

## Physiological quality of cowpea seeds after application of chitosan-based bioproduct

### *Qualidade fisiológica de sementes do feijão-caupi após aplicação de bioproduto à base de quitosana*

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**ABSTRACT:** The use of biological methods in the agricultural field has instigated studies in the development of products based on biopolymers. Chitosan is a biopolymer and important alternative to the agrochemicals used in large cultures, presenting favorable characteristics to the development of the plants, to the environmental issues, and to the agricultural sector economy. Therefore, the present study aimed to evaluate the effect of the application of a chitosan-based bioproduct in the germination and vigor of cowpea seeds. A completely randomized design was adopted with four replications of 50 seeds, with the exception of the mass and length tests, carried out in five replications of 20 seeds. The chitosan-based product was tested in the concentrations of 0.0 (witness); 0.5; 1; 2.5; 5; and 10% and physiological quality parameters, emergency, vigor, leaf pigments contents and electrolyte extravasation were evaluated. The results indicated that the treatments coated with the bioproduct significantly increased the length of the seedling, shoots and radicle, shoot and seedling fresh masses, and seedlings dry mass emerged in a bed. We concluded that the application of the bioproduct, at FTSeed concentrations at 3 and 3.25%, as a coating in cowpea seeds induced the seedlings growth, acting as a biostimulant. The principal component analysis (PCA) showed a correlation between biochemical tests and germination potential in the field, with emphasis on the 5% treatment.

**Keywords:** Biopolymer; Biostimulant; Pre-treatment; String bean; *Vigna unguiculata*.

**RESUMO:** O uso de métodos biológicos no campo agrícola tem instigado estudos no desenvolvimento de produtos à base de biopolímeros. A quitosana é um biopolímero e importante alternativa aos agroquímicos utilizados nas grandes culturas, apresentando características favoráveis ao desenvolvimento das plantas, às questões ambientais, e à economia do setor agrícola. Diante disso, o presente estudo objetivou avaliar o efeito da aplicação de um bioproduto à base de quitosana na germinação e vigor de sementes de feijão-de-corda. Foi adotado um delineamento experimental inteiramente casualizado com quatro repetições de 50 sementes, com exceção dos testes de massa e comprimento, realizados em cinco repetições de 20 sementes. Testou-se o produto à base de quitosana nas concentrações de 0,0 (testemunha); 0,5; 1; 2,5; 5; e 10% e foram avaliados parâmetros de qualidade fisiológica, emergência, vigor, teores de pigmentos foliares e extravasamento de eletrólitos. Os resultados indicaram que os tratamentos revestidos com o bioproduto aumentaram significativamente o comprimento da plântula, parte aérea e radícula, massas frescas da parte aérea e da plântula, e a massa seca da plântula emergida em canteiro. Concluiu-se que a aplicação do bioproduto, nas concentrações de FTSeed a 3 e 3,25%, como revestimento em sementes de feijão-de-corda induziu o crescimento das plântulas, atuando como um bioestimulante. A análise de componentes principais (ACP) apontou correlação entre os testes bioquímicos e potencial germinativo em campo, com destaque para o tratamento a 5%.

**Palavras-chave:** Biopolímero; Bioestimulante; Feijão-caupi; Pré-tratamento; *Vigna unguiculata*.

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## INTRODUCTION

*Vigna unguiculata* (L.) Walp, is a leguminous belonging to the Fabaceae family, of African origin and countries to the west of Africa (Freire-Filho, 1988). It arrived in Brazil by the middle of the 16<sup>th</sup> century, where it obtained success in great cultivation, especially in the Brazilian Northeast due to its ability to tolerate drought and adapt to edaphoclimatic conditions, besides its relevant role in the food and nutritional security of the Brazilian population (Freire-Filho, 1988; Boukar, 2019; Costa; Souza; Silva, 2020).

The constant increase in grains production combined with the socioeconomic importance of bean cultivation in the country requires farmers to have a better productive potential and crop yield (Quadros; Rossi; Caraviani, 2013). It is fundamental the investment in technologies that target the quality and viability of the seeds, as well as, better plant development and production (Quadros; Rossi; Caraviani, 2013; Cassol; Fantinel; Silva, 2020).

The use of coatings in seeds has as objective to improve the physical, physiological and phytosanitary attributes (Malerba; Cerana, 2018). When associated with biopolymers allow the joint use with fungicides, bioactive and substances that favor the physiological quality, as well as, avoid the loss of the active products due to the friction with planters and reduce the contact of the operators with them (Benatto-Junior *et al.*, 2012; Malerba; Cerana, 2018).

Among the biopolymer, the chitosan stands out, a low-cost poly(1,4)-2-amino-2-deoxy- -D glucose, safe, renewable, biocompatible and biodegradable obtained from the deacetylation of chitin, the second most abundant organic polymer in the world, it is found in the crustaceans exoskeleton, insects and in the cell wall of fungi (Kurita, 2006; Berger, Stamford; Stamford, 2011; Malerba; Cerana, 2018).

