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Research article

Physiological potential and health quality of corn seeds coated with chitosan

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ABSTRACT: Seed coating is a common practice in treating corn seeds and polymers are used to improve seed performance in terms of physical, physiological, and health quality. However, adverse environmental impacts caused by using non-renewable and non-biodegradable polymers are driving the search for alternatives to overcome these effects, such as natural-based polymers. This study evaluated the effect of chitosan coating formulations (0.6-3.4 g 100 mL^{-1} chitosan and 0-0.60 g glycerol g^{-1} chitosan) on the quality of corn seeds (*Zea mays* L.) regarding physical aspects (visual and morphological aspect, water content, and 1,000-seed mass), physiological potential (germination test, germination speed index, seedling length, cold test, seedling emergence, seedling emergence speed index, seedling height, and root and shoot dry matter), and health quality (Blotter test). Chitosan coatings associated with glycerol did not interfere with the water content, 1,000-seed mass, germination and emergence percentages, cold test, and root dry matter. Conversely, higher biopolymer concentrations can reduce germination speed index, emergence speed index, seedling height, and shoot dry matter. Thus, coating with chitosan 2 % and 0.30 g glycerol g^{-1} chitosan showed promising results in terms of physical aspects with no damage to the physiological potential of corn seeds while reducing the occurrence of *Penicillium* spp.
Keywords: *Zea mays* L., biopolymer, germination, film-coating

Introduction

The changing scenario of global climate imposes an urgent need to increase food production sustainably. However, food production faces challenges such as water crisis, depletion of soil fertility, water contamination, and emergence of new pests, which have significantly affected crop productivity and threaten economic gains and food security (Pathak et al., 2018). Harmful environmental effects, such as soil contamination, are caused by the indiscriminate use and disposal of petroleum-synthetic fossil-based materials, which are non-renewable and mostly non-biodegradable (Otoni et al., 2017).

Natural-based biodegradable alternatives are proposed for several applications in agriculture. In this sense, chitosan is a polysaccharide obtained by the deacetylation of chitin, which is a biodegradable, bioactive, and biocompatible biopolymer found in exoskeletons of arthropods and crustaceans. Chitosan elicitor properties play an essential role in defense responses in plant tissues (Hemantaranjan et al., 2014), synthesis of secondary metabolites (Malerba and Cerana, 2016), and control of phytopathogenic diseases (Akter et al., 2018). In agriculture, chitosan can be applied in the osmotic conditioning of seeds (Ling et al., 2022), fertilizers (Cerri et al., 2020), control of phytopathogenic diseases (Akter et al., 2018), biological control (Chandrika et al., 2019), and seed coating (Haas et al., 2018).

Corn cultivation has an immense value for human and animal nutrition and for bioenergy generation. Brazil is the world's third-largest corn producer, behind the United States and China. Global corn production averaged 1.22 billion tons in 2020-2022 and is projected to reach 1.36 billion tons by 2032 (OECD-FAO, 2023).

The seed coating technology has attracted attention, as it enhances seed value and promotes the mechanization of the planting process (Sou et al., 2017). Seed coating increases seed performance in terms of physical, physiological, and health quality (Avelar et al., 2012; Rocha et al., 2019). The treatment of corn seeds is widely used to ensure quality during storage and prior to planting (Silva et al., 2020). Seed treatment involves the application of synthetic products with a fungicidal effect that limit the spread of pathogens and ensures the emergence of seedlings in adverse environmental conditions. In this sense, the present study evaluated the potential use of chitosan in the coating process of corn seeds, considering the physical aspects, physiological potential, and health quality of the seeds.

Materials and Methods

Material

Corn seeds (*Zea mays* L.) of the Al-Piratininga variety were acquired from Coordenadoria de Desenvolvimento Rural Sustentável (CDRS, São Paulo, Brazil), with no previous treatment. The seeds were stored in a well-ventilated environment without direct sunlight. Chitosan, with an average molar mass of 3.7×10^5 g $gmol^{-1}$ and degree of deacetylation of 97 %, was purchased from Polymar. Glacial acetic acid and glycerol were purchased from Synth. All the other reagents were of analytical grade. The experiment was carried out at Universidade Federal de São Carlos, Centro de Ciências Agrárias, in the municipality of Araras (22°21′ S, 47°27′ W, 692 m altitude), São Paulo, Brazil.

Evaluation of chitosan-based formulations for corn seed coating

The effects of chitosan (0.6-3.4 g 100 mL^{-1}) and glycerol (0-0.6 g g^{-1} chitosan) concentrations in the seed coating solution on the studied responses (physical aspects and physiological potential of seeds) were evaluated by a Rotatable Central Composite 2^2 full factorial Design with three replicates at the central point and four axial points. Equation $\alpha = (2^n)^{1/4}$ was used to determine the distance of the axial points ($\alpha = 1.41$), and the number of axial points corresponds to 2 × n, where "n" is the number of independent variables (Khuri and Cornell, 1987). The experiment comprised 11 treatments and the statistical analysis was performed using Statistica (Statsoft, v. 7). The values of the real and coded levels of the variables are shown in Table 1. In addition to the proposed treatments, responses of uncoated seeds (control) were also evaluated.

