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The effect of chitosan in preventing the adverse effects of aging on the germination and seedling development of corn plant[1]

O efeito da quitosana na prevenção dos efeitos adversos do envelhecimento na germinação e no desenvolvimento de plantas de milho

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ABSTRACT - In this study, in which the effect of chitosan coating on the aging process of a seed was investigated, the rapid aging times applied did not have a negative effect on the Gmax value of the seeds. Therefore, the effect of chitosan and aging treatments on seed germination and germination time at this stage could not be observed. In the examinations for seedling development, the values obtained on the 14 th, 30 th and 60 th days were determined to be between 9.558-13.910 cm, 12.868-21.410 cm and 34.458- 66.243 cm for root length; 1.55-2.49 cm, 3.198-4.770 cm and 11,843-18,442 cm for seedling length; 0.173-0.280 g, 0.690-1.570 g and 2.508-5.903 g for fresh root weight; 0.170-0.240 g, 0.653-1.608 g and 3.413-16.273 g for fresh shoot weight; 0.018-0.030 g, 0.090-0.170 g and 0.248-0.553 g for dry root weight; 0.018-0.020 g, 0.083-0.188 g and 0.338-1.543 g for dry shoot weight; 0.150-0.250%, 0.600-1.418% and 2.163-5.358% for root moisture content; 0.150-0.220%, 0.570-1.420% and 3.075-14.728% for shoot moisture content, respectively. EC values were determined as 0.640-0.930 µS $cm^{-1}g^{-1}$ on the 30th day and 0.230-0.641 µS $cm^{-1}g^{-1}$ on the 60th day. Considering the general effects of the applications, the best results were obtained in A2B2 applications in the 14-day period, A1B1 in the 30-day period and A2B2 in the 60-day period. According to these results, it was observed that chitosan periodically increased the seedling growth in the maize plant. More research is needed on the effects of chitosan applications on germination and seedling growth.

Key words: *Zea mays*. Bio-stimulant. Storage life. Stamina. Seedling vigor.

RESUMO - Neste estudo, em que se investigou o efeito do recobrimento de quitosana no processo de envelhecimento de uma semente, os tempos rápidos de envelhecimento aplicados não tiveram efeito negativo sobre o valor Gmax das sementes. Portanto, o efeito da quitosana e dos tratamentos de envelhecimento na germinação das sementes e no tempo de germinação nesta fase não pode ser observado. Nos exames para o desenvolvimento das mudas, os valores obtidos no 14º, 30º e 60º dias foram determinados entre 9,558-13,910 cm, 12,868-21,410 cm e 34,458-66,243 cm para o comprimento da raiz; 1,55-2,49 cm, 3,198-4,770 cm e 11.843-18.442 cm para o comprimento da muda; 0,173-0,280 g, 0,690-1,570 ge 2,508-5,903 g para o peso fresco da raiz; 0,170-0,240 g, 0,653-1,608 ge 3,413-16,273 g para peso de rebento fresco; 0,018-0,030 g, 0,090-0,170 ge 0,248-0,553 g para o peso seco da raiz; 0,018-0,020 g, 0,083-0,188 ge 0,338-1,543 g para peso de rebento seco; 0,150-0,250%, 0,600-1,418% e 2,163-5,358% para o teor de umidade da raiz; 0,150-0,220%, 0,570-1,420% e 3,075-14,728% para o teor de umidade do rebento, respectivamente. Os valores de CE foram determinados como 0,640-0,930 µS cm-1g-1 no 30º dia e 0,230-0,641 µS cm-1g-1 no 60º dia. Considerando os efeitos gerais das aplicações, os melhores resultados foram obtidos nas aplicações de A2B2 no período de 14 dias, A1B1 no período de 30 dias e A2B2 no período de 60 dias. De acordo com esses resultados, observou-se que a quitosana aumentou periodicamente o crescimento das mudas na planta de milho. Mais pesquisas são necessárias sobre os efeitos das aplicações de quitosana na germinação e no crescimento das mudas.