Recent studies have pointed out chitosan as an alternative to toxic and chemical products used in the agriculture (Zerpa *et al.*, 2017; Lima; Bonilla; Lucena, 2022; Lima *et al.*, 2022; Lima *et al.*, 2023). In the case of seeds, its application can be as seed coating or imbibition, as biostimulant, in the growth and plant production, in the reduction of phytopathogens growth, induction of defense mechanisms, incorporating higher crop yield, length and mass variables (Mesa; Pedroso; Arrebato, 2015; Crini *et al.*, 2019).

Knowing this, the present study aimed to evaluate the application effect of a chitosan-based bioproduct in the germination and vigor of cowpea seeds.

## 2 MATERIAL AND METHODS

The study was conducted at the Plant Ecophysiology Laboratory (ECOFISIO) and its field experimental area, both located at the State University of Ceará (UECE), Campus Itaperi, Fortaleza, Ceará, Brazil. The field experiment site is characterized by a Subhumid Hot Tropical climate; average temperature of 26 to 28°C; annual rainfall of 1,378 mm concentrated from January to May; Metropolitan watershed; relief of Coastal Tablelands; Neosols soil; vegetation of the Vegetation Complex of the Coastal Zone (IPECEDATA, 2024). The cowpea Mighty BRS cultivar was used (*Vigna unguiculata* (L.) Walp), submitted to the application of FTSeed, product provided and developed by the Fertsan Company, whose main components are soluble salts of chitosan polymeric derivatives, polysaccharides mix, urea, saccharides, organic acid mix, preservative and water.

Before starting the sows, the seeds were disinfected with 5% sodium hypochlorite commercial solution (NaClO) (2.5% a.i.) for five minutes. Then they were dried in towel paper and subsequently submitted to the treatments by imbibition in 30 minutes in the FTSeed solution, with the recommendation

of 10ml/Kg, according to the treatments: T1 (witness – water immersion), T2 (FTSeed at 0.5%), T3 (FTSeed at 1%), T4 (FTSeed at 2.5%), T5 (FTSeed at 5%) and T6 (FTSeed at 10%).

The following evaluations were performed in the laboratory in the treatments mentioned above:

## 2.1 GERMINATION AND FIRST COUNT

Four repetitions of 50 seeds were distributed in two germitest paper sheets, covered by a third, and moistened with 2.5 times the weight of the paper, and made rollers that were maintained in biochemical oxygen demand germinator (BOD) in constant temperature of 25°C and 12 hour photoperiod. The first count was performed on the fifth day after sowing (DAS) and the final count at the eighth DAS (BRASIL, 2009), and the results were expressed in average percentages of normal seedlings by repetition.

## 2.2 BIOCHEMICAL TESTS

Performed in conjunction with the above tests, after the final germination count, a and b chlorophyll and carotenoid levels were determined using 0.1 g of fresh primary leaves, macerated with 0.08 g of calcium carbonate and 2.8 mL of acetone at 80%, after filtered directly in a volumetric balloon of 10 mL the volume was completed with acetone at 80%. With this extract were performed the absorbance readings in the spectrophotometer at 648,6 and 663.2 nm, to estimate the “a” (Ca) and “b” (Cb) chlorophyll content, and for the carotenoids content were performed the readings in 646.8, 663.2 and 470 nm, according to Lichtenthaler (1987). “A” and “b” chlorophyll and carotenoids values were calculated through the following equations (1, 2 and 3) established by Lichtenthaler (1987):

$$a \text{ chlorophyll: } 12.25 \times A_{663.2} - 2.79 \times A_{646.8}$$

$$b \text{ chlorophyll: } 21.50 \times A_{646.8} - 5.10 \times A_{663.2}$$

$$\text{Carotenoids: } \{1000 \times A_{470} - (1.82 \times Ca - 85.02 \times Cb)\} / 198$$

## 2.3 ELECTROLYTES EXTRAVASATION

Conducted in conjunction with the above tests, after the final germination count, 0.1 g of fresh leaves placed in test tubes containing 10 mL of distilled water were used. Then, the tubes were closed and kept to rest for 24h in BOD at 25°C. After this period, the initial conductivity (C1) was read using a benchtop conductivity meter, soon after they were placed in water bath at 80°C for 60 minutes, and when cooled the final conductivity was measured (C2). The relative permeability was calculated by the equation  $[C1 / (C1 + C2)] \times 100$ , according to Tarhanen *et al.* (1999).

