Research, Society and Development, v. 11, n. 7, e21911729688, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i7.29688

Biopolymers in the preservation of rhizobacteria cells and efficiency in soybean inoculation

Biopolímeros na preservação de células de rizobactérias e eficiência na inoculação de soja

Biopolímeros en la conservación de células de rizobactérias y eficacia en la inoculación de soja

Received: 04/27/2022 | Reviewed: 05/06/2022 | Accept: 05/17/2022 | Published: 05/21/2022

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Abstract
Soybean (*Glycine max* (L). Merr.) is among the most important legumes worldwide performing a significant role in biological fixation of nitrogen through rhizobacteria. These microorganisms that act in the plant root system are inoculated by biological products, which must contain viable cells. The viability of inoculants is a market challenge, and biopolymers have been studied for the preservation of microorganisms. Thus, this study aimed to assess the influence of adding xanthan gum and carboxymethylcellulose biopolymers on the preservation of cells of *Bradyrhizobium elkanii*, *Bradyrhizobium diazoefficiens*, *Azospirillum* sp., and *Pseudomonas fluorescens*, and inoculation of soybean seeds. The inoculants were produced and stored added with biopolymers. Soybean seeds were inoculated at 0, 90 and 210 inoculant storage days and sown in pots of 1 L. 50 days after emergence for assessing physiological parameters of stomatal conductance (gs - mol H_20 m^{-2} s^{-1}), CO_2 assimilation rate (A - µmol CO_2 m^{-2} s^{-1}), transpiration rate (E - mmol H_2O m^{-2} s^{-1}), in addition to morphological parameters of plant height (H), fresh mass of aerial part (FMAP), root fresh mass (RFM), number of nodules (NNo), fresh mass of nodules (FMNo), dry mass of aerial part (DMAP), root dry mass (RDM), and dry mass of nodules (DMNo). The use of biopolymers proved efficient at preserving the cells of the microorganisms tested at 210 storage days through the responses obtained from an increase in aerial and root plant biomass resulting from a more efficient nodulation in the inoculant with biopolymer.
Keywords: Inoculant; Preservative; *Glycine max* (L.) Merr.

Resumo

A soja (*Glycine max* (L). Merr.) está entre as leguminosas mais importantes do mundo, desempenhando um papel significativo na fixação biológica de nitrogênio por meio de rizobactérias. Esses microrganismos que atuam no sistema radicular das plantas são inoculados por produtos biológicos, que devem conter células viáveis. A viabilidade de inoculantes é um desafio de mercado, e os biopolímeros vêm sendo estudados para a preservação de microrganismos. Assim, este trabalho teve como objetivo avaliar a influência da adição de goma xantana e biopolímeros de carboximetilcelulose na preservação de células de *Bradyrhizobium elkanii*, *Bradyrhizobium diazoefficiens*, *Azospirillum* sp. e *Pseudomonas fluorescens*, e inoculação de sementes de soja. Os inoculantes foram produzidos e armazenados adicionados de biopolímeros. Sementes de soja foram inoculadas aos 0, 90 e 210 dias de armazenamento do inoculante e semeadas em vasos de 1 L. 50 dias após a emergência para avaliação dos parâmetros fisiológicos de condutância estomática (gs - mol H_20 m^{-2} s^{-1}), taxa de assimilação de CO_2 (A - µmol CO_2 m^{-2} s^{-1}), taxa de transpiração (E - mmol H_2O m^{-2} s^{-1}), além de parâmetros morfológicos de altura de planta (H), massa fresca da parte aérea (FMAP), massa fresca de raiz (RFM), número de nódulos (NNo), massa fresca de nódulos (FMNo), massa seca da parte aérea (DMAP), massa seca de raiz (RDM) e massa seca de nódulos (DMNo). O uso de biopolímeros mostrou-se eficiente na preservação das células dos microrganismos testados aos 210 dias de armazenamento através das respostas obtidas a partir do aumento da biomassa aérea e radicular resultante de uma nodulação mais eficiente no inoculante com biopolímero.

Palavras-chave: Inoculante; Conservante; *Glycine max* (L.) Merr.

Resumen

La soja (*Glycine max* (L). Merr.) es una de las leguminosas más importantes del mundo y desempeña un papel importante en la fijación biológica de nitrógeno por parte de las rizobacterias. Estos microorganismos que actúan sobre el sistema radicular de las plantas son inoculados por productos biológicos, los cuales deben contener células viables. La viabilidad de los inoculantes es un desafío de mercado y se han estudiado los biopolímeros para la conservación de microorganismos. Así, este trabajo tuvo como objetivo evaluar la influencia de la adición de biopolímeros de goma xantana y carboximetilcelulosa en la conservación de células de *Bradyrhizobium elkanii*, *Bradyrhizobium diazoefficiens*, *Azospirillum* sp. y *Pseudomonas fluorescens*, e inoculación de semillas de soja. Los inoculantes se produjeron y almacenaron con la adición de biopolímeros. Semillas de soya fueron inoculadas a los 0, 90 y 210 días de almacenamiento del inoculante y sembradas en macetas de 1 L. 50 días después de la emergencia para evaluar los parámetros fisiológicos de conductancia estomática (gs - mol H_20 m^{-2} s^{-1}), tasa de asimilación de CO_2 (A - µmol CO_2 m^{-2} s^{-1}), tasa de transpiración (E - mmol H_2O m^{-2} s^{-1}), además de parámetros morfológicos de altura de la planta (H), masa fresca de la parte del brote (FMAP), raíz masa fresca (RFM), número de nódulos (NNo), masa fresca de nódulos (FMNo), masa seca aérea (DMAP), masa seca de raíces (RDM) y masa seca de nódulos (DMNo). El uso de biopolímeros demostró ser eficiente en la preservación de las células de los microorganismos probados a los 210 días de almacenamiento a través de las respuestas obtenidas del incremento de biomasa aérea y radicular producto de una nodulación más eficiente en el inoculante con biopolímero.