Preparation of chitosan solutions for coating corn seeds

The coating solutions were prepared by adding glycerol (0-0.6 g g^{-1} chitosan) in an aqueous solution of acetic acid (1.5 %) at room temperature under constant mechanical stirring (16.7 s^{-1}) (Fisatom, model 713) before the addition of chitosan (0.6-3.4 g 100 mL^{-1}). The solution was kept under constant stirring for 1 h until the material was completely dissolved. The pH solution was measured at room temperature in a pH meter (Digimed DM-22) and adjusted to 4.7 by adding dropwise sodium hydroxide (1 mol L^{-1}). The solutions were kept in a refrigerator in closed glass containers until use.

Table 1 – Full factorial experimental design matrix 2^2 with axial and central points for the formulation of chitosan coatings on corn seeds.

Treatment	X_1	X_2
	g 100 mL^{-1}	g g^{-1} chitosan
T1	1.0 (–1)	0.09 (–1)
T2	3.0 (+1)	0.09 (–1)
T3	1.0 (–1)	0.51 (+1)
T4	3.0 (+1)	0.51 (+1)
T5	0.6 (–1.41)	0.30 (0)
T6	3.4 (+1.41)	0.30 (0)
T7	2.0 (0)	0.0 (–1.41)
T8	2.0 (0)	0.60 (+1.41)
T9	2.0 (0)	0.30 (0)
T10	2.0 (0)	0.30 (0)
T11	2.0 (0)	0.30 (0)

X_1 = chitosan concentration; X_2 = glycerol concentration. Values in parentheses are coded levels of the variables.

Corn seeds coating

Corn seeds were immersed in coating solutions using tweezers, removed immediately, and 50 seeds were placed in each Petri dish (150 × 15 mm), spaced approximately 0.5 cm. Four plates of each treatment were prepared, totaling 200 seeds per treatment. The plates were dried in a forced air oven at 38 °C for 6 h. After drying, the coated seeds were removed from the plates and stored in a cold chamber at 10-12 °C and relative humidity at 30-40 % for at least seven days before the evaluations.

Physical evaluation of seeds after chitosan application

Visual appearance of the coating

Forty seeds from each treatment were immersed in a 1 % methylene blue aqueous solution and removed immediately to assess the homogeneity of the coating. Then, the seeds were immersed twice in 70 % ethanol to remove dye excess and dried on paper towels. The presence or absence of coloration in the region close to the radicle (tip cap) and percentage of coloration in the cotyledon and endosperm of the seeds were evaluated (Figures 1 A-C) and classified as follows: Absent, in which there was no tissue staining; Light, in which the cotyledon showed coloration up to < 50 % and one or both sides of the endosperm with coloration up to 25-50 %; Moderate, in which the cotyledon showed ≥ 50 % coloration, or < 50 % cotyledon and one or both sides of the endosperm with > 50 % coloration; and Intense, where 100 % staining was observed in all tissues (Figures 1 D-G). The results were expressed as a percentage of seeds for each class described.

Scanning Electron Microscopy

The surface and cross-section morphologies of the coated seeds were evaluated by Scanning Electron Microscopy (SEM) using a Thermo Fischer Scientific® Prisma E scanning microscope in a high vacuum mode with an

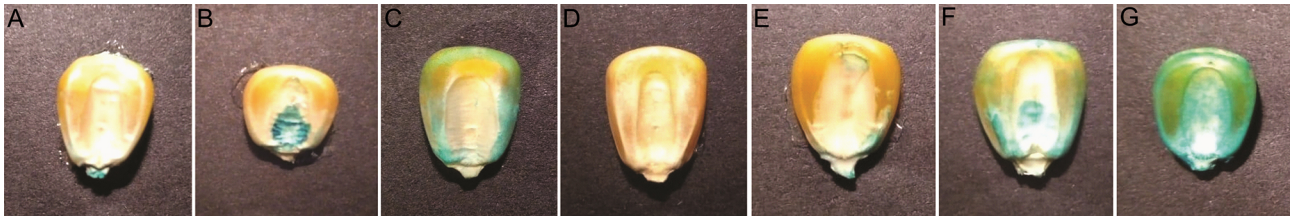

Figure 1 – A) Staining intensity in the tip cap, B) cotyledon, C) endosperm of chitosan-coated corn seeds, D) classified as Absent, E) Light, F) Moderate, and G) Intense and Control.

accelerating voltage of 20.00 kV. Cross-sections were prepared by breaking the seeds with a blade in two halves. Seed samples were mounted on aluminum stubs and fixed by double-sided adhesive carbon tape.

Water content and 1,000-seed mass

The water content of the seeds was determined gravimetrically by drying at 105 °C for 24 h, in triplicate, according to standard method (MAPA, 2009a). The 1,000-seed mass was determined by weighing four replicates of 100 seeds of each treatment, where the averages were compared with the water content of the respective treatment and the values were adjusted considering 13 % of water content (MAPA, 2009a).

Evaluation of the physiological potential of seeds

The germination test was performed according to the Rules for Seed Analysis (MAPA, 2009a). Four replications of 50 seeds from each treatment were placed on two sheets of Germitest paper, moistened with water (corresponding to 2.5 times the paper mass), and covered with an additional sheet to make the rolls. The rolls were kept in a Biochemical Oxygen Demand (BOD) germination chamber at 25 °C for seven days. The first count after four days and the final germination percentage after seven days were considered. The Germination Speed Index (GSI) was obtained through daily evaluation of germination, according to the equation described by Maguire (1962). After the final count (7^{th} day), root and shoot measurements of normal seedlings were performed using a ruler (precision ± 0.1 cm).