Palavras-chave: *Zea mays*. Bioestimulante. Tempo de armazenamento. Resistência. Vigor de mudas.

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INTRODUCTION

Chitosan (Cht) is one of the most significant and crucial carbohydrate biopolymers on earth after cellulose (EL HADRAMI *et al.*, 2010). It exhibits important effects in the control of antibacterial, antifungal and antiviral diseases as it strengthens the plant's natural defense with its high nitrogen content amino groups and high biocompatibility, biodegradability and antimicrobial properties (EL HADRAMI *et al*., 2010). It increases the interaction between plants and microorganisms (KAUR; DHILLON, 2014). In addition, it allows the nitrogen and phosphorus content to increase in plant root and shoot structures with its use as a biofertilizer, thus promoting growth and causing a beneficial effect on yield (AL-GAZALI *et al*., 1997; NGUYEN *et al*., 2019).

Herbal products continue their respiratory and other metabolic activities even after harvest. As a result, undesirable results are encountered, such as aging, rotting and mold. Thanks to edible Cht coatings, it has been observed that products extend fruit color, quality and shelf life (ILIĆ *et al*., 2018; SAAVEDRA *et al*., 2016; SABIR *et al.*, 2019; SHIEKH *et al.*, 2013). On the other hand, Seeds experience profound decreases in germination rate and vigor when exposed to fungal infection and water loss due to long-term storage. Cht coatings reduce respiration and perspiration (TIAN *et al*., 2019), prevent physiological and biochemical changes, oxidation reactions (ADILETTA *et al*., 2018), loss of essential oils, and protect seeds from mechanical and microbial damage (SHIEKH *et al*., 2013).

Because corn is preferable for both consumers and producers, research continues without slowing down, and breeding studies can obtain new varieties. Therefore, it is essential to preserve the current maize collections in gene banks as appropriately as possible to ensure continued and sustainable use of genetic diversity in plant breeding studies for both current and future generations. Maintaining seed viability over extended time in gene banks is critical in conserving plant genetic resources (DESHEVA; PETROVA; DESHEY, 2017).

Seed quality is often reflected by the seed viability, which refers to the aggregated characteristics of seed activities resulting from germination in more comprehensive environments for the storage life of seeds (GU *et al.*, 2017). This determines the potential for rapid and uniform seedling emergence (MONDO *et al.*, 2013; WOLTZ; TEKRONY, 2001) and the probability of developing vigorous seedlings in any field conditions (ASSOCIATION OF OFFICIAL SEED ANALYSTS, 1983, 2002).

Generally, low germination rate and high susceptibility of seeds and seedlings produce various negative consequences in the germination process. This situation leads to irregular shoot and root growth, which leads to a decrease in yield (MARCOS-FILHO, 2005).

Plant researchers are currently using chemicals on different maize genotypes, and some of them have proven to be more efficient than others (ALI; ASHRAF, 2011; SOUZA *et al*., 2013). In addition, different concentrations of the Cht chemical were applied to the corn plant and its positive and negative effects were investigated. However, the issue of how chitosan affects the aging process of corn seeds remains a mystery.

Due to the importance of the corn crop and the good results obtained with the Cht application, the contribution of chitosan to the storage life of the seed has been wondered. This study aimed at performing the physicochemical characterization of this biopolymer and evaluate its effects on germination, early growth, cell cycle, and root morphology for plant growth in different aging conditions of the maize plant.

MATERIAL AND METHODS

Maize seeds were provided by Kahramanmaraş Sütçü İmam University in Kahramanmaraş/Turkey. Chitosan (C3646-25G from shrimp shells, ≥ 75%, deacetylated) was obtained from Sigma-Aldrich company.

Preparation of Chitosan Coating Solutions: Chitosan (Cht) solutions were prepared by dissolving 1 g of chitosan flakes in distilled water (80 mL) containing 2.5 mL of HCI (10 N). To completely dissolve the chitosan, the solution was stirred overnight at room temperature using a magnetic stirrer. After pH adjustment to 5.6 with 0.1 ml of NaOH (2N), the final volume was brought to 100 mL by the addition of distilled water (EL GHAOUTH *et al*., 1992).