## 2.4 LENGTH AND MASS

Five repetitions of 20 seeds were used by treatment, distributed in two rows of 10 seeds intercalated under two germitest paper sheets and covered with a third sheet. All the sheets were previously moistened with water in the quantity 2.5 times the weight of the dry paper. Rolls were created with the germitest paper sheets and placed in polyethylene bags and were maintained in biochemical oxygen demand germinator (BOD) in constant temperature of 25°C and 12 hours photoperiod. To determine the length of normal seedlings, it was measured at the eighth DAS the shoot length (SL) in the border region between the

hypocotyl and the radicle, the radicle length (RL) and its sum, obtaining the value of the seedling length. (SeL) (Krzyzanowski *et al.*, 2020).

Together with this test, the measurement of the shoots (SFM), the radicles (RFM) and the seedlings (SeFM) fresh mass was performed, obtaining the average weight of fresh matter by seedlings, expressed in g/seedlings of each repetition (Krzyzanowski *et al.*, 2020).

## 2.5 FIELD TEST

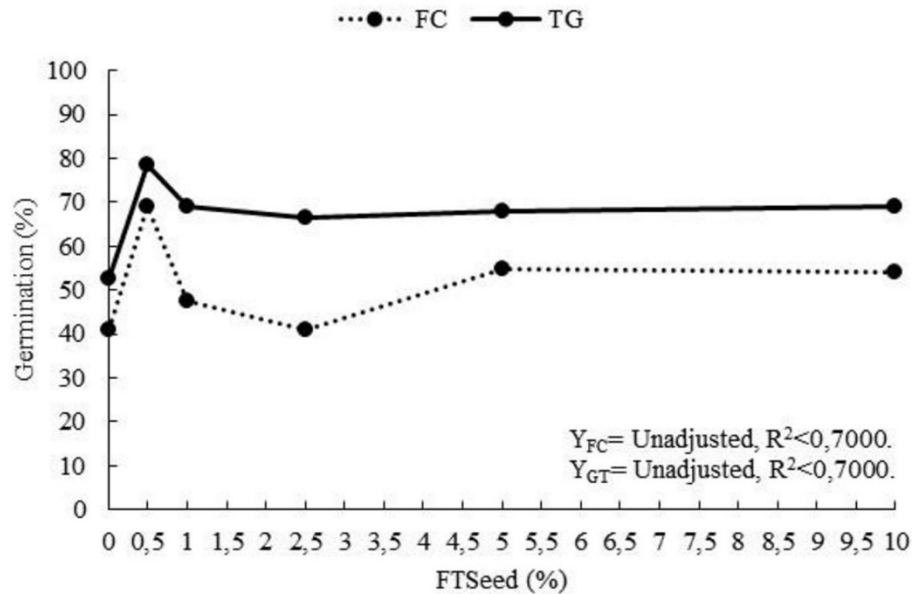
Performed in beds, the seeds were distributed in four repetitions (rows) of 50 seeds for each treatment, and the rows spaced in 10 cm and with the aid of a stick were buried at the depth of 3 cm. The seedlings were treated with application of Matathion 50CE, due to the presence of ants in the beds. The irrigation was performed daily with equivalent of 10 mm of water per bed, with the exception of rainy days. During the eight days of experiment, the seedlings that emerged were counted to evaluate the emergence speed index (ESI), first emergence count (FEC) (five DAS) and emergence in the bed (EB) (eighth DAS). In the eighth DAS, the plants without leaf damage were sectioned close to the soil and the measurements of the length, fresh and dry mass of the shoot were performed (Krzyzanowski *et al.*, 2020).

## 2.6 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experiment was performed in a completely randomized design with six treatments: for germination test, first count, biochemistry, emergence speed index, first emergence count and emergence in the bed were performed four repetitions of 50 seeds; for length and mass measurements were performed five repetitions of 20 seeds. The results were submitted to the analysis of variance observing the significance by F test, which when significant and with determination coefficients higher than 0.70, adjustments were performed by means of polynomial regressions of up to 3<sup>o</sup> degree. The ESTAT software (System for statistical analysis) was used for these calculations (UNESP, 1994). The Paleontological Statistics (PAST) software (Hammer; Harper; Ryan, 2001) was used for principal component analysis (PCA), in order to determine the components that explain the variance of the data in which no fit was obtained in the equations.

## 3 RESULTS AND DISCUSSION

In the evaluation of the first count (FC) and germination test (GT) there was significant statistical variation among the evaluated treatments (Figure 1), although the determination coefficient was lower than 0.70 ( $R^2=0.0492$  and  $R^2=0.2058$ , respectively). In both tests there was a growth trend in T2 (FTSeed at 0.5%), reaching 69% and 78.5% of germination, respectively.