The cold test was conducted in a paper roll with soil with four replicates of 50 seeds from each treatment placed on two sheets of Germitest paper previously moistened with a volume of water 2.5 times the paper mass, covered with a thin layer of sieved soil (60 mL) from a crop site and a third sheet to make the rolls, which were kept in sealed plastic bags in a BOD germination chamber at 10 °C for seven days (Krzyzanowski et al., 2020). After the cooling period, the rolls were placed unsealed in the BOD chamber at 25 °C, and the percentage of normal seedlings was quantified after seven days.

The seedling emergence test was conducted to assess the growth of seedlings in the soil by sowing four replicates of 50 seeds from each treatment in 30.3 × 22.1 × 7.5 cm trays filled with coarse sand and sieved clayey soil from a crop site in a 2:1 ratio. Before sowing, the soil field capacity was quantified. Sowing was done at a maximum depth of 1 cm and the trays were irrigated with a water volume corresponding to 60 % of the field capacity. The percentage of seedlings that emerged each day was calculated until stabilization, which occurred nine days after sowing. The Emergence Speed Index (ESI) was obtained at the end, according to the equation described by Maguire (1962).

The height of normal seedlings was measured at the end of the emergence test using a ruler (precision ± 0.1 cm), considering from the substrate level to the tip of the highest leaf. The values of root dry matter (RDM) and shoot dry matter (SDM) were determined at the end of the emergence test by removing the seedlings from the trays and subjecting them to washing, followed by separation of seed, root, and shoot. Roots and shoots from each treatment were placed separately in paper bags and taken to an oven at 65 °C for four days. After drying, the material was weighed on an analytical balance (Krzyzanowski et al., 2020).

Evaluation of the health quality of seeds

The Blotter test was adapted from the Seed Health Analysis Manual (MAPA, 2009b). Eight replicates of 25 chitosan-coated seeds were placed on three discs of moistened filter paper (water volume equivalent to 2.5 times the paper mass) in sterilized Petri dishes and kept sealed in a chamber under a photoperiod of 12 h at 20 °C for seven days. After this period, seeds were examined under a stereomicroscope (30× - 80×), and an optical microscope (400×) was used to confirm the identification. The results were expressed in the percentage of occurrence of the fungi observed.

Statistical analysis

The experiment was carried out under a completely randomized design. The analysis of variance (ANOVA) and the Tukey test were performed to determine differences between averages at a significance level of 5 % using the software Statistica (Statsoft, version 7). The means for the Blotter test were compared using the t-Student test ($p < 0.05$).

Results

Physical evaluation of seeds after chitosan application

The seed coloring test with methylene blue solution confirmed the presence and effectiveness of the coating. Higher chitosan concentrations (T2, T4, T6, and T8) showed better quality of corn seed coating, considering the highest percentage of seeds classified as slightly colored. Coatings with lower concentrations of chitosan (T1, T3, and T5) had significant failures. The SEM revealed that the treatment with the highest chitosan concentration (T6, Figure 2D) had a thicker layer, promoting uniform coating around the seed, followed by treatment T9 (Figure 2C). Treatments T1 (Figure 2A) and T5 (Figure 2B), with lower chitosan concentrations, presented a thinner coating with coverage failures.

Treatments with higher concentrations of chitosan and glycerol (T4 and T6) had a higher water content ($p < 0.05$) (Table 2). As for the 1,000-seed mass, treatments did not differ from the control, despite showing a similar trend in terms of the water content (Table 2).

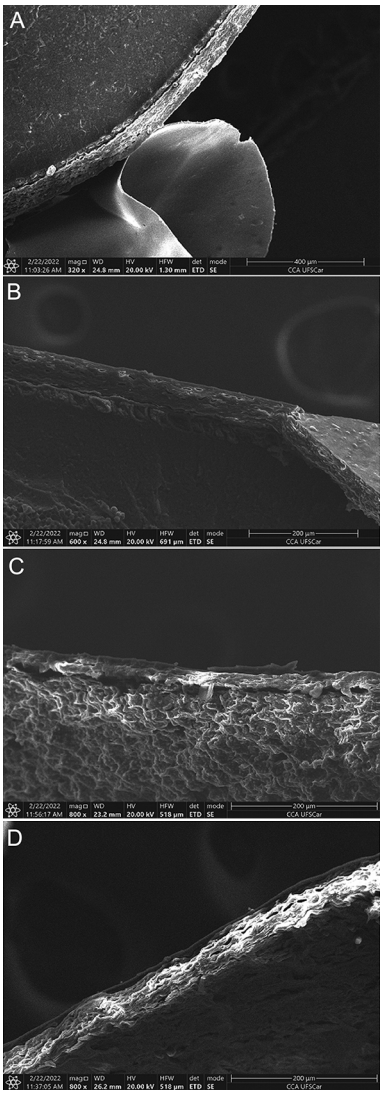

Figure 2 – Scanning Electron Microscopy (SEM) micrographs of chitosan-based coatings on corn seeds: A) view of coating in contact with the seed pericarp in T1 (1/0.09), magnification 320×; B) layer surrounding the seed in T5 (0.6/0.30), magnification 600×; C) T9 (2.0/0.30), magnification 800×; and D) T6 (3.4/0.30), magnification 800×.

