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Germination performance and vigor of tobacco seeds coated with a chitosan-based bioproduct

Desempenho germinativo e vigor de sementes de fumo revestidas com bioproduto à base de quitosana

Rendimiento germinativo y vigor de semillas de tabaco recubiertas con bioproducto a base de quitosano

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Abstract
The biopolymer coating use on seeds has aroused the interest of the agricultural sector due to its low cost, reduction of impacts on the environment and improvements in productivity and yield of major crops, being chitosan an important polymer for application in seeds. Considering this knowledge, the present study aimed to evaluate the application effect of a bioproduct based on chitosan, from the parameters of physiological quality, vigor, and biochemistry in tobacco seeds. Seeds of the Virginia variety were submitted to treatments: 0.0 (witness); 0.5; 1; 2.5; 5; and 10% were used to obtain first count values, germination test, seedling length and fresh mass, and chlorophyll and carotenoid contents. The bioproduct had a positive effect on chlorophyll a, b, and carotenoids at 10% FTSeed concentration and seedling length under 5.25% FTSeed. The study pointed out that the bioproduct has a favorable action on the development and leaf pigments, being an alternative biostimulant with less impact on the environment for the agricultural sector.
Keywords: *Nicotiana tabacum* L.; Biopolymer; Biostimulant; Coating.

Resumo
O uso de revestimento de biopolímeros em sementes têm despertado o interesse do setor agrícola devido ao baixo custo, redução dos impactos ao ambiente e melhorias na produtividade e rendimento das grandes culturas, sendo a quitosana um importante polímero para essas finalidades. Sabendo disso, o presente estudo objetivou avaliar o efeito da aplicação de um bioproduto à base de quitosana, a partir dos parâmetros de qualidade fisiológica, vigor e bioquímica em sementes de fumo. Foram utilizadas sementes da variedade Virginia submetidas aos tratamentos: 0,0 (testemunha); 0,5; 1; 2,5; 5; e 10%, para obtenção de valores de primeira contagem, teste de germinação, comprimento e massa fresca de plântula, e teores de clorofila e carotenoides. O bioproduto desempenhou efeito positivo sobre a clorofila a, b e carotenoides na concentração de FTSeed a 10% e comprimento das plântulas sob FTSeed a 5,25%. O estudo apontou que o bioproduto desempenha ação favorável sobre o desenvolvimento e pigmentos foliares, sendo um bioestimulante alternativo e de menor impacto ao ambiente para o setor agrícola.
Palavras-chave: *Nicotiana tabacum* L.; Biopolímero; Bioestimulante; Revestimento.

Resumen
El uso de recubrimientos de biopolímeros en semillas ha despertado el interés del sector agrícola por su bajo costo, reducción de impactos ambientales y mejoras en la productividad y rendimiento de grandes cultivos, siendo el quitosano un polímero importante para estos fines. Sabiendo esto, el presente estudio tuvo como objetivo evaluar el efecto de la aplicación de un bioproducto a base de quitosano, a partir de los parámetros de calidad fisiológica, vigor y bioquímica en semillas de tabaco. Se utilizaron semillas de Virginia sometidas a los siguientes tratamientos: 0,0 (testigo); 0,5; 1; 2,5; 5; y 10%, para obtener valores de primer conteo, prueba de germinación, longitud de plántula y masa fresca, y contenido de clorofila y carotenoides. El bioproducto tuvo un efecto positivo sobre la clorofila a, b y los carotenoides a una concentración de FTSeed al 10 % y la longitud de las plántulas bajo FTSeed al 5,25 %. El estudio apuntó que el bioproducto tiene una acción favorable sobre el desarrollo y pigmentos foliares, siendo un bioestimulante alternativo y con menor impacto ambiental para el sector agrícola.
Palabras clave: *Nicotiana tabacum* L.; Biopolímero; Bioestimulante; Revestimiento.

1. Introduction

The culture of tobacco, *Nicotiana tabacum* L., is of great relevance to Brazil, one of the main worldwide producers and exporters. Its seeds are used to produce new seedlings for extraction of the leaves, which is the crop's profitable product (Hirsch & Landau, 2020, Boettcher et al., 2020).