Palabras clave: Inoculante; Preservativo; *Glicina max* (L.) Merr.

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the most important legumes worldwide, a scenario in which Brazil is among the five main producers that combined represent 90% of productivity. Additionally, it performs a significant role in sustainable agriculture and economy due to its nitrogen fixation capacity combined with rhizobacteria promoting symbiosis between plant and several microorganisms, like *Bradyrhizobium* sp., *Bacillus* sp., *Azospirillum* sp., and *Pseudomonas* sp. (Zhao et al., 2018; Pawar et al., 2018).

The use of plant growth promoting bacteria (PGPB) is an alternative to reduce the use of chemical fertilizers due to their ability to colonize the plant root system and improve its performance (Berendsen et al., 2012; Nehra et al., 2016). Inoculants require a method that is able to preserve cell viability for long periods. According to Berninger et al. (2017), several methods can be applied, such as the addition of protecting substances as trehalose, gum arabic, carboxymethylcellulose, alginate, xanthan gum, among others.

The use of exopolysaccharides in the formulation of inoculants has been tested due to their ability to protect rhizobial cells against stress factors such as salinity, drying, and pH (Qurashi & Sabri, 2012; Tewari & Arora, 2014). Tests on the combination of PGPB and carboxymethylcellulose in sorghum seedlings have been conducted and confirmed their root and aerial part growth promoters, in addition to improving germination index (Widawati & Suliasih, 2018). Several cell protectors

have been tested; Santhosh (2015) demonstrated that inoculants produced without the addition of protectors were not able to endure 150 storage days, while inoculants produced with polyvinylpyrrolidone, polyethylene glycol, and gum Arabic can preserve viable cells until 180 days.

Thereby, this study aimed to verify the efficiency of using carboxymethylcellulose and xanthan gum biopolymers at preserving the viability of cells of *Bradyrhizobium elkanii*, *Bradyrhizobium dayzoefficies*, *Azospirillum* sp., and *Pseudomonas fluorescens* over 210 shelf days for soybean culture.

2. Methodology

We performed our study at the region located at 11º48'29" S, 48º56'39" W, 280 m of altitude and is characterized as B1A'wa', humid, mega thermal climate, with mild water deficiency in the winter (Souza et al., 2019). We used pots with a capacity of 1.0 L, filled with soil collected from a crop area classified as middle-texture Red-Yellow Dystrophic Latosol (Santos et al., 2018), at a depth of 0-20 cm, presenting the following chemical features: Ca 1.2 cmol dm^{-3}; Mg 0.6 cmol dm^{-3}; Al 0.0 cmol dm^{-3}; H+Al 2.2 cmol dm^{-3}; K 0.07 mg dm^{-3}; M.O. 1.6 dag kg^{-1}; C.O. 0.9 dag kg^{-1}; CTC (T) 4.07 cmol dm^{-3}, SB 1.87 cmol dm^{-3}, and pH 5.6. The following physical features were found: 60% sand, 5% silte, and 35% clay. The soil was neither corrected nor fertilized at any moment in the experiment aiming to assess the true potential of the inoculants tested.

The experiment was based on a completely randomized design with three treatments and five repetitions: one treatment without addition of biopolymer to the inoculant, one added with carboxymethylcellulose, and another added with xanthan gum. The inoculants were produced in the laboratory, using MS2 culture medium (16 mL L^{-1} soybean molasses and 4.0 g L^{-1} yeast extract) for the microorganisms *Bradyrhizobium elkanii*, *Bradyrhizobium diazoefficiens*, and *Azospirillum* sp., and MS3 culture medium (5.0 g L^{-1} peptone, 5.0 g L^{-1} yeast extract, 3.0 g L^{-1} sodium chloride, 3.0 g L^{-1} magnesium sulfate, 0.5 g L^{-1} potassium sulfate, and 16 mL L^{-1} soybean molasses) for *Pseudomonas fluorescens*. Fermentation was followed by adding preservatives, with 15 mL of sterile preservative solution (0.1 g biopolymer in 100 mL distilled water) in 150 mL of fermented inoculum, stored in Falcon tubes at 5 °C for 210 days.