The interaction term (+ 2.91) had an effect on the water content, indicating that glycerol concentration influences the behavior of chitosan concentration (Figure 3). The positive effect indicates that an increase in chitosan concentration from 0.6 to 3.4 g 100 mL^{-1} and glycerol from 0 to 0.60 g g^{-1} chitosan tends to increase the water content (Figure 3).

Evaluation of the physiological potential of seeds

Chitosan coating did not promote a difference in seed germination at the final count (Table 3), with germination values ranging from 94 % to 99.5 %.

Table 2 – Water content and 1,000-seed mass results for chitosan-based coatings on corn seeds.

Treatment (chitosan/glycerol)	Water content*	1,000-seed mass**
	g 100 g^{-1}	g
T1 (1.0/0.09)	10.20 ± 0.1^{abc}	426.5 ± 7.7^{a}
T2 (3.0/0.09)	10.07 ± 0.1^{bc}	432.0 ± 3.4^{a}
T3 (1.0/0.51)	10.01 ± 0.3^{c}	434.4 ± 3.0^{a}
T4 (3.0/0.51)	10.62 ± 0.1^{a}	438.4 ± 3.4^{a}
T5 (0.6/0.30)	10.35 ± 0.2^{abc}	429.4 ± 3.5^{a}
T6 (3.4/0.30)	10.49 ± 0.2^{ab}	433.9 ± 6.0^{a}
T7 (2.0/0)	10.30 ± 0.2^{abc}	431.6 ± 5.7^{a}
T8 (2.0/0.60)	10.15 ± 0.1^{bc}	433.8 ± 3.4^{a}
T9 (2.0/0.30)	10.13 ± 0.2^{bc}	429.4 ± 3.1^{a}
T10 (2.0/0.30)	10.16 ± 0.2^{abc}	429.2 ± 4.1^{a}
T11 (2.0/0.30)	10.01 ± 0.1^{c}	433.2 ± 8.0^{a}
Control	9.96 ± 0.1^{c}	427.5 ± 4.1^{a}

Mean ± standard deviation of three* and four** experimental determinations; same letters in the column indicate no difference ($p < 0.05$) between the means by the Tukey test.

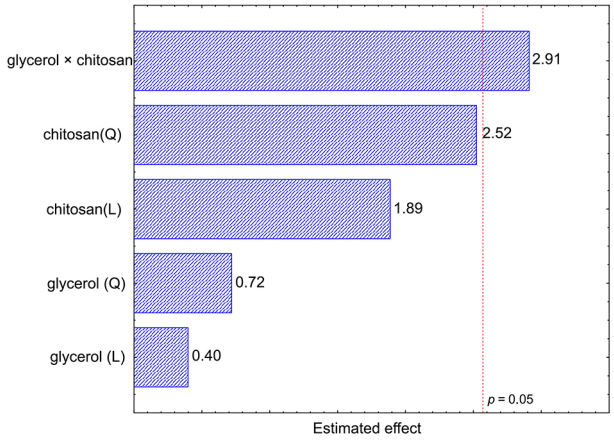

Figure 3 – Pareto graph for the effect of chitosan and glycerol concentrations on water content. (L) – linear term and (Q) – quadratic term.

The germination speed index (GSI) reduced with the increase in chitosan concentration from 0.6 to 3.4 g 100 mL^{-1}, except for treatments T8 (2/0.60), T9 (2/0.30) and T10 (2/0.30), which did not differ from the control (Table 3). The treatment with the highest chitosan concentration (T6) presented the lowest GSI value (12.6) compared to the control (15.6). The highest GSI values were obtained at intermediate chitosan concentrations and higher glycerol concentrations (Figure 4).

Root length and total (root + shoot) length were smaller than the control, except for treatments T1 (1/0.09) and T2 (3/0.09). Treatment T2 showed a shoot length 16 % greater compared to control ($p < 0.05$) (Table 3). The linear term of glycerol concentration (–3.03) negatively affected shoot length, indicating that the increase in glycerol concentration from 0 to 0.6 g g^{-1} chitosan caused a decrease in seedling shoot length (Figure 5). Despite the lower GSI of chitosan-coated seeds, shoot development

Table 3 – Germination percentage (first and final count), germination speed index (GSI), root length, shoot length, and total seedling length for chitosan-based coatings on corn seeds.