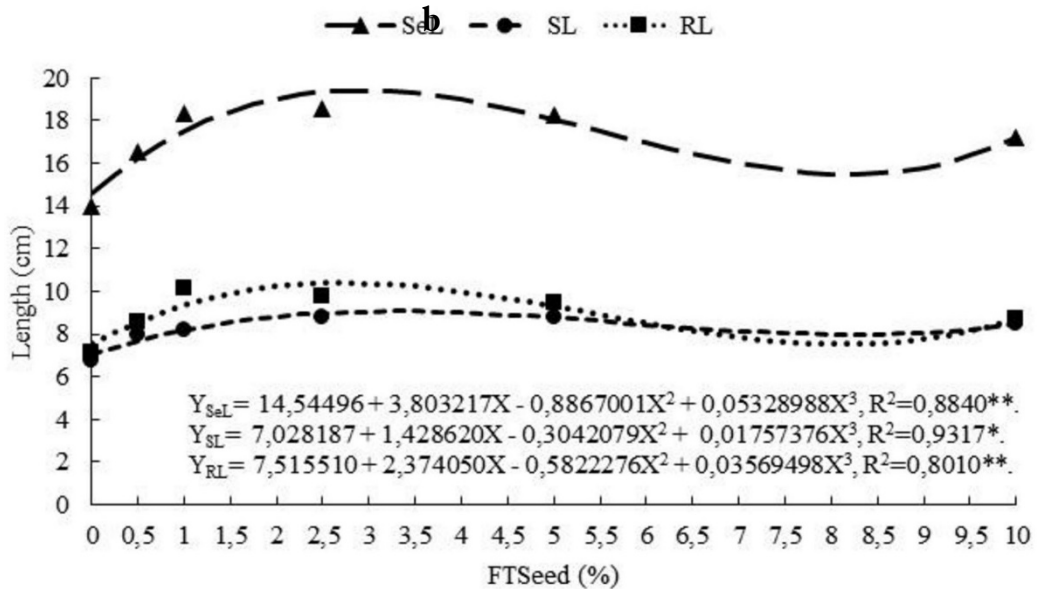
**Figure 1.** Germination test (GT) and first count (FC) of the cowpea. T1 – Witness (FTSeed at 0%). T2 – FTSeed at 0.5%. T3 – FTSeed at 1%. T4- FTSeed at 2.5%. T5 – FTSeed at 5%. T6 – FTSeed at 10%. \*, \*\*Significant, respectively, at the level of 5 and 1% of probability, by the F test. ns Non-significant

The seed germination rate can be influenced positive or negatively due to the treatments based on chitosan, and its concentrations. Basil seeds, cowpea and cucumbers treated with different concentrations of chitin and chitosan, performed higher germination rates in the treatment with lower concentration of the product (0.1%), as well as, the result of the present study (Kanawi; Haydar; Radhi, 2021).

The different types of application of chitosan-based products can also add better seed germination rates, such as the use of silver chitosan nanoparticles (Ag-CS Nps) in the germination of chickpea seeds (Anusuya; Banu, 2016). Also in the pre-treatment for 30 minutes with the chitosan extracted with 0.5% HCl in cowpea seeds, which besides providing higher germination rate still conferred antifungal action (Burrows *et al.*, 2007).

Although the coating with FTSeed showed a coefficient of determination lower than 0.70 in germination rates, it did not negatively affect the first count and germination rate of the cowpea, discarding its toxicity on the seed, similar to what was observed in studies with chitosan coating in *Phaseolus vulgaris* ‘negro Jamapa’ and ‘Pinto Americano’, and *Zea mays* (Ruiz-de-la-Cruz *et al.*, 2017; Godínez-Garrido *et al.*, 2021).

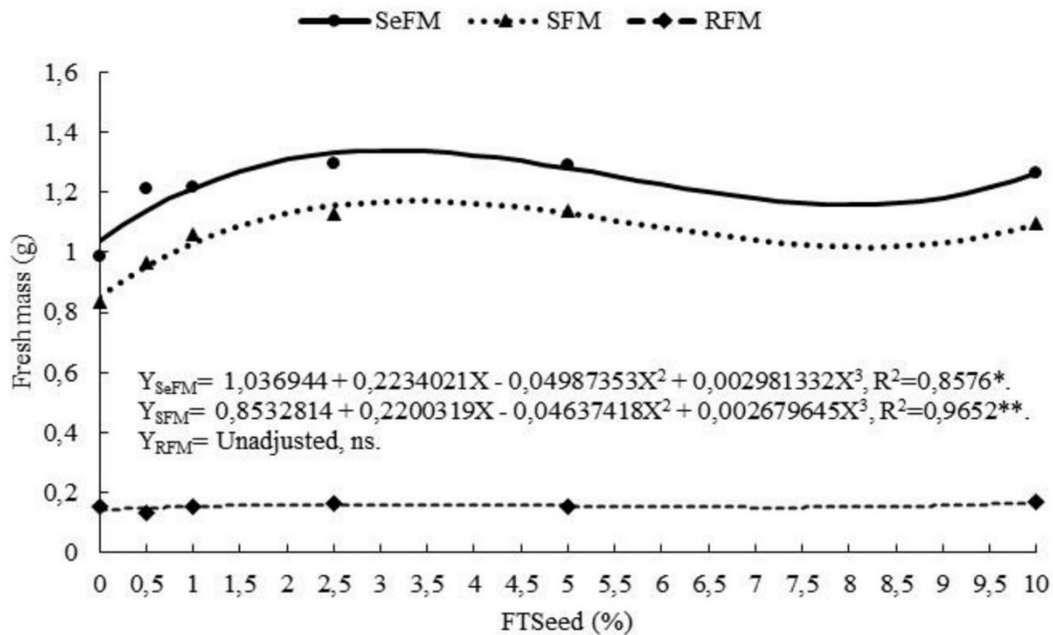
When analyzing Figure 2, we observe a cubic behavior of the seedlings (SeL), the shoot (SL) and the root (RL) lengths. With the increase of FTSeed concentrations there was an increase of the lengths reaching the estimated maximum point in the concentrations of 3 (19.4131 cm), 3.25 (9.0612 cm) and 2.75% (10.3834 cm) of the bioproduct, respectively, followed by a reduction of all lengths.