Since all tests were performed to assess the preservation of rhizobacteria cells viability using biopolymers, we used the inoculant produced on three different storage dates: 0, 90 and 210 days.

Preparing the seeds for planting

We used a soybean culture from cultivar CZ 37D43 IPRO and seed inoculation proceeded as follows: 40 µL of inoculant produced in 20 g of seed were used for the experiment with *Bradyrhizobium elkanii* and *Bradyrhizabium diazoefficiens*, diluting in 1 mL of distilled water to inoculate all seeds uniformly. Likewise, we initially inoculated the seeds for the experiment with *Azospirillum* sp. but using the commercial *Bradyrhizobium*. Drying was followed by inoculation with *Azospirillum* sp. with 100 µL of inoculant. Likewise, we performed an inoculation with commercial *Bradyrhizobium* for the experiment with *Pseudomonas fluorescens* followed by inoculation with 100 µL *Pseudomonas fluorescens*.

Inoculation was followed by sowing five seeds per pot. Emergence occurred five days after sowing and thinning 12 days after emergence, leaving only two plants per pot for the assessments.

Assessments

All assessments were carried out 50 days after sowing (DAS) determining the physiological and morphological parameters of soybean plants. The physiological assessments were performed using the open system of photosynthesis with CO_2 analyzer and water vapor through infrared radiation (InfraRed Gas Analyzer - IRGA, model LCiSD, ADC System®) on the plant leaves without injuries. The photosynthetically active radiation on leaf surface (Qleaf) for the analyses were in

average 1778.44 µmol photons m^{-2} s^{-1} at mean leaf temperature of 39 ºC. 10 readings per repetition were performed for the assessment to generate more accurate data, and the physiological variables analyzed were stomatal conductance (g_s - mol H_20 m^{-2} s^{-1}), CO_2 assimilation rate (A - µmol CO_2 m^{-2} s^{-1}), and transpiration rate (E - mmol H_2O m^{-2} s^{-1}).

The morphological analyses assessed the plant height (H) parameter using a millimeter ruler. Subsequently, the plants were washed in running water to collect the desired material followed by the quantification of fresh mass of aerial part (FMAP), root fresh mass (RFM), number of nodules (NNo), and fresh mass of nodules (FMNo) on analytical balance (0.001 g). Next, the plants were placed in properly identified paper bags in drying oven at 65 °C for 72 hours to assess dry mass of aerial part (DMAP), root dry mass (RDM), and dry mass of nodules (DMNo).

Statistical analysis

All data collected were subjected to analysis of variance and test of means (Tukey at 5% significance). Subsequently, the whole dataset was subjected to multivariate analysis using main components technique (PCA - Principal Component Analysis) (Hair et al., 2009). The eigenvectors were used to assess the relevance of each variable and treatment in the two first components, as well as the relation among the variables. These values were used as correlation coefficient (Gomes et al., 2004). All analyses and charts were developed on R software version 4.0 (Team, 2020) using packages MASS (Ripley et al., 2020), ExpDes.pt (Ferreira et al., 2018), FactoMineR (Husson et al., 2020), ggplot2 (Wickham et al., 2020), and SigmaPlot software version 14.0.

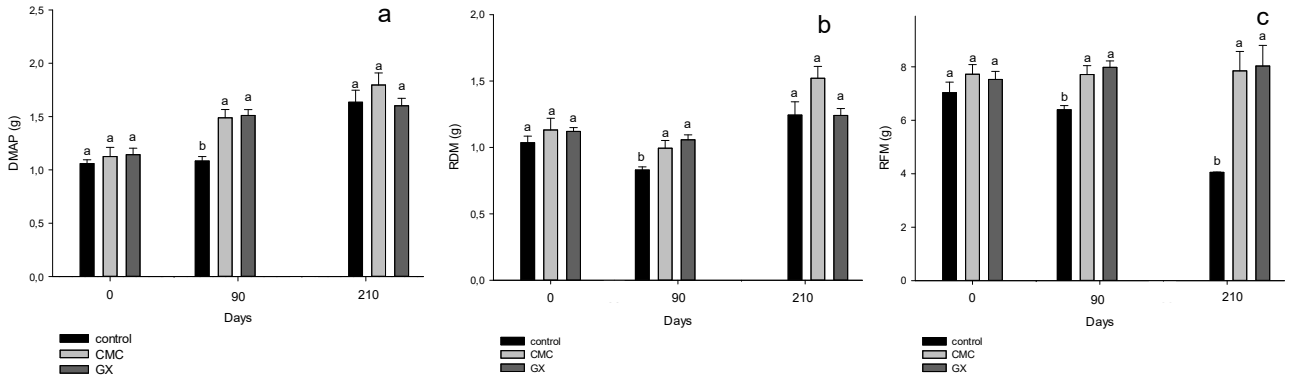
3. Results and Discussion

Bradyrhizobium elkanii

The inoculation of soybean seeds with *Bradyrhizobium elkanii* added with polymers proved efficient for the inoculant storage period (Figure 1) both for fresh mass and dry mass. Figures 1A and 1B, 90 days, show the efficiency of preservatives at preserving the viability of *Bradyrhizobium elkanii* cells, thus generating better nodulation due to a larger number of preserved cells, hence promoting better plant development presenting difference in dry mass of both aerial part (DMAP) and root (RDM), in addition to difference in root fresh mass (RFM) (Figure 1C) at 90 and 210 days, indicating that preserving the microorganism cells ensured its efficiency at forming higher plant biomass, especially in the root system.