Chitosan priming and aging of corn seeds: Primarily, all seeds of each application were surface disinfected by soaking them into 1% Sodium Hypochlorite solution for 5 min and then rinsed five times with sterile distilled water. Then, half of the seeds were then dried for 24 h before being soaked in the chitosan coating solutions for 5 min.

Soaked and unsoaked corn seeds were divided into five groups and subjected to accelerated aging as per International Seed Testing Association (2006) procedure to obtain low vigor (0, 24, 48, 72 and 96 h) seed lot for use in the experiment.

According to this, Factor A1: Seeds are not priming with chitosan; Factor A2: Seeds are priming with chitosan; Factor B: Seeds are aging: B1: 0 h (Control), B2: 24 h, B3: 48 h, B4: 72 h and B5: 96 h.

Experimental Design: Seeds were planted into 5.5-cm-deep flat cells (75 cm^3) for 14 DAS, 9 × 9 × 16 cm volume plastic cups for 30 and 60 DAS containing perlite, peat, sand and red garden soil in the ratio of 1:1:1:1. The seedlings were grown in a natural photoperiod in an unheated glass greenhouse. During the experiment, the seedlings were watered as needed but not fertilized. During the growing period, temperatures in the greenhouse ranged between 20–35 °C during the day and 10–24 °C at night. The procedures were repeated four times in the greenhouse, and all of the cups were arranged in a completely random pattern.

Observations were taken in 3 different periods (14 th, 30 th and 60 th days after planting, DAS) to examine the effects of chitosan on germination and seedling growth; emergences for all samples were determined at the 8 th and 14 th DAS. When the percentage of emergence had stabilized (14 th DAS) in all treatments, seedlings were used to assess the final germination percentage (%) after 8 and 14 days respectively, germination rate (GR), mean germination time (MGT) (PANUCCIO *et al*., 2014). The time (days) it took the ultimate germination percentage to progress from 10% to 90% was used to calculate germination uniformity (FAROOQ *et al*., 2005).

After fourteen, thirty, and sixty days, the seedlings were carefully picked, rinsed with water to remove soil particles, and evaluated for several growth characteristics. The root, shoot, and total seedling length were all measured to determine the growth response. Seedlings were clipped at the root-shoot junction, and the roots and shoots were measured with a ruler (in millimeters) (CHOU; LIN, 1976). An analytical balance was used to determine the fresh weight of the roots and shoots. The plant pieces were dried in an oven at 65 °C for 48 hours until they reached a consistent dry mass, and they were measured on an analytical balance (BUSH, 1995). Then, these values were used to calculate the root-shoot moisture content percentage.

Furthermore, the electrical conductivity measurement used to detect damage in the leaf tissues of plants Kaya, Ak and Hıggs (2003) was made according to the specified method. According to this; 2 plants were selected from each replication of the treatments, 1 cm diameter discs were cut from the last developed leaves, placed in 20 ml dH_2O and shaken in a shaker for 24 hours, then the electrical conductivity of the soaking water was measured, and the permeability of the cell membrane (EC1) was determined. Afterward, the samples were kept in an autoclave set at 121 ºC for 20 minutes, the leaf tissue was completely broken down, and the electrical conductivity (EC2) of the obtained water was measured again. The EC1/EC2 ratio was calculated to arrive at the relative electrical conductivity data. If the value obtained after the calculation is high, the amount of damage in the cell is high (the cell membranes have lost their functionality), and if it is low, the amount of damage is low (the cell membranes are not damaged and are functional). The electrical conductivity readings were taken with a “Hanna Instruments, pH and EC Combo Tester” conductivity meter, and the mean values of the leaf samples were expressed as $S.cm^{-1}g^{-1}$.