Treatment (chitosan/glycerol)	Germination		GSI	Length		
	1st count	Final count		Root	Shoot	Total
	%			cm		
T1 (1/0.09)	94.0 ± 1.6^{a}	98.5 ± 1.9^{a}	14.6 ± 0.3^{bcd}	10.9 ± 0.5^{ab}	6.7 ± 0.7^{ab}	17.6 ± 1.2^{ab}
T2 (3/0.09)	95.0 ± 2.6^{a}	97.0 ± 2.0^{a}	14.2 ± 0.4^{cde}	12.4 ± 0.2^{a}	7.2 ± 0.2^{a}	19.6 ± 0.3^{a}
T3 (1/0.51)	94.5 ± 3.4^{a}	99.5 ± 1.0^{a}	14.4 ± 0.5^{bcde}	9.4 ± 0.5^{bc}	6.5 ± 0.7^{ab}	15.9 ± 1.1^{bc}
T4 (3/0.51)	94.5 ± 2.5^{a}	98.0 ± 1.6^{a}	14.0 ± 0.4^{def}	7.5 ± 0.7^{d}	6.1 ± 0.3^{b}	13.6 ± 0.8^{d}
T5 (0.6/0.30)	90.0 ± 2.0^{a}	97.3 ± 2.3^{a}	13.2 ± 0.1^{fg}	7.7 ± 0.2^{cd}	6.1 ± 0.3^{b}	13.8 ± 0.2^{cd}
T6 (3.4/0.30)	81.5 ± 2.5^{b}	94.5 ± 1.9^{a}	12.6 ± 0.3^{g}	8.1 ± 0.1^{cd}	6.5 ± 0.4^{ab}	14.6 ± 0.4^{cd}
T7 (2/0)	89.0 ± 2.6^{a}	94.0 ± 1.6^{a}	13.6 ± 0.3^{ef}	8.7 ± 0.8^{cd}	6.6 ± 0.2^{ab}	15.3 ± 0.9^{cd}
T8 (2/0.60)	90.5 ± 5.0^{a}	97.5 ± 3.8^{a}	15.0 ± 0.5^{abc}	7.6 ± 1.2^{cd}	6.1 ± 0.1^{b}	13.7 ± 1.2^{cd}
T9 (2/0.30)	91.5 ± 5.0^{a}	98.0 ± 2.8^{a}	14.7 ± 0.4^{abcd}	8.5 ± 0.8^{cd}	6.1 ± 0.2^{b}	14.6 ± 0.9^{cd}
T10 (2/0.30)	94.5 ± 1.0^{a}	98.5 ± 1.9^{a}	15.2 ± 0.2^{ab}	8.2 ± 1.0^{cd}	5.9 ± 0.1^{b}	14.1 ± 1.1^{cd}
T11 (2/0.30)	92.0 ± 2.8^{a}	98.0 ± 0.0^{a}	14.6 ± 0.2^{bcd}	8.1 ± 0.8^{cd}	6.2 ± 0.1^{b}	14.3 ± 1.0^{cd}
Control	95.5 ± 2.5^{a}	97.0 ± 3.5^{a}	15.6 ± 0.4^{a}	12.6 ± 0.8^{a}	6.2 ± 0.2^{b}	18.8 ± 0.9^{a}

Mean ± standard deviation of four experimental determinations; same letters in the column indicate no difference ($p < 0.05$) between the means by the Tukey test.

was not affected at chitosan concentrations up to 3 g 100 mL^{-1} with a low glycerol concentration.

The cold test showed that none of the treatments affected seed vigor (Table 4). Chitosan and glycerol concentrations showed no effects within the evaluated ranges.

There was no difference for seedling emergence (Table 5) and no effect of chitosan and glycerol concentrations within the ranges studied. However, the ESI differed from the control in all treatments, except those with the lowest chitosan concentrations (T1 and T5) and glycerol concentrations up to 0.30 g g^{-1} chitosan. The treatment with the highest chitosan concentration (T6) presented the lowest ESI value. In contrast, the highest ESI values were verified for chitosan concentrations up to 2 g 100 mL^{-1} and glycerol concentrations up to 0.4 g g^{-1} chitosan (Figure 6).

The treatment with the highest chitosan concentration (T6) reduced seedling height by 19 % compared to the control, indicating a similar behavior to the ESI (Table 5). Treatments with chitosan 3 and 2 g 100 mL^{-1} and higher glycerol concentration (0.51 and 0.60 g g^{-1} chitosan, respectively) also differed from the control.

RDM did not vary in relation to the control and no effect was observed in relation to the variables evaluated. SDM showed a trend like that observed for ESI and height in which the treatments with higher chitosan concentrations had lower values compared to the control. However, there was no effect of chitosan concentrations from 0.6 to 3.4 g 100 mL^{-1} and glycerol concentrations from 0 to 0.6 g g^{-1} chitosan on SDM.

T1 (1.0/0.09) showed the best results on the physiological potential, with no interference on the parameters of viability and vigor evaluated for corn seeds, except for the GSI; however, T1 presented lower quality when visually evaluated by staining. Regarding to seedling length, T1 (1.0/0.09) and T2 (3.0/0.09) were adequate; nevertheless, considering the importance of

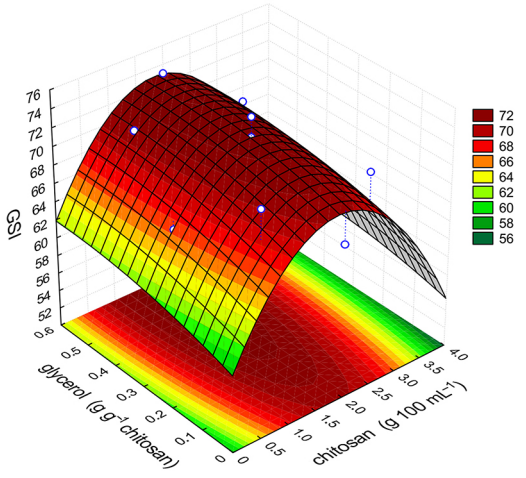

Figure 4 – Response surface for the effect of chitosan and glycerol concentrations on the germination speed index (GSI).