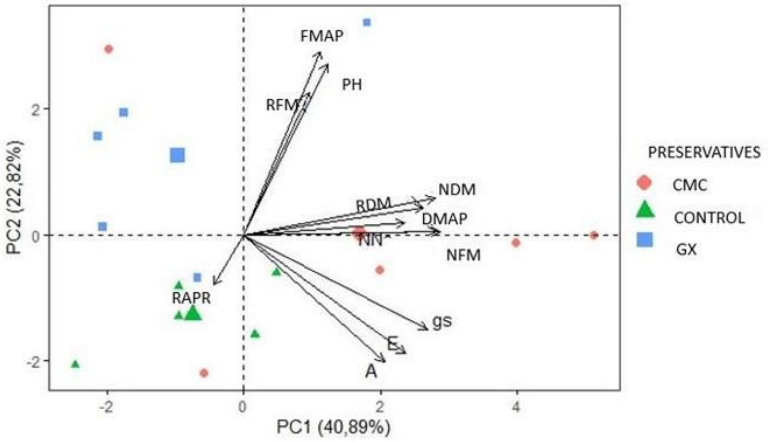
Figure 2 represents the main components analysis, which correlates all variables. According to Hair et al. (2009), the adequate number of components must be represented by a minimum percentage of 80%. However, for such percentage to be reached, more than two main components must be addressed, which would hamper the interpretation of results due to the use of more than two dimensions. Thereby, we used only PC1 and PC2, with 63.71% of total variation, which indicated the inter-relations of preservatives with the microorganism through the responses obtained from the physiological and morphological variables of the plant.

Figure 1. Dry mass of aerial part (DMAP) (A), root dry mass (RDM) (B), and root fresh mass (FDM) (C) of soybean plants (*Glycine max* (L.) Merr.)) inoculated with *Bradyrhizobium elkanii*. Means followed by the same lower case letter at each assessment time do not differ according to the Tukey test ($p < 0.05$).



Source: Authors.

Figure 2. Biplot of the first and second components generated from the response variables of soybean (*Glycine max* (L.) Merr.) inoculated with *Bradyrhizobium elkanii* at 210 storage days added or not with biopolymer. PC1: first component; PC2: second component; H: height of plants; RFM: root fresh mass; RDM: root dry mass; FMAP: fresh mass of aerial part; DMAP: dry mass of aerial part; NNo; number of nodules; FMNo: fresh mass of nodules; DMNo; dry mass of nodules; APRR: aerial part-root relation; E: transpiration rate; gs: stomatal conductance; A: CO_2 assimilation rate.



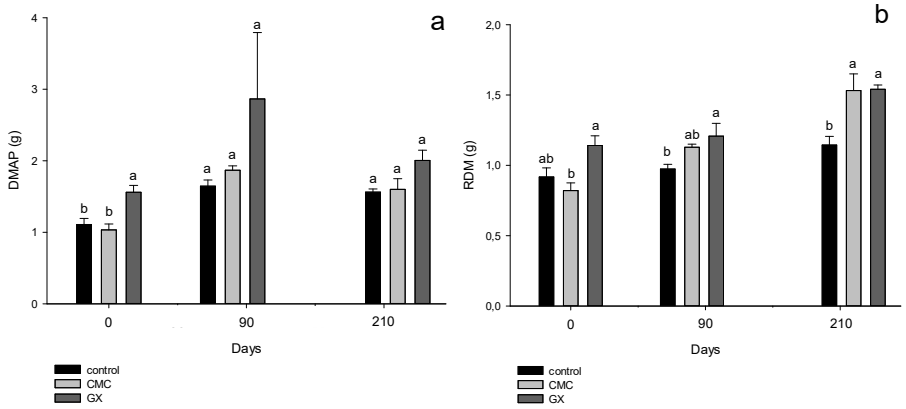
Source: Authors.

The variables of DMAP, RDM, NNo, FMNo, DMNo were more positively sensitive in the experiment, which are grouped closer to the carboxymethylcellulose treatment, confirming its efficiency at preserving cells after 210 days. Both preservatives had close Euclidian distance, with 2.76 for XG and 2.54 for CMC, opposite to the witness, thus justifying its performance at preserving the inoculant quality. Regarding the physiological analyses, all variables analyzed were close both to the witness and the carboxymethylcellulose treatment, indicating that the use of xanthan gum may have negatively affected the preservation of cell number, decreasing the microorganism efficiency at promoting nodulation. This scenario explains the results of morphology, which are directly related to lower physiological efficiency due to the lower nodulation provided by the cells preserved with xanthan gum.

