamento da Safra Brasileira: Grãos-Safra 2022/23, sétimo levantamento. Conab. Brasília, DF. https://n9.cl/1lryh
4. Conceição, P.M. and H.D. Vieira. 2008. Qualidade fisiológica e resistência do recobrimento de sementes de milho. Revista Brasileira de Sementes 30(3): 48-53.
5. Ibanhes Neto, H.F., A.C. Silva, C.H. Sumida, M.S. Gouveia, V. Pellizzaro and L.S.A. Takahashi. 2021. Physiological potential of green bean seeds treated with *Bacillus subtili*s. Journal of Seed Science 43: e202143016.
6. Lizárraga-Sánchez, G.J., K.Y. Leyva-Madrigal, P. Sánchez-Peña, F.R. Quiroz-Figueroa and I.E. Maldonado-Mendoza. 2015. *Bacillus cereus sensu lato* strain B25 controls maize stalk and ear rot in Sinaloa, Mexico. Field Crops Research 176(1): 11-21.
7. Machado, J.C., A.Q. Machado, E.A. Pozza, C.F. Machado and W.L.A. Zancan. 2013. Inoculum potential of *Fusarium verticillioides* and performance of maize seeds. Tropical Plant Pathology 38(3): 213-217.
8. Marcos-Filho, J. 2015. Fisiologia de Sementes de Plantas Cultivadas. Abrates. Londrina, Paraná. 659 p.
9. Meneguzzo, M.R., I.R. Carvalho, S.M. Dellagustin, F. da Motta Xavier, A.P. Nadal, V.P. Gonçalves et al. 2020. Length of soybean and corn seedlings influenced by seed vigor and size. Revista de Ciências Agrárias 43(2): 193-201.
10. Nguyen, P.A., C. Strub, A. Fontana and S. Schorr-Galindo. 2017. Crop molds and mycotoxins: Alternative management using biocontrol. Biological Control 104(1): 10-27.
11. Obrzut, V.V, A.F. Mógor, S.M. Mazaro, and G. Mógor. 2021. Initial growth, production in consecutive years and biochemical changes on tomato cultivars in organic system with application of *Bacillus subtilis*. IDESIA (Chile) 39(2): 47-55.
12. Oliveira, A.B. and E. Gomes-Filho. 2010. Efeito do condicionamento osmótico na germinação e vigor de sementes de sorgo com diferentes qualidades fisiológicas. Revista Brasileira de Sementes 32(3): 25-34.
13. Rojas-Sánchez, B., G. Santoyo, P. Delgado-Valerio and M. Rocha-Granados. 2022. *Bacillus* spp. endófitos promueven diferencialmente el crecimiento de tres variedades de zarzamora (*Rubus* subgénero *Eubatus*). Bioagro 34(2): 99-110.
14. Saeed, M.F., A. Jamal, I. Ahmad, S. Ali, G.M. Shah, S.K. Husnain et al. 2020. Storage conditions deteriorate cotton and wheat seeds quality: an assessment of farmers' awareness in Pakistan. Agronomy 10(9): 1246.
15. Sena, D.V.D.A., E.U. Alves and D.S.D. Medeiros. 2015. Vigor de sementes de milho cv. 'Sertanejo' por testes baseados no desempenho de plântulas. Ciência Rural 45(1l): 1910-1916.
16. Silva, K.M.J., R.G.V. Pinho, E.V.R.V. Pinho, R.M. Oliveira, H.O. Santos and T.S. Silva. 2020. Chemical treatment and size of corn seed on physiological and sanitary quality during storage. Journal of Seed Science 42: e202042010.

17. Silva, M.A.D., V.R.S. Barboza, J.N. Silva, E.P. Gonçalves and J.S. Viana. 2022. Physiological potential of stored *Schinopsis brasiliensis* Engler diaspores. Revista Ciência Agronômica 53: e20207649.

18. Tavanti, T.R., R.F.R. Tavanti, F.S. Galindo, I. Simões, L.S. Dameto and M.E. SÁ. 2020. Yield and quality of soybean seeds inoculated with *Bacillus subtilis* strains. Revista Brasileira de Engenharia Agrícola e Ambiental 24(1): 65-71.