Among the techniques to reduce the use of agrochemicals in major crops there is the use of biodegradable and biocompatible nature polymers, especially if they are low cost, show promising results and reduce the damage caused to ecosystems (Malerba & Cerana, 2018, Reyes-Pérez et al., 2018). Their use as seed coating or covering has drawn attention of researchers and farmers due to the observed effects, acting as biostimulants in germination and plant growth, defense against fungi and pathogens, field results in mass and plant height, increased productivity, and crop yield, among others (Benatto-Junior et al., 2012, Terry Alfonso et al., 2017, Reyes-Pérez et al., 2018).

Chitosan presents itself as one of the biopolymers of great study in the agricultural sector, mainly in applications as coating, a bioproduct component or formulation of gels together with other compounds aiming to favor the physiological performance of seeds (Zerpa et al., 2017, Masjuan et al., 2018, Tovar et al., 2020). Characterized by being renewable, with low cost, biodegradable, biocompatible and non-toxic, this biopolymer is composed of glucosamine and N - acetyl glucosamine subunits linked by (1-4) glycosidic bonds, and obtained from the deacetylation of chitin, a polysaccharide found in the exoskeleton of crustaceans and insects (Pereira et al., 2020, Sarwar et al., 2020).

Considering this, the present study aimed to evaluate the effect of the application of a chitosan-based bioproduct, from the parameters of physiological quality, vigor, and biochemistry in tobacco seeds.

2. Methodology

The study was carried out in the Plant Ecophysiology Laboratory (ECOFISIO) at the State University of Ceará (UECE), Itaperi Campus, located in the State of Ceará, Brazil. The Virginia variety of tobacco (*Nicotiana tabacum* L.) was used, supplied by the company ProfiGen do Brasil Ltda. The product applied was FTSeed, developed and supplied by the company Fertsan, whose main components are soluble salts of polymeric derivatives of chitosan, mixture of polysaccharides, urea, saccharides, mixture of organic acids, preservative, and water.

The seeds were soaked for 30 minutes in the FTSeed solution, with a recommendation of 10µL per 100 seeds, 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%).

2.1 Length and mass

The seeds were distributed in five repetitions of 20 seeds per treatment arranged in Petri dishes with two sheets of

filter paper moistened with 0.2% potassium nitrate solution (KNO3) to break seed dormancy (Brasil, 2009) in an amount three times the weight of dry paper, repeating the moistening of the paper on the eighth day after sowing (DAS) with water in the same amount. These were kept in a germinator of biochemical oxygen demand (BOD) at an alternating temperature of 20 ºC – 30 ºC and photoperiod of 12 h.

At sixteenth DAS, normal seedling lengths were determined, and normal seedling fresh mass and dry mass values were determined using an oven at 80 °C for 24 h, obtaining the average weight of fresh and dry matter per seedling, expressed in g/plants from each repetition (Krzyzanowski et al., 2020).

2.2 Germination, first count and germination speed index

The seeds were distributed in four repetitions of 50 seeds arranged in Petri dishes with two sheets of filter paper moistened with 0.2% potassium nitrate solution (KNO3) to break seed dormancy (Brasil, 2009), in an amount of three times the weight of dry paper, repeating the moistening of the paper on the eighth day after sowing with water in the same amount. These were kept in a germinator of BOD at an alternating temperature of 20 ºC – 30 ºC and a 12-hour photoperiod. The first count was performed on the seventh DAS and the germination test on the sixteenth DAS (Brasil, 2009), the results were expressed as average percentages of normal seedlings per repetition. The germinated seeds were also counted daily to obtain the germination speed index, considering as normal the seedlings with the presence of cotyledons, hypocotyl, and radicle (Krzyzanowski et al., 2020).

2.3 Biochemical tests

At the 16th DAS the chlorophyll a, b and carotenoid contents were determined using leaves from normal seedlings, macerated with 0.08 g of calcium carbonate and 2.8 mL of 80% acetone, after filtering directly into a 10 mL volumetric flask, the volume was completed with 80% acetone. With this extract absorbance, readings were taken in the spectrophotometer at 648.6 and 663.2 nm, to estimate the chlorophyll "a" and "b" content, and for the carotenoid content readings, these were taken at 646.8, 663.2 and 470 nm, according to Lichtenthaler (1987). The chlorophyll a, b, and carotenoid values were calculated using the following equations established by Lichtenthaler (1987):