Bradyrhizobium diazoefficiens

Soybean inoculation with *Bradyrhizobium diazoefficiens* demonstrated that the treatment with xanthan gum was greater than the others only at time 0; additionally, for the aerial part, adding biopolymers showed no difference for all 210 storage days (Figure 3A). As for the root, we found that the use of biopolymer was efficient in all assessment periods, where at time 0 the use of xanthan gum was more efficient than carboxymethylcellulose. However, at 90 days, these biopolymers reached similar results, which were better than the witness, while at 210 inoculant storage days, its use with biopolymers proved efficient at developing soybean roots due to higher nodulation in relation to the use of inoculant without any preservation method of bacterial cells (Figure 3B).

Figure 3. Dry mass of aerial part (DMAP) (A) and root dry mass (RDM) (B) of soybean plants (*Glycine max* (L.) Merr.) inoculated with *Bradyrhizobium diazoefficiens*. Means followed by the same lower case letter at each assessment time do not differ according to the Tukey test ($p < 0.05$).



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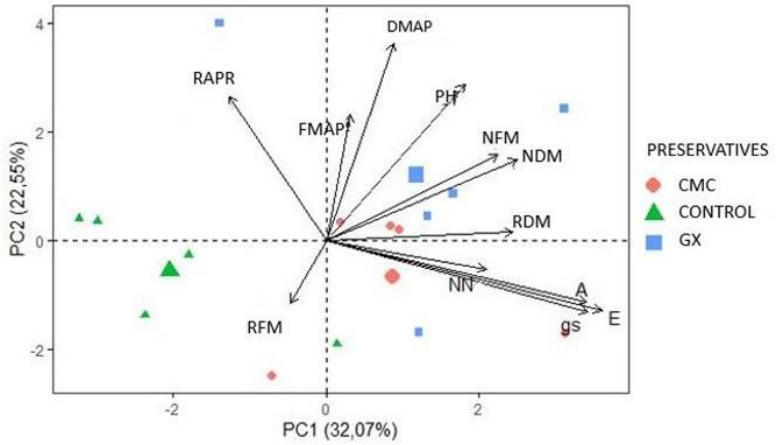
The main components analysis for this experiment also used PC1 and PC2, with 54.62% of total variation (Figure 4). We observed that the treatments added with biopolymers are opposite to the witness, thus justifying their influence on good results.

This analysis indicated that the plants had more active metabolism when inoculated with the microorganism preserved with carboxymethylcellulose due to its performance in the roots producing nodules, which generated sharper physiological response. However, regarding nodulation, although the CMC treatment produced more nodules, the treatment with xanthan gum generated larger nodules, since both fresh and dry masses were higher, thus promoting better results of plant development.

Euclidian distance values were 2.93 and 3.68 for CMC and XG, respectively, where the variables that most contributed were those represented in the first quadrant, while the negatively correlated variables were those arranged in opposite directions to the origin of the figure, showing that both treatments were significant in relation to the witness, which is represented in the opposite quadrants (negative).

The *Bradyrhzobium* species form symbiotic relations with legumes by performing nitrogen fixation in the plant root through the formation of nodules. According to Polenko et al. (1987) and Son et al. (2006), co-inoculation may increase both the number and mass of nodulation, enhancing the availability of nutrients to soybean. Thereby, the use of biopolymers proves relevant for preserving inoculants due to their efficiency at increasing both number and mass of nodules without requiring co-inoculation.

Figure 41. Biplot of the first and second components generated from soybean response variables (*Glycine max* (L.) Merr.) inoculated with *Bradyrhizobium dayzoefficies* aos 210 storage days added or not with biopolymer. PC1: first component; PC2: second component; H: height of plants; RFM: root fresh mass; RDM: root dry mass; FMAP: fresh mass of aerial part; DMAP: dry mass of aerial part; NNo; number of nodules; FMNo: fresh mass of nodules; DMNo; dry mass of nodules; APRR: aerial part-root relation; *E*: transpiration rate; *gs*: stomatal conductance; *A*: CO_2 assimilation rate.

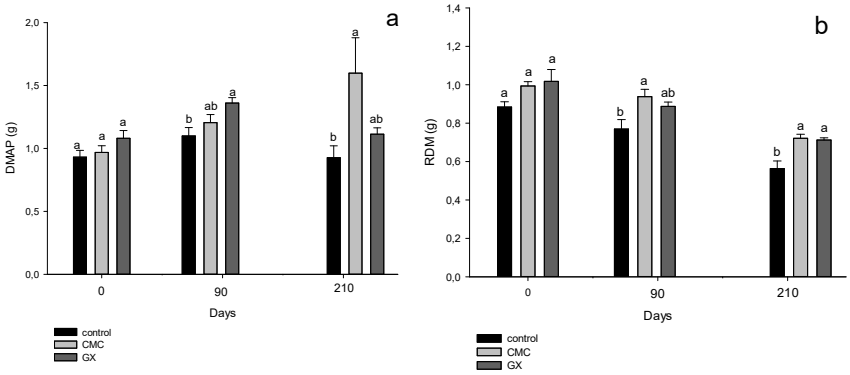


Source: Authors.