**Figure 2.** Radicle (RL), shoot (SL) and seedlings (SeL) length of cowpea. T1 – Witness (FTSeed at 0%). T2 – FTSeed at 0.5%. T3 – FTSeed at 1%. T4- FTSeed at 2.5%. T5 – FTSeed at 5%. T6 – FTSeed at 10%. \*, \*\*Significant, respectively, at the level of 5 and 1% of probability, by the F test. ns Non-significant

The application of the product on the seeds enabled a better development of the structures when compared to the witness, corroborating with Zerpa *et al.* (2017). They report that the application of chitosan is able to promote a better root development and strengthen the vigor of the plants by the SAR mechanism (Systemic Acquired Resistance), in which from the contact between the polymer and the plant, this triggers a defense mechanism, increasing the development of its roots aiming at higher absorption of nutrients and strengthening.

For the results of Figure 3, the seedlings (SeFM) and of the shoot (SFM) fresh mass had cubic increase, which according to the increase of the FTSeed concentrations reached estimated maximum values of 3 (1.3388 g) and 3.25% (1.1705 g), respectively. The radicle fresh mass (RFM) was not statistically significant (Figure 3).

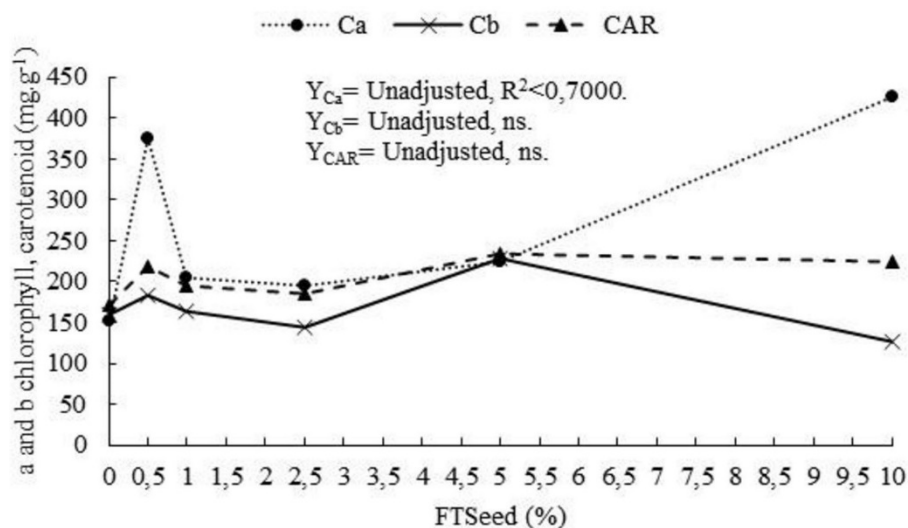


**Figure 3.** The radicle (RFM), the shoot (SFM) and the seedlings (SeFM) fresh mass of cowpea. T1 – Witness (FTSeed at 0%). T2 – FTSeed at 0.5%. T3 – FTSeed at 1%. T4- FTSeed at 2.5%. T5 – FTSeed at 5%. T6 – FTSeed at 10%. \*, \*\*Significant, respectively, at the level of 5 and 1% of probability, by the F test. ns Non-significant

Although chitosan increases the root development in several species, such as soybean and rice (Menéndez; Rodríguez; Hernández, 2020; Zerpa *et al.*, 2017), no difference was observed in the treatments roots mass despite the existence of differences in length among the radicles formed by the treatments with the bioproduct in the present study.