Statistical Analysis: Overall, two-way ANOVA (chitosan doses and aging as factors) and non-metric multidimensional scaling (NMDS) with Bray–Curtis similarity as the similarity measure was used to examine effects. We used analysis of variance (ANOVA) to compare all treatments and Duncan for post-hoc comparisons. The calculations were performed using Statistical Analysis Software version 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS AND DISCUSSION

Evaluations related to seedling emergence parameters are elaborated in Table 1. In this study in which the effect of Cht coating on the aging process of the seed was investigated, it was observed that the rapid aging times applied did not have a negative effect on the Gmax value of the seeds, and the seeds of all lots showed 100% germination. Although differences were observed in other parameters related to the germination times, these differences were not statistically significant ($p < 0.05$). Therefore, the effect of chitosan on the germination and germination times of the seeds at this stage could not be observed.

Table 1 - Seedling emergence amount and seedling emergence time values of applications

	Factors	A1		A2		Mean	
Gmax	B1	100.00	± 0.00	100.00	± 0.00	100.00	± 0.00
	B2	100.00	± 0.00	100.00	± 0.00	100.00	± 0.00
	B3	100.00	± 0.00	100.00	± 0.00	100.00	± 0.00
	B4	100.00	± 0.00	100.00	± 0.00	100.00	± 0.00
	B5	100.00	± 0.00	100.00	± 0.00	100.00	± 0.00
	Mean	100.00	± 0.00	100.00	± 0.00	100.00	± 0.00
	F Value	F value could not be calculated because the results obtained from all samples were the same					

Continuation Table 1

T10	B1	1.02	± 0.14	1.04	± 0.12	1.03	± 0.12
	B2	1.02	± 0.14	0.80	± 0.00	0.91	± 0.15
	B3	1.24	± 0.30	0.92	± 0.06	1.08	± 0.26
	B4	1.04	± 0.24	1.24	± 0.30	1.14	± 0.27
	B5	1.03	± 0.22	1.01	± 0.23	1.02	± 0.21
	Mean	1.07	± 0.21	1.00	± 0.22	1.03	± 0.22
	F Value	A: 1.141; B: 1.477; A × B: 2.115					
T50	B1	5.08	± 0.73	5.22	± 0.62	5.15	± 0.63
	B2	5.08	± 0.73	4.00	± 0.00	4.54	± 0.75
	B3	6.21	± 1.51	4.58	± 0.28	5.39	± 1.33
	B4	5.21	± 1.22	6.21	± 1.51	5.71	± 1.38
	B5	5.17	± 1.11	5.03	± 1.17	5.10	± 1.06
	Mean	5.35	± 1.07	5.01	± 1.10	5.18	± 1.09
	F Value	A: 1.140; B: 1.463; A × B: 2.100					
T90	B1	10.00	± 2.31	10.75	± 1.89	10.38	± 2.00
	B2	10.00	± 2.31	7.20	± 0.00	8.60	± 2.13
	B3	9.11	± 0.75	8.75	± 1.50	8.93	± 1.11
	B4	9.93	± 2.71	9.78	± 1.65	9.85	± 2.08
	B5	9.68	± 1.80	8.93	± 1.68	9.30	± 1.66
	Mean	9.74	± 1.89	9.08	± 1.81	9.41	± 1.85
	F Value	A: 1.318; B: 1.220; A × B: 1.043					
T10-90	B1	8.99	± 2.17	9.71	± 1.78	9.35	± 1.87
	B2	8.99	± 2.17	6.40	± 0.00	7.70	± 1.98
	B3	7.87	± 0.51	7.83	± 1.45	7.85	± 1.00
	B4	8.89	± 2.48	8.54	± 1.62	8.71	± 1.95
	B5	8.64	± 1.73	7.92	± 1.57	8.28	± 1.57
	Mean	8.67	± 1.77	8.08	± 1.69	8.38	± 1.74
	F Value	A: 1.213; B: 1.242; A × B: 1.046					
MGT	B1	9.20	± 0.69	9.35	± 0.57	9.28	± 0.59
	B2	9.20	± 0.69	8.00	± 0.00	8.60	± 0.79
	B3	8.65	± 0.34	8.75	± 0.30	8.70	± 0.30
	B4	9.20	± 1.10	9.05	± 0.53	9.13	± 0.80
	B5	8.80	± 0.57	8.65	± 0.50	8.73	± 0.50
	Mean	9.01	± 0.69	8.76	± 0.60	8.89	± 0.65
	F Value	A: 1.764; B: 1.980; A × B: 1.700					