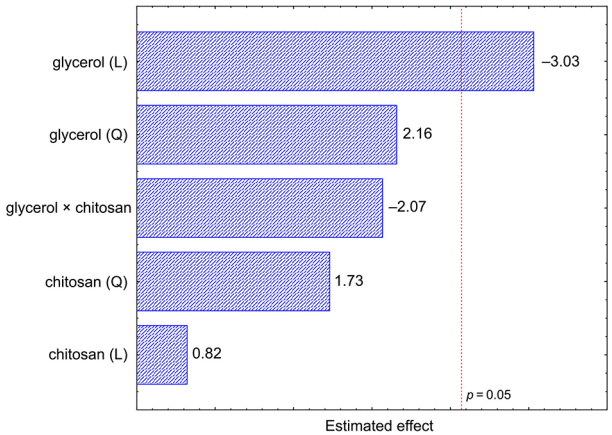

Figure 5 – Pareto graph for the effect of chitosan and glycerol concentrations on shoot length. (L) – linear term and (Q) – quadratic term.

the variable GSI in field conditions, the most interesting treatments were T8 (2.0/0.60) and T9 (2.0/0.30).

The coating with chitosan solution associated to glycerol did not influence the water content, 1,000-seed mass, germination, emergence percentage, cold test or RDM. The treatments with the most significant potential for corn seed coating were, respectively: T1 (1.0/0.09) > T9, T10, T11 (2.0/0.30) > T8 (2.0/0.60) > T2 (3.0/0.09) > T5 (0.6/0.30).

Evaluation of health quality of seeds

The Blotter test was performed with uncoated seeds (control) and seeds coated in the selected condition (T9 = 2.0/0.30), considering the physical aspects and the physiological potential of the seeds after the treatment. The results indicate that the coating with chitosan 2 % inhibited the growth of *Penicillium* spp., differing from the control (Figure 7). There was no difference between treatments for the occurrence of *Aspergillus* spp. and *Fusarium* spp.

Table 4 – Germination results after cold test for chitosan-based coatings on corn seeds.

Treatment (chitosan/glycerol)	Cold test
	%
T1 (1.0/0.09)	95.0 ± 1.2[a]
T2 (3.0/0.09)	89.5 ± 6.8[a]
T3 (1.0/0.51)	90.0 ± 5.9[a]
T4 (3.0/0.51)	91.0 ± 4.2[a]
T5 (0.6/0.30)	91.0 ± 4.8[a]
T6 (3.4/0.30)	90.5 ± 1.0[a]
T7 (2.0/0)	95.0 ± 2.0[a]
T8 (2.0/0.60)	95.0 ± 2.6[a]
T9 (2.0/0.30)	87.0 ± 5.3[a]
T10 (2.0/0.30)	92.0 ± 4.3[a]
T11 (2.0/0.30)	90.7 ± 4.1[a]
Control	90.0 ± 1.6[a]

Mean ± standard deviation of four experimental determinations; same letters in the column indicate no difference ($p < 0.05$) between the means by the Tukey test.

Discussion

The coating process involves wetting the material surface and spreading the covering solution, followed by possible adhesion (Casariego et al., 2008). Chitosan has excellent capacity to form films and coatings but tends to be brittle. The rigidity of the polymer matrix is mainly determined by the strength of polymer-polymer interactions, which can be controlled by adding a plasticizer, such as glycerol (Rodríguez-Núñez et al., 2014). Glycerol, one of the most used plasticizers, is a hydrophilic molecule of low molar mass that can easily fit between polymer chains and

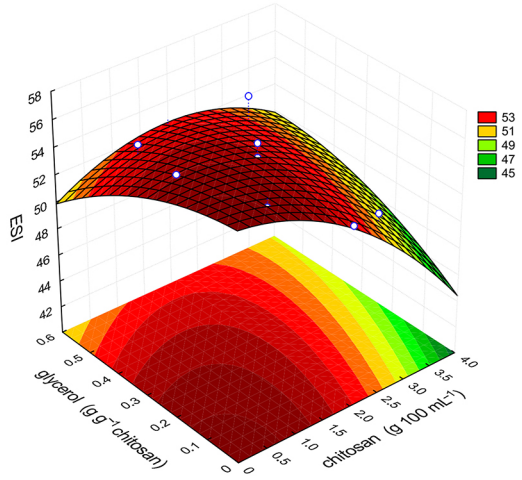

Figure 6 – Response surface for the effect of chitosan and glycerol concentrations on the emergence speed index (ESI).

Table 5 – Emergence percentage, emergence speed index (ESI), seedling height, and root and shoot dry matter values for chitosan-based coatings on corn seeds.