Chlorophyll a: 12,25 x A663,2 – 2,79 x A646,8

Chlorophyll b: 21,50 x A646,8 – 5,10 x A663,2

Carotenoids: {1000 x A470 – (1,82 x Ca – 85,02 x Cb)} /198

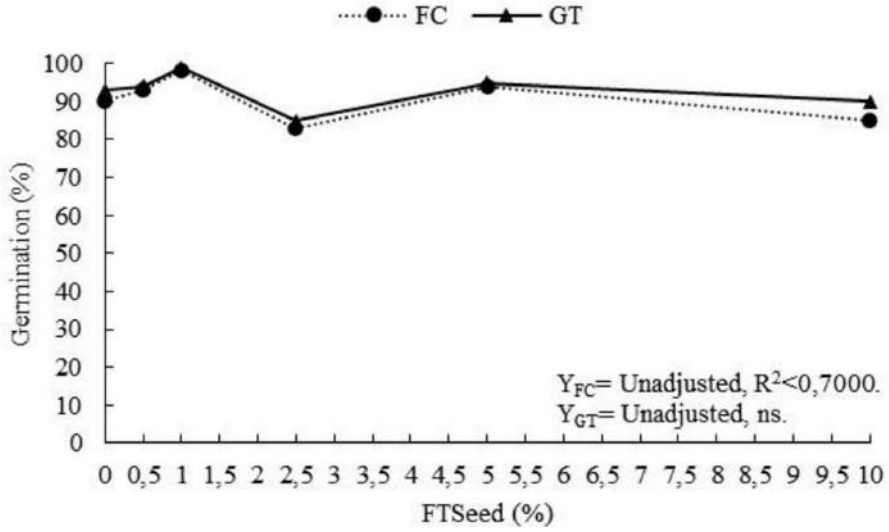
2.4 Experimental design and statistical analysis

The experimental design was entirely randomized, and the results were submitted to variance analysis observing the significance by the F test, which when significant and with determination coefficients greater than 0.70, adjustments were made by means of polynomial regressions of up to the 3rd degree. The software ESTAT (Statistical Analysis System) was used for these calculations.

3. Results and Discussion

The first count (FC) of tobacco seedlings showed statistically significant variation among the treatments evaluated, although the coefficient of determination was less than 0.70 (R^2=0.1931), and oscillations were observed according to the variation of concentrations, showing a higher percentage under the concentration of 1% FTSeed (98%). The germination test (GT) showed no significant difference (Figure 1).

Figure 1 - First count (FC) and germination test (GT) of tobacco.



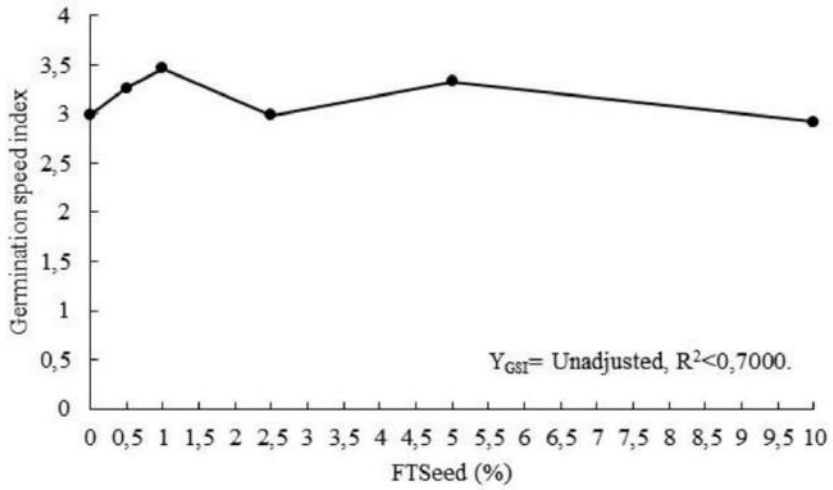
T1 - Witness (0% FTSeed). T2 - FTSeed at 0.5%. T3 - FTSeed at 1%. T4- FTSeed at 2.5%. T5 - FTSeed at 5%. T6 - FTSeed at 10%. *, **Significant, respectively, at the level of 5 and 1% of probability, by the F test. ns Non-significant. Source: Authors.