* : $p < 0.05$ and ** : significant at $p < 0.01$ significance level; A: Chitosan; B: Aging

The accelerated aging test is one of the most sensitive and efficient methods used to evaluate seed vigor, allowing a direct relationship between seed behavior and seedling emergence in the field (MARCOS-FILHO, 2015).

In the rapid aging test applied in this study, it was observed that the differences between the aging times were not at a level that would lead to an adequate difference in the germination parameters. For this reason, the effects of the Cht coating process before the aging process could not be observed. However, Reyes-Pérez *et al.* (2021) reported that Cht application increased seed germination from 11.66% to 16.67% and increased seedling emergence performance. In addition, some other studies have shown that it increases the germination rate in rice seeds (ABIRAMI *et al.*, 2021) and peanuts (ZHOU *et al.*, 2002) and supports seedling development in wheat seeds (HAMEED *et al.*, 2014).

Another important parameter when evaluating the physiological quality of the seeds is the seedling emergence test. Healthy seeds that have matured can quickly and adequately form seedlings even under unfavorable conditions. In studies using the seedling emergence test, it was observed that the viability status of different types of seeds significantly affected the seedling percentage, seedling emergence uniformity and speed (DUTRA; TEÓFILO, 2007).

The use of viability testing to determine the performance of seedlings is based on the principle that seeds with high viability will produce more vigorous seedlings, reflecting the efficiency of repair mechanisms, mobilization of reserves and synthesis of new tissues during germination. Accordingly, the irregular emergence of the seedlings may cause delays in the development of plants and irregular growth in various phenological stages, leading to losses in production (MARCOS-FILHO, 2015).

The values of the seedling viability parameters obtained 14 days after the sowing of the chitosan coating and aging times in the seedlings are given in Table 2. Accordingly, the difference created by the Cht application on root length was statistically significant at a $p < 0.01$ significance level and it was seen that Cht increased root length. It was noted that the rapid aging process had no statistically significant effect on root length, while the Cht x Aging interaction caused quite significant differences.

While the A factor did not affect shoot length, it caused significant differences in the B factor. On the other hand, the differences observed in the A × B interaction were significant at a $p < 0.01$ significance level. The highest shoot length value was obtained in the applications at B2 and B3 levels, and they were in the same group statistically. The lowest body length was obtained from the B5 application. Accordingly, it was observed that 24-hour and 48-hour aging applications promoted shoot length in seedlings but had a negative effect on increasing durations.

It was determined that A, B and A x B interaction on root fresh weight property caused statistically significant ($p < 0.01$) differences. It was noted that FRW values varied between 0.200 - 0.223 g according to the coating condition, and the FRW value was higher in Cht coated seeds. On the other hand, FRW aging times varied between 0.190 - 0.248 g. It was determined that the highest FRW value was obtained in B2 and B1 applications, respectively, and they were in the same group statistically. Other applications had lower values and were in the same group among themselves.

While the differences caused by the A and A x B interaction factors on the FSW feature were significant at the $p < 0.05$ level, they were significant at the $p < 0.01$ level for the B factor, in the A factor differences, the FSW values varied between 0.199 - 0.210 g, and the highest value was obtained from the A2 application, FSW values varied between 0.180 - 0.229 g under the efficacy of B factor, and the highest values were obtained from B3 and B2 applications, respectively, the lowest value was recorded in the B5 application.

While only the B factor caused a statistically significant ($p < 0.05$) difference in terms of DRW, it was determined to be insignificant in terms of A and A × B interaction, in terms of the B factor, it was observed that DRW values varied between 0.018 - 0.030 g, the highest value was obtained from B1 and the lowest value from B3 application, the differences observed between DSW values, which is another feature, were not statistically significant ($p < 0.05$) for any factor.