***Azospirillum* sp.**

The inoculation of soybean seeds with *Azospirillum* sp. showed that the use of biopolymers was efficient at both the preservation and viability of cells by promoting plant development due to beneficial effects of seed inoculation, leading the plant to better develop its aerial part and root (Figure 5).

Figure 5. Dry mass of aerial part (DMAP) (A) and root dry mass (RDM) (B) of soybean plants (*Glycine max* (L.) Merr.) inoculated with *Azospirillum* sp. Means followed by the same lower case letter at each assessment time do not differ according to the Tukey test ($p < 0.05$).



Source: Authors.

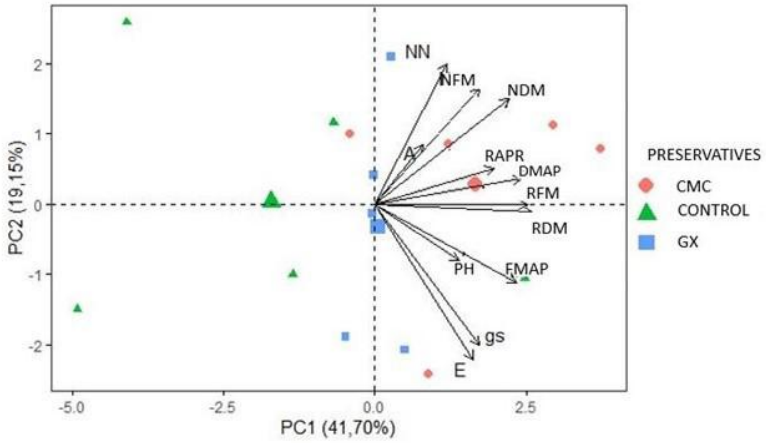
Dry mass of aerial part (Figure 5A) showed statistical difference at 90 days, when the use of inoculant with xanthan gum was able to preserve a larger number of cells that were more efficient at forming nodules, hence developing the aerial part. However, at 210 days, cell number reached a sharp decrease since the use of carboxymethylcellulose was able to preserve the viable cells for plant nodulation, which generated better results of dry mass in relation to the witness. As for the root (Figure 5B), the results were also better with the use of inoculant preserved with biopolymer, which was greater with the

presence of capoxymethyl cellulose at 90 days, and with two biopolymers at preserving the viability of *Azospirillum* sp. cells in relation to the witness at 210 days, since the plants responded positively.

Azospirillum braziliense is one of the most studied plant growths promoting bacteria due to its ability to increase productivity, especially for enhancing plant root by absorbing water and minerals (Fibach-Paldi et al. 2012).

The main components analysis for the use of *Azospirillum* sp. also used PC1 and PC2, with 60.85% of total variation (Figure 6). We found that the treatments with biopolymers were also significantly more positive than the witness, thus corroborating the need of biopolymers to provide the microorganism with potential to act on plant development through its performance in the rhizosphere.

Figure 6. Biplot of the first and second components generated from soybean response variables (*Glycine max* (L.) Merr.) inoculated with *Azospirillum* sp. aos 210 storage days added or not with biopolymer. PC1: first component; PC2: second component; H: height of plants; RDM: root fresh mass; RFM: root dry mass; FMAP: fresh mass of aerial part; DMAP: dry mass of aerial part; NNo; number of nodules; FMNo: fresh mass of nodules; DMNo; dry mass of nodules; APRR: aerial part-root relation; *E*: transpiration rate; *gs*: stomatal conductance; *A*: CO_2 assimilation rate.



Source: Authors.

The main components analysis on the performance of biopolymers at ensuring the *Azospirillum* sp. viability indicated significant influence of their use as preservatives, since the witness was completely opposite to all variables analyzed. The use of biopolymers positively influenced the microorganism with a 210-day preservation period, thus ensuring a larger number of cells and better efficiency at promoting plant development based on the combination of *Azospirillum* sp. and *Bradyrhizobium* and providing better nodulation. The Euclidian distance from the control was longer in the CMC treatment, 3.39, and 1.8 for the XG. DMAP RFM, RDM, APRR, and DMNo are the vectors grouped with closer proximity to the CMC, which are the variables of greater representativeness for identifying differences among plants, thus ensuring a greater efficiency of this preservative.

Bulegon et al. (2016) found that *Azospirillum braziliense* stimulates the nodulation of soybean culture and enhances the efficiency of using carbon assimilated by the plant, which is in line with our results, where biopolymers were able to preserve a large number of *Azospirillum* sp. cells at 210 storage days, thus providing better nodulation and efficiency at producing plant biodry mass through carbon, which was better assimilated by plant upon a larger number of bacteria present in its rhizosphere.