Despite the low coefficient of determination in the first count, a high germination value can be observed, corroborating the results in seeds of *Lycopersicon esculentum* L. (tomato), *Capsicum annuum* L. (pepper) and *Solanum melongena* L (eggplant), where the application of chitosan (50mg/L), chitin + chitosan (100mg/L) and chitin (25mg/L) or chitin + chitosan (25mg/L), respectively, showed significantly positive effects on seed germination, exceeding values of 80% of germinated seedlings (Amine et al., 2020).

As observed in Figure 2, the germination speed index (GSI) showed statistically significant variation among the treatments evaluated. Although the determination coefficient was less than 0.70 (R^2=0.2853), having fluctuations among the treatments evaluated, being the application of FTSeed at 1% (3.46) responsible for obtaining more vigorous plants, but in higher concentrations, these values decay. There was a similar result to works with rice, cucumber, and corn, in which the application of chitosan on the seeds played a positive effect on their germination speed (Mesa, Pedroso, & Arrebato, 2015, Masjuan et al., 2018, Guan et al., 2009).

Although the germination test did not differ significantly among treatments, the GSI showed positive results to the use of the bioproduct, similar to the work of Tovar et al. (2020) with corn seeds treated with moringa with chitosan and moringa with chitosan and iron nanoparticles, which even without showing differences in the germination test compared to the control, the use of nanoparticles favored the GSI, accelerating the metabolism and germination of corn.

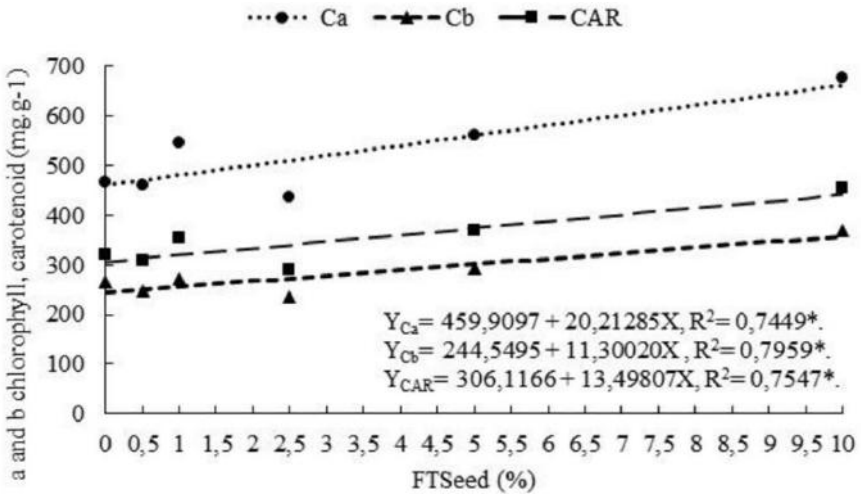
Figure 2 - Germination speed index (GSI) of tobacco.



T1 - Witness (0% FTSeed). T2 - 0.5% FTSeed. 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. Source: Authors.

The chlorophyll (Ca and Cb) and carotenoids (CAR) contents of tobacco (Figure 3) showed linear growth under application of the bioproduct, reaching their maximum estimated point under the concentration of FTSeed at 10% (662.04 $mg.g^{-1}$, 357.55 $mg.g^{-1}$, 441.09 $mg.g^{-1}$, respectively). Studies with wheat seeds after application of chitosan nanoparticles (CSNPs) pointed out positive effects on chlorophyll contents, thus there is an improvement in the photosynthetic capacity of the plant and consequently in the growth rate, as observed with FTSeed coating (Li et al., 2019).

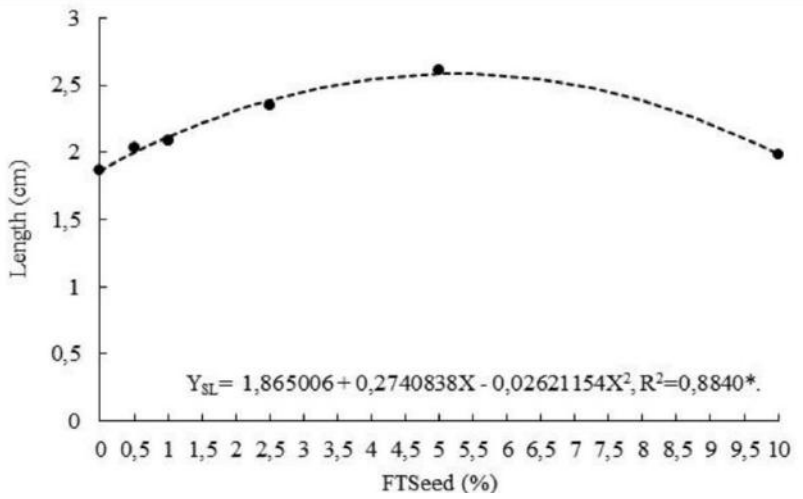
Figure 3 - Chlorophyll a (Ca), b (Cb) and carotenoid (CAR) of tobacco.