Table 2 - 14 th-day root length (RL) of the seedlings of the treatments; shoot length (SL); fresh root weight (FRW); fresh shoot weight (FSW); dry root weight (DRW); dry shoot weight (DSW); root moisture content (RMC) and shoot moisture content (SMC) values

		A1		A2		Mean	
RL	B1	10.558	± 0.94	12.058	± 0.19	11.31	± 1.02
	B2	12.890	± 0.48	10.813	± 0.63	11.85	± 1.23
	B3	9.558	± 0.78	13.910	± 0.52	11.73	± 2.41
	B4	12.113	± 0.11	11.478	± 1.58	11.80	± 1.09
	B5	11.435	± 0.21	12.268	± 0.00	11.85	± 0.66
	Mean	11.310 b	± 1.31	12.110 a	± 1.31	11.71	± 1.35
	F Value	A: 1146**; B: 0.76; A × B: 21.28**					

Continuation Table 2

SL	B1	2.223	± 0.11	1.823	± 0.07	2.023 b	± 0.23
	B2	2.320	± 0.08	2.143	± 0.10	2.231 a	± 0.13
	B3	1.878	± 0.06	2.490	± 0.14	2.184 a	± 0.34
	B4	1.958	± 0.16	1.743	± 0.10	1.850 c	± 0.17
	B5	1.558	± 0.18	1.890	± 0.13	1.724 d	± 0.23
	Mean	1.99	± 0.30	2.02	± 0.30	2.00	± 0.29
	F Value	A: 0.66; B: 26.53**; A × B: 25.55**					
FRW	B1	0.180	± 0.01	0.280	± 0.02	0.230 a	± 0.06
	B2	0.258	± 0.03	0.238	± 0.02	0.248 a	± 0.03
	B3	0.173	± 0.03	0.220	± 0.01	0.196 b	± 0.03
	B4	0.190	± 0.01	0.198	± 0.01	0.194 b	± 0.01
	B5	0.200	± 0.02	0.180	± 0.01	0.190 b	± 0.02
	Mean	0.200 b	± 0.02	0.223 a	± 0.02	0.212	± 0.03
	F Value	A: 14.04**; B: 14.06**; A × B: 13.90**					
FSW	B1	0.188	± 0.01	0.210	± 0.02	0.199 b	± 0.02
	B2	0.210	± 0.02	0.220	± 0.01	0.215 a	± 0.02
	B3	0.218	± 0.01	0.240	± 0.01	0.229 a	± 0.01
	B4	0.210	± 0.02	0.190	± 0.01	0.200 b	± 0.02
	B5	0.170	± 0.01	0.190	± 0.01	0.180 c	± 0.01
	Mean	0.199 b	± 0.01	0.210 a	± 0.01	0.205	± 0.02
	F Value	A: 6.54*; B: 14.62**; A × B: 3.53*					
DRW	B1	0.030	± 0.01	0.030	± 0.02	0.030 a	± 0.02
	B2	0.028	± 0.01	0.028	± 0.01	0.028 ab	± 0.01
	B3	0.018	± 0.01	0.018	± 0.01	0.018 b	± 0.00
	B4	0.028	± 0.01	0.020	± 0.01	0.024 ab	± 0.01
	B5	0.020	± 0.01	0.020	± 0.01	0.020 ab	± 0.01
	Mean	0.023	± 0.01	0.023	± 0.01	0.024	± 0.01
	F Value	A: 0.23; B: 2.14*; A × B: 0.23					
DSW	B1	0.018	± 0.01	0.018	± 0.01	0.018	± 0.01
	B2	0.020	± 0.01	0.018	± 0.01	0.019	± 0.01
	B3	0.018	± 0.01	0.020	± 0.01	0.019	± 0.01
	B4	0.020	± 0.01	0.020	± 0.01	0.020	± 0.01
	B5	0.020	± 0.01	0.018	± 0.01	0.019	± 0.01
	Mean	0.019	± 0.01	0.019	± 0.01	0.019	± 0.01
	F Value	A: 0.04; B: 0.11; A × B: 0.15					
RMC	B1	0.150	± 0.00	0.250	± 0.05	0.200 ab	± 0.06
	B2	0.230	± 0.03	0.210	± 0.03	0.220 a	± 0.03
	B3	0.158	± 0.02	0.203	± 0.01	0.180 bc	± 0.03
	B4	0.163	± 0.01	0.178	± 0.01	0.170 c	± 0.01
	B5	0.180	± 0.03	0.160	± 0.01	0.170 c	± 0.02
	Mean	0.176 b	± 0.04	0.200 a	± 0.04	0.190	± 0.04
	F Value	A: 10.23**; B: 6.68**; A × B: 9.03**					