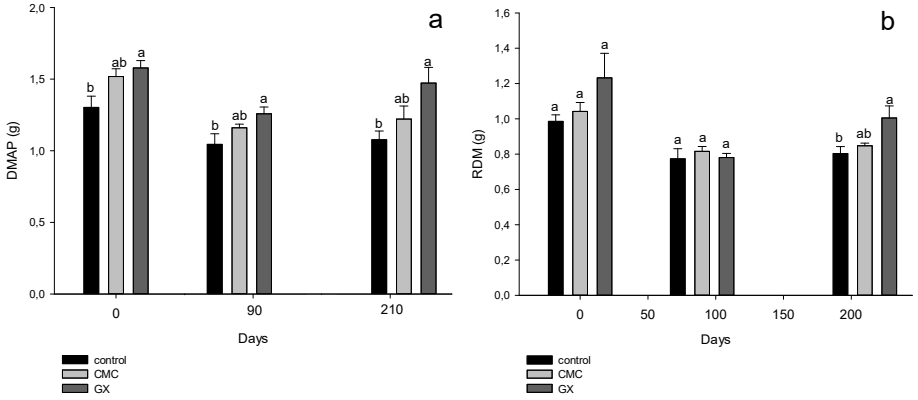
Gopal and Baby (2016) tested the addition of chemical products in the liquid formulation of *Azospirillum* and observed longer shelf periods, but also a decrease in the volume of UFC mL^{-1} in all treatments. Trehalose reached $6x10^8$ UFC mL^{-1} in 7 storage months, probably due to its water retention capacity that provides resistance against drying.

In addition to the use of preservatives to ensure microorganism viability, low oxygenation rate during storage is also associated with useful life of *Azospirillum* sp., since, according to Carrasco-Espinosa et al. (2015), aerobial fermentations are less efficient for this microorganism.

Pseudomonas fluorescens

The use of inoculant based on *Pseudomonas fluorescens* in soybean also showed good results when preserved with biopolymers during 210 shelf days (Figure 7). Figure 7A demonstrates that xanthan gum proved efficient at preserving *Pseudomonas fluorecens* cells in all assessment periods, which resulted in more efficient symbiosis between microorganism and plant, thus ensuring better results for the aerial part.

Figure 7. Dry mass of aerial part (DMAP) (A) and root dry mass (RDM) (B) of soybean plants (*Glycine mmax* (L.) Merr.) inoculated with *Pseudomonas fluorescens.* Means followed by the same lower-case letter at each assessment time do not differ according to the Tukey test ($p < 0.05$).

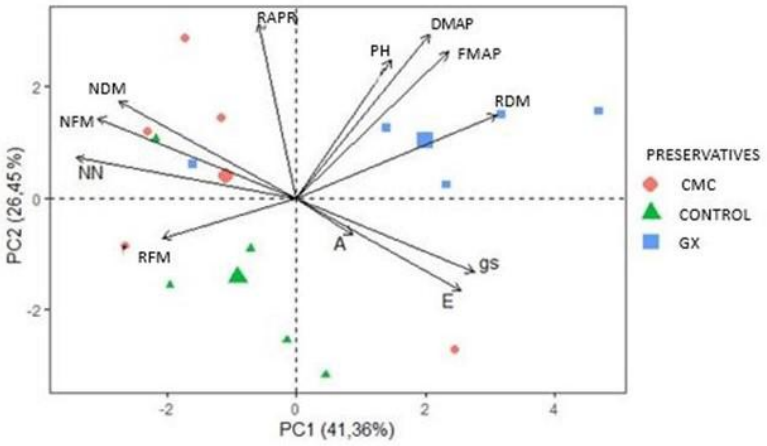


Source: Authors.

Figure 7B shows that only at 210 days there was a difference in the interaction of the microorganism added with xanthan gum in relation to the witness; in addition, the presence of the biopolymer in *Pseudomonas fluorescens* cells generated a better response of the plant due to the larger number of viable cells of the microorganism present in the inoculant.

The main components analysis for the use of *Pseudomonas fluorescens* also used PC1 and PC2, with 67.81% of total variation (Figure 8), showing greater influence of XG biopolymer in relation to biodry mass production due to a more efficient nodulation, since the vectors corresponding to dry mass of aerial part and root dry mass had a lower angle in relation to the XG treatment, thus indicating greater affinity of the cells with this biopolymer, hence providing better performance in the system root.

Figure 8. Biplot of the first and second components generated from soybean response variables (*Glycine max* (L.) Merr.) inoculated with *Pseudomonas fluorescens* aos 210 storage days added or not with biopolymer. PC1: first component; PC2: second component; H: height of plants; RFM: root fresh mass; RDM: root dry mass; FMAP: fresh mass of aerial part; DMAP: dry mass of aerial part; NNo; number of nodules; FMNo: fresh mass of nodules; DMNo; dry mass of nodules; APRR: aerial part-root relation; *E*: transpiration rate; *gs*: stomatal conductance; *A*: CO_2 assimilation rate.



Source: Authors.

The variables analyzed responded better to the use of bacterial cells preserved with biopolymer by providing better nodulation; however, it is clear that xanthan gum was more efficient due to its better results of dry mass both for aerial part and root. The means values of number of nodules for the CMC treatment were higher; however, the ratio of dry mass per number of nodules of XG was higher, resulting in the position of the vectors of physiological responses. This better corresponds to the XG treatment since the microorganism preserved by this biopolymer provided the plant with more nutrients by performing more gas exchanges and carbon absorption for its metabolism, hence accumulating more biomass.