T1 - Witness (0% FTSeed). T2 - 0.5% FTSeed. 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. Source: Authors.

The length of the tobacco seedlings (SL) increased quadratically according to the concentrations evaluated, reaching the maximum point estimated with the concentration of FTSeed at 5.25% (2.58 cm) (Figure 4). The coating of seeds with chitosan-based products shows promising results on the length of their seedlings, with significant differences over the control groups, as pointed out in experiments with eggplant and tobacco (Amine et al., 2020, González Gómez et al., 2020). Moreover, it is worth noting its direct relationship with the high rate of leaf pigments with the application of FTSeed, where the increase in photosynthetic capacity acts to increase seedling growth (Zeng & Luo, 2012).

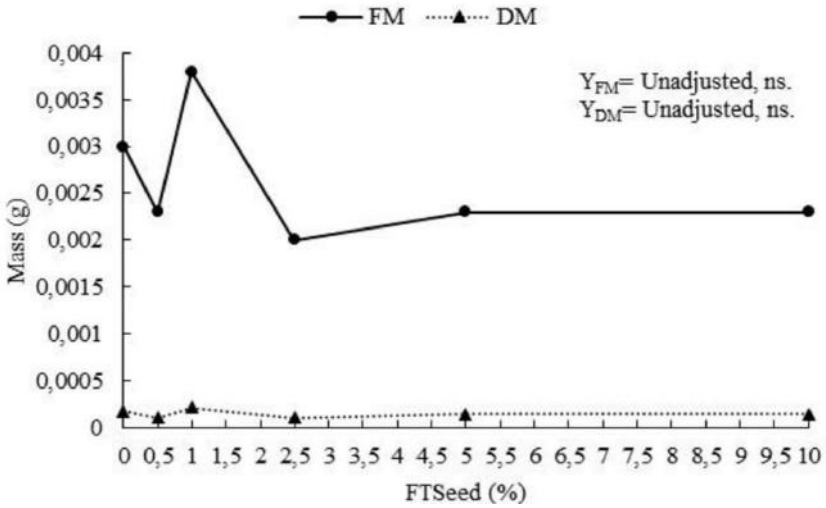
Figure 4 - Seedling length (SL) of tobacco.



T1 - Witness (0% FTSeed). T2 - 0.5% FTSeed. 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. Source: Authors.

The evaluation of the fresh mass (FM) and dry mass (DM) of the seedlings did not show significant differences among treatments (Figure 5). This differs from the results of the application of QuitoMax® on tomato seeds combined with foliar spraying in beds, where there was a difference among the treatments with the product, obtaining higher values of fresh and dry mass compared to the control (Terry Alfonso et al., 2017), as well as in tobacco seeds, where the application of the same product on the seeds and in joint application with sprinkling of seedlings, resulted in greater fresh mass of seedlings treated with QuitoMax®, differing from the control (González Gómez et al., 2020).

Figure 5 - Seedling fresh mass (FM) and dry mass (DM).



T1 - Witness (0% FTSeed). T2 - 0.5% FTSeed. 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. Source: Authors.

4. Conclusion

It was concluded that the application of the chitosan-based bioproduct (FTSeed) on the seeds of Nicotiana tabacum var. Virginia, provided greater seedling length at the estimated concentration of 5.25%, while the highest chlorophyll a, b and carotenoid contents were achieved at the estimated concentration of 10%, although the highest coefficient of determination was

obtained for seedling length (R2=0.9895, $p < 0.05$). In general, the chitosan-based bioproduct acted as a biostimulant in seedling growth and in the photosynthetic pigments' formation of tobacco.

Furthermore, the present work can provide parameters for subsequent studies with the use of chitosan in tobacco and in other species of commercial interest, aiming to improve plant performance, increasing production and consequently the productivity of tobacco leaves, in addition to generating less environmental impact on the agricultural sector and encourage the use of new bioproducts to replace agrochemicals.

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