Continuation Table 2

SMC	B1	0.170	± 0.00	0.193	± 0.02	0.181 b	± 0.02
	B2	0.190	± 0.03	0.203	± 0.01	0.196 ab	± 0.02
	B3	0.200	± 0.01	0.220	± 0.01	0.210 a	± 0.02
	B4	0.190	± 0.02	0.170	± 0.01	0.180 b	± 0.02
	B5	0.150	± 0.01	0.173	± 0.01	0.161 a	± 0.02
	Mean	0.180 b	± 0.02	0.192 a	± 0.02	0.19	± 0.02
	F Value	A: 5.20*; B: 10.64**; A × B: 2.57					

* : $p < 0.05$ and ** : significant at $p < 0.01$ significance level; A: Chitosan; B: Aging

When the RMC values were examined, it was determined that the A, B and A × B interaction effects caused significant differences. Accordingly, the RMC values varied between 0.176 - 0.200 g under the efficacy of the A factor, and the highest value was obtained from the A2 application. On the other hand, RMC values varied between 0.170 - 0.220 g under the efficacy of factor B. The highest RMS value was obtained from the B2 application, while the lowest was obtained from the B4 and B5 applications. There was no statistical difference between B4 and B5 applications, and they were in similar group.

It was noted that the SMC values were significantly affected by the A factor at the 5% significance level and the B factor at the 1% significance level and caused differences. On the other hand, the A × B interaction was determined to be insignificant. It was observed that the SMC values varied between 0.180 - 0.192 g under the efficacy of the A factor, and the highest value was obtained from the A2 application. It was determined that the SMC values varied between 0.180 - 0.210 g with the effect of the B factor, the highest values were observed in B3 and B5 applications, and the lowest values were observed in B4 and B1 applications.

30 days after planting, the observations of the plants were repeated and the results obtained by making EC analysis are given in Table 3. Accordingly, it was determined that the differences observed in RL values with the effect of A, B and A × B interaction were statistically significant. The RL value in the A factor varied between 16.633 - 18.428 cm, and the highest value was observed in the A2 application. On the other hand, under the effect of the B factor, RL values changed between 14.389 (B1) -19.531 (B2) cm. While the values obtained from the B3 and B4 applications were in the same group as B1, it was noted that an intermediate value was obtained in the B5 application.

When the data were evaluated in terms of SL features, it was observed that the effect of factor B ($p < 0.01$) and A × B interaction ($p < 0.05$) caused significant differences. While SL values varied between 4.044 and 4.208 cm under the A factor effect, they changed between 3.394 - 4.759 cm under the B factor effect. In the B factor, it was observed that the lowest SL values were obtained from B1 and the highest value from B2.

When the FRW feature was examined, it was seen that all factors and interactions had a significant ($p < 0.01$) effect on the values obtained. While the FRW value in the A factor varied between 0.858 - 1.113 g, it ranged between 0.885 - 1.371 g in the B factor. The highest value in terms of the A factor was obtained from A1. The highest value was recorded in B1, the lowest value in B2 application in the B factor.