Another factor is the longer Euclidian distance for XG (3.82) than for CMC (1.83); in addition, DMAP, RDM, and H vectors correspond to the use of XG, thus confirming its efficiency at preserving the cells of the viable microorganism for plant growth promotion. Praveen Biradar and Santhosh (2018) used xanthan gum as adjuvant in an inoculant based on *Pseudomonas fluorescens* to assess its useful life and reached higher values of UFC mL^{-1} than by using carboxymethylcellulose. Such data are similar to our findings, where XG indirectly influenced more the plant morphological response, probably due to the larger number of cells present in the inoculant used after 210 storage days.

Rhizocompetence is the ability of a microorganism derived from an inoculant to resist adversities in the soil, such as competition for nutrients, space, and metabolites secreted by other microorganisms. Therefore, it is important to formulate a product with a culture medium that provides all necessary nutrients and protects the microorganism from such adversities, thus ensuring longer shelf period with an increase in UFC mL^{-1} and cell viability (Sahu and Brahmaprakesh, 2016).

According to Bhattacharyya and Jha (2012), rhizobacteria have been constantly used to substitute agrochemical products, and several studies have demonstrated that the substances produced by microorganisms in the plant root act both directly and indirectly on the plant metabolism. For Widawati1 and Suliasih (2018), using carboxymethylcellulose in inoculant based on *Azospirillum* may maximize the germination index of sorghum seeds, as well as increase root growth and sprouts *in vitro*, which was also observed in our research, where the use of CMC provided better efficiency for the microorganism to act on the soybean plant root (Figure 5B, Figure 6).

According to Nimnoi et al. (2014), in addition to favoring lower production costs, the use of PGPB also improves nodulation by rhizobial bacteria, since the experiment with *Pseudomonas fluorescens* showed that using microorganism added with biopolymers positively influenced plant nodulation, hence producing higher plant biodry mass.

França et al. (2013) also tested the use of CMC for preserving cells of *Rhizobium tropici* and *Bradyrhizobium japonicum*, which were able to survive for 180 days added with biopolymer, indicating the viability of using CMC. Mohamed et al. (2019) used some polymeric additives in rhizobial inoculant preservation and found a large number of cells over two storage months; however, they reported that cell survival depended both on the type of additive and strain used, thus associating with the results of this study, where CMC was more efficient for *Bradyrhizobium elkanii,* while XG was more efficient for *Bradyrhizobium diazoefficiens*.

According to Pioneer (2004), an adequate inoculation must present between 10 and 30 nodules per plant, and the values obtained in this study were equal or superior to the recommended for treatments with preservatives. Such outcomes are also similar to the findings by Braccini et al. (2016), who obtained 18.48 nodules per plant by using *Bradyrhizobium japonicum* with liquid inoculation in seeds, and 16.48 nodules per plant using *Bradyrhizobium japonicum* and *Azospirillum braziliense* also inoculated in seeds.

Photosynthesis efficiency is extremely relevant to plants; according to Silva et al. (2019), photosynthesis rate is the relation of the number of fixed CO_2 molecules per unit of leaf area per time unit, which is associated with the plant ability to carry out gas exchanges. In this study, it was determinant for plant biomass production, where better nodulation results generated improved outcomes in plant morphology.

A means of relating the influence of microorganisms on plant metabolism is through morphological responses, which can be associated with physiological parameters such as transpiration rate (*E*), stomatal conductance (*gs*), and CO_2 assimilation rate (*A*) (Taiz et al., 2017). Thereby, we found that the main components analyses (Figures 2, 4, 6, and 8), like the use of biopolymers, significantly responded to microorganisms' viability since the angle formed in the figures represented by the vectors of physiological features significantly corresponded to the treatments with xanthan gum and carboxymethylcellulose. In addition, these variables also appeared at most times angularly opposite to the witness.

Stomatal conductance (*gs*) and carbon assimilation (*A*) depend on internal and external carbon concentrations in the leaves. According to Kaschuk et al. (2010), approximately 14% of the carbon assimilated by the plant is transferred to diazotrophic rhizobacteria to perform exchange of nitrogen compounds. This can be observed in the treatments using biopolymer, thus corroborating that both CMC and XG were able to store more cells of the microorganisms and maintain them viable, which is associated with a better efficiency of the bacteria in the plant root, producing better nodulation and influencing metabolism and gas exchanges.

Our study demonstrates significant difference in the inoculation of soybean seeds with inoculants preserved with biopolymers by influencing on morphological and physiological features of soybean plants, thus confirming the preservation potential of cell number and viability even after 210 storage days.

4. Conclusion

Using carboxymethylcellulose and xanthan gum for preserving cells of *Bradyrhizobium elkanii*, *Bradyrhizobium diazoefficiens*, *Azospirillum* sp., and *Pseudomonas fluorescens* proved efficient at preserving the viability of microorganisms, hence better nodulation, and efficiency of growth plant promotion of aerial part and root of soybean plants even after 210 storage days.

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