Treatment (chitosan/glycerol)	Emergence	ESI	Height	Dry matter	
				Root	Shoot
	%		cm	g	
T1 (1/0.09)	98.0 ± 2.8[a]	13.9 ± 0.2[ab]	13.4 ± 0.8[ab]	2.72 ± 0.2[a]	2.25 ± 0.1[ab]
T2 (3/0.09)	97.0 ± 2.6[a]	12.9 ± 0.3[bc]	13.0 ± 0.5[abc]	2.96 ± 0.3[a]	2.18 ± 0.0[b]
T3 (1/0.51)	97.0 ± 2.6[a]	13.5 ± 0.4[bc]	13.1 ± 0.5[ab]	2.98 ± 0.3[a]	2.21 ± 0.3[ab]
T4 (3/0.51)	96.0 ± 1.6[a]	13.6 ± 0.5[bc]	12.7 ± 0.4[bc]	2.88 ± 0.3[a]	2.26 ± 0.1[ab]
T5 (0.6/0.30)	97.5 ± 2.5[a]	13.9 ± 0.3[ab]	13.7 ± 0.4[ab]	2.59 ± 0.1[a]	2.23 ± 0.1[ab]
T6 (3.4/0.30)	97.5 ± 1.0[a]	12.5 ± 0.3[c]	11.8 ± 0.6[c]	2.56 ± 0.1[a]	2.17 ± 0.2[b]
T7 (2/0)	97.5 ± 3.0[a]	13.5 ± 0.4[bc]	13.4 ± 0.5[ab]	2.85 ± 0.3[a]	2.26 ± 0.2[ab]
T8 (2/0.60)	94.5 ± 2.5[a]	12.9 ± 0.5[bc]	12.5 ± 0.5[bc]	2.72 ± 0.2[a]	2.18 ± 0.2[b]
T9 (2/0.30)	98.0 ± 1.6[a]	13.5 ± 0.4[bc]	13.5 ± 0.6[ab]	2.76 ± 0.2[a]	2.35 ± 0.2[ab]
T10 (2/0.30)	96.5 ± 1.9[a]	13.6 ± 0.6[bc]	13.1 ± 0.3[ab]	2.69 ± 0.5[a]	2.21 ± 0.1[ab]
T11 (2/0.30)	100.0 ± 2.0[a]	13.9 ± 0.3[b]	13.4 ± 0.6[ab]	3.16 ± 0.3[a]	2.34 ± 0.2[ab]
Control	98.5 ± 1.0[a]	14.8 ± 0.8[a]	14.1 ± 0.2[a]	3.16 ± 0.0[a]	2.60 ± 0.1[a]

Mean ± standard deviation of four experimental determinations; same letters in the column indicate no difference ($p < 0.05$) between the means by the Tukey test.

reduce intermolecular interactions, which preferentially occur between the OH groups of glycerol and the hydroxyl and amino groups of chitosan (Pavinatto et al., 2020; Wahba, 2020).

The higher water content obtained in T4 and T6 may be related to the hydrophilic characteristics of chitosan and glycerol, providing greater absorption and retention of water molecules in the biopolymeric matrix formed around the seed. Coating failures at lower chitosan concentrations can be attributed to the lower physical barrier around the seed. Regarding the visual analysis of seed coating, only the control showed 100 % evaluated in the Intense color class (I), demonstrating that the seeds of all treatments were coated to a greater or lesser degree (Table 6).

Seed germination is a critical step, as the seedling is susceptible to external agents and adverse edaphoclimatic conditions during this period due to its reduced root system and scarce leaf area. The values obtained for germination were considered high (> 90 %), indicating that the seed lot used was of good quality. Moreover, the use of chitosan coating did not affect seed viability.

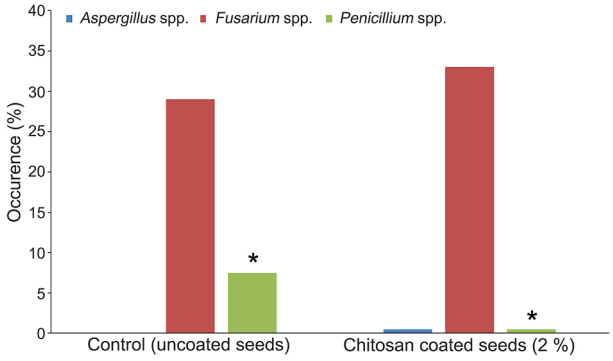

Figure 7 – Occurrence (%) of *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. in uncoated corn seeds (control) and corn seeds with chitosan coating (2 g 100 mL^{-1} and glycerol 0.30 g g^{-1} chitosan). *Means with differences by the t-Student test ($p < 0.05$).

Table 6 – Classification of the visual appearance of chitosan-based coatings on corn seeds according to color intensity.

Treatment (chitosan/glycerol)	Visual aspect			
	A	L	M	I
	---------- % ----------			
T1 (1/0.09)	0	52.5	47.5	0
T2 (3/0.09)	2.5	77.5	20.0	0
T3 (1/0.51)	0	60.0	40.0	0
T4 (3/0.51)	0	82.5	17.5	0
T5 (0.6/0.30)	0	37.5	62.5	0
T6 (3.4/0.30)	0	72.5	27.5	0
T7 (2/0)	0	62.5	37.5	0
T8 (2/0.60)	0	85.0	15.0	0
T9 (2/0.30)	0	72.5	27.5	0
T10 (2/0.30)	0	70.0	30.0	0
T11 (2/0.30)	0	77.5	22.5	0
Control	0	0	0	100.0

A = absent; L = light; M = moderate; I = intense.

Seed coating with biopolymers promoted increases in soybean seed germination and seedling productivity (Zeng et al., 2012) and corn seed germination (Vercelheze et al., 2019). Corn seeds coated with low chitosan concentrations improved the germination percentage and seedling size (Mohamed et al., 2020). Chitosan coating did not affect corn seed germination (Lizárraga-Paulín et al., 2013; Peña-Datoli et al., 2016), which is consistent with the results of the present study, as the seed treatment should not harm the parameters of seed viability and vigor.