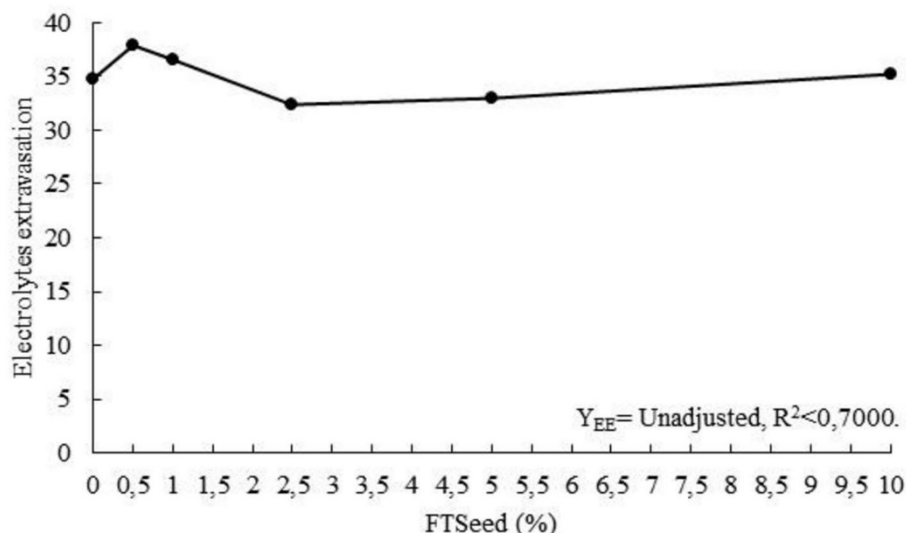
The results are similar to Anusuya and Banu (2016) study, who report higher values of the seedlings length and seedlings fresh mass in chickpea seeds after application 0.1%, p/v of Ag-CS Nps, compared to the control. The possible explanation for the increase of these length rates can be due to the increase of the absorption of inorganic nutrients, favoring the break of organic substances and consequently the photosynthetic rate (Anusuya; Banu, 2016).

There was no significant difference for the analyzed variables of b chlorophyll (Cb) and carotenoid (CAR). For a chlorophyll (Ca) there was statistically significant variation among the treatments, although the determination coefficient was lower than 0.70 ( $R^2 = 0.5361$ ) (Figure 4), we observed oscillations according to the variation of the FTSeed concentrations, presenting higher Ca content under the concentration of 10% of FTSeed ( $424.956 \text{ mg.g}^{-1}$ ). It can be inferred that the application of chitosan-based products, such as FTSeed, enable the increase of the photosynthetic capacity of the seedlings acting in its growth (Zeng; Luo, 2012).



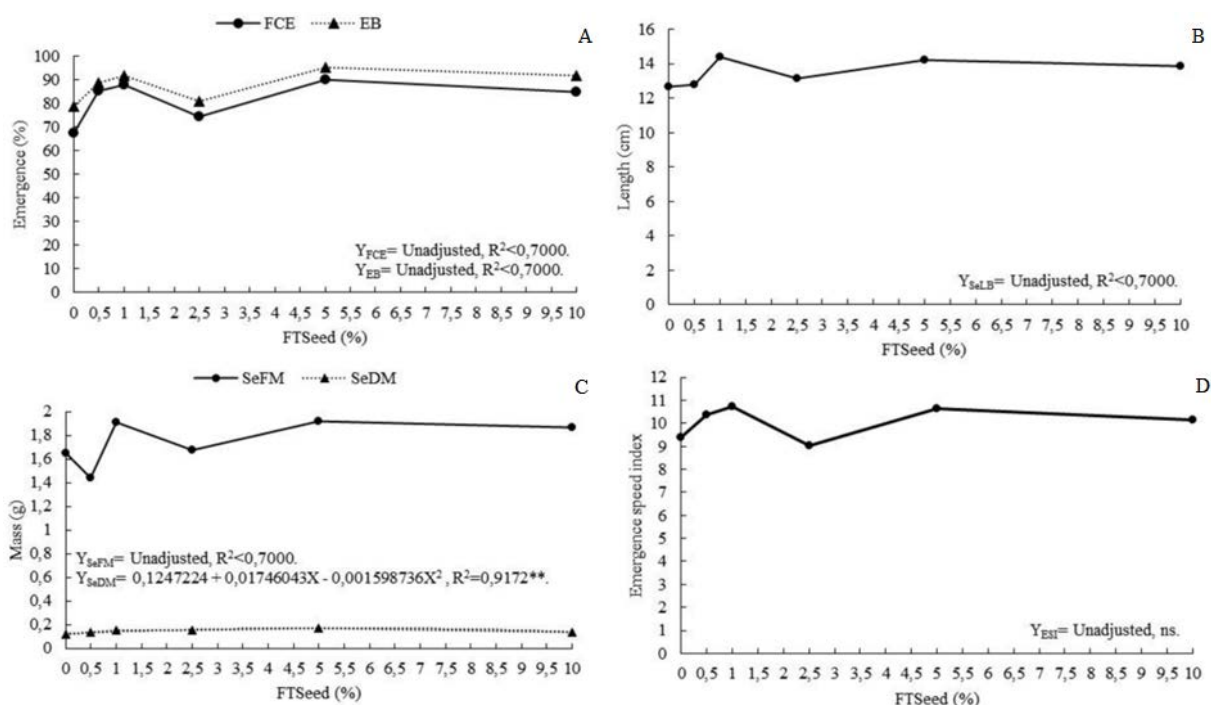
**Figure 4.** A (Ca) and b (Cb) chlorophyll, carotenoid (CAR) of the cowpea. T1 – Witness (FTSeed at 0%). T2 – FTSeed at 0.5%. T3 – FTSeed at 1%. T4- FTSeed at 2.5%. T5 – FTSeed at 5%. T6 – FTSeed at 10%. \*, \*\*Significant, respectively, at the level of 5 and 1% of probability, by the F test. ns Non-significant