Chitosan coatings at concentrations of 1 %, 3 %, and 5 % did not alter seed viability but decreased the GSI of corn seeds, possibly attributed to the physical barrier imposed by the coating at higher concentrations, requiring a longer time for the seeds to germinate (Peña-Datoli et al., 2016). The lowest ESI in T6 (3.4/0.30) reinforces the hypothesis that higher chitosan concentrations cause a physical barrier that can reduce the speed of this process despite protecting the seed with a semi-permeable film and allowing for soil water absorption (Zeng et al., 2012). This observation is further supported by the results of Godínez-Garrido et al. (2022), who identified a stimulating response in the GSI of both common bean (*Phaseolus vulgaris* L.) and sesame seeds (*Sesamum indicum* L.) treated with lower chitosan concentrations (0.1 % and 0.5 %).

The excellent film-forming properties of chitosan can improve water absorption and seed germination, providing greater seed protection and enhancing seedling growth (Oliveira et al., 2009). Low glycerol concentrations ensured root length, regardless of the chitosan concentration. Higher glycerol concentrations may have a toxic effect on shoot length; however, this effect needs further investigation since there were no differences between the treatments and control, except T2. Furthermore, it opposes the trend that an increase in glycerol concentration could increase germination speed (Figure 4). Chitosan associated with polyethylene glycol and glycerol favored germination, root and shoot length, and the vigor index of castor bean seeds, and the percentage of seed germination increased with higher glycerol concentrations from 0.75 % to 1.0 % (Chandrika et al., 2019), diverging from the results of the present study.

The cold test results indicate that coating corn seeds with chitosan solution does not affect seedling development under environmental stress conditions (Table 4). Chitosan (2 %) ensured the viability of corn seeds and did not change the phenological parameters of the seedlings (Lizárraga-Paulín et al., 2011). Chitosan-treated pepper seeds submitted to a cold test exhibited an increase in the percentage of emergence and growth of seedlings, suggesting that an increase in chitinase and glucanase activities induced systemic resistance of seeds to adverse conditions (Samarah et al., 2020).

Fusarium spp., *Aspergillus* spp., and *Penicillium* spp. are among the main fungal genera responsible for the deterioration of corn seeds (Silva et al., 2020). Lentil seeds (*Lens esculenta*) immersed in chitosan solution (0.1 %) with subsequent drying showed reduced *Penicillium* spp. growth and mycotoxin production, while there was no difference for *Aspergillus* spp. and *Fusarium* spp. (Abd-Allah and Hashem, 2006). Due to the polycationic characteristic of chitosan, contact with the fungal wall can lead to leakage of cell contents and delay or inhibit the synthesis of mRNA and proteins (Lee et al., 2016).

The application of low molar mass chitosan solutions (0.5, 1.0, 2.0, and 4.0 g L^{-1}) reduced the mycelial growth of *Fusarium equiseti* in Jatropha (*Jatropha curcas* L.) seeds (Pabón-Baquero et al., 2015). Some authors have reported that different molar masses and concentrations of chitosan affect distinct effects on antifungal activity and that the presence of short chains in chitosan oligomers promoted greater inhibition of fungal growth compared to high molar mass chitosan (Lee et al., 2016). In the present study, there was no interference of the 2 g 100 mL^{-1} chitosan solution for the control of *Aspergillus* spp. and *Fusarium* spp. in corn seeds, which the use of chitosan of medium molar mass may have caused.

Higher glycerol concentrations may be associated to a tendency to reduce shoot length. In comparison, higher chitosan concentrations may promote a reduction in the GSI, the ESI, seedling height, and SDM values.

The data gathered in the present work suggest that treatments with higher chitosan concentrations affected the vigor of corn seeds due to the physical barrier imposed by the coating without affecting seed viability, represented by the seed germination percentage. As expected, the effect of higher chitosan concentrations on plant growth is likely due to a reduction in the speed of seedling development, not to a toxic effect of the coating on plant tissue. The coating did not affect the vigor of seed resistance parameters to environmental stresses.

Chitosan coating in the selected condition (chitosan 2 g 100 mL^{-1} and glycerol 0.30 g g^{-1} chitosan) showed excellent results in terms of physical aspects without compromising the physiological potential of corn seeds while reducing the occurrence of *Penicillium* spp. Chitosan 2 g 100 mL^{-1} did not affect seed quality; therefore, it can act as a loading matrix for active compounds, such as nutrients and essential oils, which may be promising in future studies.

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Authors' Contributions

Conceptualization: Zacharias MB, Forti VA, Silva MA. **Data curation:** Zacharias MB, Forti VA, Silva MA. **Formal analysis:** Zacharias MB, Forti VA, Silva MA. **Funding acquisition:** Silva MA. **Investigation:** Zacharias MB, Forti VA, Silva MA. **Methodology:** Zacharias MB, Forti VA, Silva MA. **Project administration:** Silva MA. **Resources:** Forti VA, Silva MA. **Supervision:** Forti VA, Silva MA. **Writing-original draft:** Zacharias MB. **Writing-review & editing:** Zacharias MB, Forti VA, Silva MA.

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