The evaluation of the electrolytes extravasation (EE), according to Figure 5, had statistically significant variation, despite the determination coefficient was lower than 0.70 ( $R^2 = 0.4871$ ), being observed variations among the applied treatments, where the EE was reduced under the concentration of FTSeed at 2.5% (32.425), lower than the value obtained in the witness (34.72). Thus, the FTSeed coating at 2.5% was efficient in reducing the leak of electrolytes and maintaining the integrity of the leaf membranes (Bajji *et al.*, 2002).



**Figure 5.** Electrolytes extravasation (EE) of the cowpea. T1 – Witness (FTSeed at 0%). T2 – FTSeed at 0.5%. T3 – FTSeed at 1%. T4- FTSeed at 2.5%. T5 – FTSeed at 5%. T6 – FTSeed at 10%. \*, \*\*Significant, respectively, at the level of 5 and 1% of probability, by the F test. ns Non-significant

The first count in the emergence (FCE) and the emergence in bed (EB) had statistically significant variation (Figure 6A), although the determination coefficient was lower than 0.70 ( $R^2=0.2453$  and  $R^2=0.2497$ ). The fluctuations are observed according to the variation of the FTSeed concentrations, reaching its maximum point with the concentration of FTSeed at 5% (90 and 95.5%, respectively).



**Figure 6.** First count in the emergence (FCE) and the emergence (EB) (A), seedlings length (SeLB) (B), seedlings fresh (SeFM) and dry (SeDM) mass (C), and emergence speed index (ESI) (D) in bed of cowpea. T1 – Witness (FTSeed at 0%). T2 – FTSeed at 0.5%. T3 – FTSeed at 1%. T4- FTSeed at 2.5%. T5 – FTSeed at 5%. T6 – FTSeed at 10%. \*, \*\*Significant, respectively, at the level of 5 and 1% of probability, by the F test. ns Non-significant

These results are related to the study of Zeng, Luo and Tu (2012), where after the use of 5% chitosan coating in soybean seeds, better results were observed in the field emergence, exceeding 90% of emerged seedlings.



According to Figure 6B, the seedlings length emerged in the bed (SeLB) there was statistically significant variation, despite the determination coefficient was lower than 0.70 ( $R^2=0.3868$ ). An increase of the length is observed under the concentration of FTSeed at 1% (14.3985 cm).

Corroborating the results, in a study with chitosan coating on sesame (*Sesamum indicum* L.) and bean (*Phaseolus vulgaris* L.) seeds, it was observed that treatments formulated with chitosan stimulated bean stem growth by up to 30% compared to control and twice as much in sesame (Godínez-Garrido *et al.*, 2022).

According to Figure 6C, the seedlings fresh mass (SeFM) emerged in a bed obtained statistically significant variation, although the determination coefficient was lower than 0.70 ( $R^2=0.3943$ ). The mass had fluctuations among the evaluated treatments, and it was observed higher mass increase in the concentration of FTSeed at 5% (1.922 g).

For the seedlings dry mass (SeDM) emerged in a bed had quadratic behavior in response to the variation of the concentrations (Figure 6C), verifying a mass increase to its estimated maximum value under the concentration of 5.5% (0.1723 g) of FTSeed.

Corroborating with the results of length and mass in beds, in soybean seeds coated with different concentrations of chitosan, higher length and seedlings dry mass values were observed compared to the witness, the authors also highlight visible differences among the seedlings obtained of the coated seeds in comparison to the uncoated ones (Zeng; Luo; Tu, 2012).

It is known that vigorous seeds provide higher transfer of dry matter of the reserve tissues to the growing embryonic axis, resulting in higher mass seedlings, due to the accumulation of dry matter, and higher growth rate (Krzyzanowski *et al.*, 2020), as observed with the application of the bioproduct in the cowpea seeds.

The emergence speed index (ESI) of the cowpea seedlings did not present significant difference, as highlighted in Figure 6D. This result is similar to those obtained in the study of Orzali, Forni and Riccioni (2014). They performed the immersion of wheat seeds, of the Simeto and Cresco cultivars, in different chitosan concentrations, which did not present significant changes in the germination speed when compared to the control. The authors point that this result reflects the nontoxicity of the chitosan to the seeds of wheat, as well as, in the cowpea seeds of the present study.

From PCA, with the data in which no adjustment was obtained in the equations, five components were obtained, however the first two components together, account for 76.85% of the variance of the analyzed data, presenting an eigenvalue greater than 1 in all components, therefore, only the representativeness of the two components will be taken into consideration (Table 1).

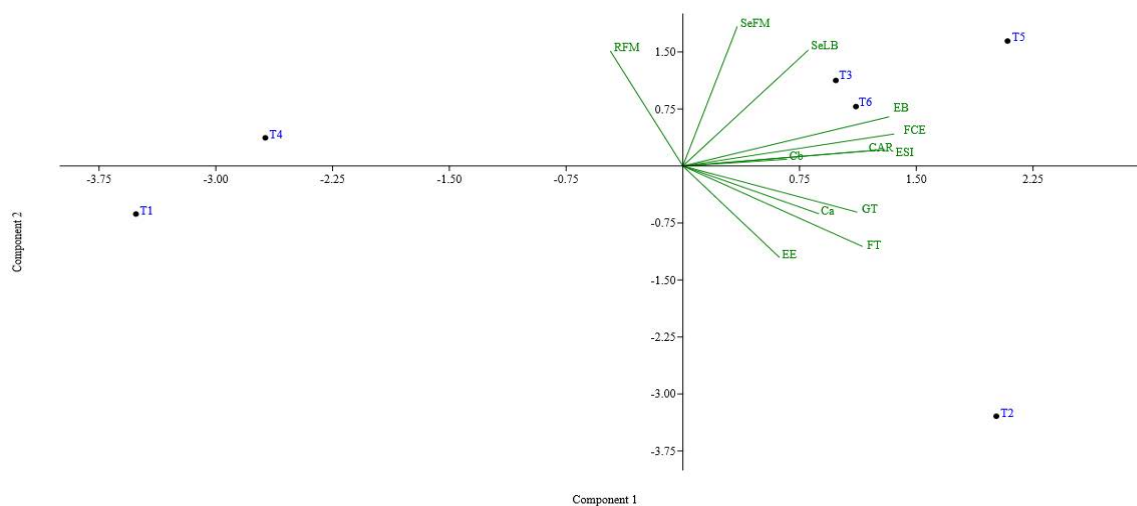
**Table 1.** Principal Component Analysis (PCA) of chitosan concentrations applied to cowpea

Principal Component	Eigenvalue	Variance (%)
1	6,02985	50.249
2	3,19221	26.602
3	1,50544	12.545
4	0,914221	7.685
5	0,358274	2,9856

For the first component, the field germination variables (EB, FCE, ESI) and the biochemical variable CAR were significant, demonstrating that in 50.24% of cases the behavior of cowpea seeds under chitosan

concentrations is explained by these variables, however the RFM had a negative influence on the data (Figure 7). The second component RFM, SeFM, SeLB, EB, FCE, Cb, CAR and ESI were relevant and Ca, GT, FT and EE had a negative correlation.

The proximity between biochemical tests and germination potential in the field, especially in treatments T3, T5 and T6, reflects the direct influence of this treatment on the germinative development and leaf pigments of cowpea. With emphasis on T5, which is located further to the right of the graph.



**Figure 7.** Analysis of principal components of chitosan concentrations applied to cowpea.

Studies carried out with the same bioproduct indicate similar results, in which higher concentrations promoted an increase in the values of biochemical parameters (chlorophyll and carotenoids), germination (first count and percentage of germination), length and mass, as in the use of FTSeed coating in corn (4.5 to 10%) and in tobacco (5.25 and 10%) (Lima *et al.*, 2022; Lima *et al.*, 2023).

#### 4 FINAL CONSIDERATIONS

The application of the bioproduct, FTSeed, in *Vigna unguiculata* seeds resulted in improvement in the growth of the seedlings, since it increased the length and the mass, especially for the concentrations of FTSeed at 3 and 3.25%, which had estimated maximum values of seedlings and shoot length and fresh mass. The application of FTSeed at 5% had better results when evaluating the tests (FT, GT, ESI, FCE, EB, SeLB, SeFM, CAR, Ca, Cb, EE and RFM) in which there was no adjustment to the equations. Therefore, it presents important application value and an alternative to agrochemicals that can bring economic and environmental benefits.

#### 5 ACKNOWLEDGMENTS

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