The differences caused by the A, B and A × B interaction factors on the FSW feature were statistically significant at the $p < 0.01$ level, different from the 14 th-day values. In the A factor differences, the FSW values varied between 0.887 - 1.132 g, and the highest value was obtained from the A1 application. Under the efficiency of the B factor, FSW values varied between 0.818 - 1.409 g; the highest value was found in the B1 application and the lowest value in the B4 application, respectively.

In terms of DRW, only the effect of factor B caused significant ($p < 0.01$) differences. While the values in the A factor differences varied between 0.111 - 0.114 g, the values in the B factor differences varied between 0.095 - 0.161 g. While the highest value in the B factor was obtained from the B1 application and the lowest value was obtained from the B4 application, all applications except for B1 were statistically in the same group.

DSW values showed significant differences in terms of all factors and interactions of the applications. DSW values at factor A ranged from 0.11 (A2) to 0.14 (A1) g. In the B factor, these values were recorded to vary between 0.10 (B4) – 0.18 (B1) g.

Significant changes were detected in the data related to the RMC property with the effects of A, B and A × B interactions. Here, while the data with the effect of A factor changed between 0.75 - 1.00 g, it was seen that the A2 factor caused a decrease in the amount

Table 3 - 30 th-day root length (RL) of the seedlings of the treatments; shoot length (SL); fresh root weight (FRW); fresh shoot weight (FSW); dry root weight (DRW); dry shoot weight (DSW); root moisture content (RMC); shoot moisture content (SMC) and electrical conductivity (EC) values

		A1		A2		Mean	
RL	B1	19.020	± 0.91	20.043	± 0.70	19.531 a	± 0.93
	B2	12.868	± 2.60	15.910	± 1.04	14.389 c	± 2.45
	B3	19.333	± 0.51	17.433	± 1.56	18.383 a	± 1.48
	B4	16.288	± 0.82	21.410	± 3.49	18.849 a	± 3.61
	B5	15.658	± 0.34	17.343	± 1.58	16.500 b	± 1.39
	Mean	16.633 b	± 2.70	18.428 a	± 2.68	17.530	± 2.81
	F Value	A: 11.780**; B: 12.730**; A × B: 4.919**					
SL	B1	3.390	± 0.33	3.198	± 0.29	3.394 c	± 0.30
	B2	5.278	± 0.90	4.240	± 0.75	4.759 a	± 0.94
	B3	4.233	± 0.85	4.770	± 0.97	4.501 ab	± 0.90
	B4	3.788	± 0.26	4.530	± 0.39	4.159 ab	± 0.50
	B5	3.533	± 0.51	4.300	± 0.44	3.916 bc	± 0.60
	Mean	4.044	± 0.89	4.208	± 0.78	4.126	± 0.83
	F Value	A: 0.686; B: 6.560**; A × B: 3.089*					
FRW	B1	1.570	± 0.04	1.173	± 0.03	1.371 a	± 0.21
	B2	0.820	± 0.01	0.790	± 0.04	0.805 d	± 0.03
	B3	1.180	± 0.01	0.690	± 0.02	0.935 b	± 0.26
	B4	0.970	± 0.01	0.890	± 0.02	0.930 b	± 0.04
	B5	1.023	± 0.02	0.748	± 0.01	0.885 c	± 0.15
	Mean	1.113 a	± 0.26	0.858 b	± 0.18	0.985	± 0.26
	F Value	A: 1259.713**; B: 766.731**; A × B: 152.849**					
FSW	B1	1.608	± 0.02	1.210	± 0.01	1.409 a	± 0.21
	B2	0.828	± 0.01	1.040	± 0.05	0.934 c	± 0.12
	B3	1.368	± 0.01	0.653	± 0.01	1.010 b	± 0.38
	B4	0.758	± 0.01	0.878	± 0.01	0.818 e	± 0.07
	B5	1.100	± 0.01	0.653	± 0.01	0.876 d	± 0.24
	Mean	1.132 a	± 0.33	0.887 b	± 0.22	1.009	± 0.30
	F Value	A: 1685.881**; B: 1229.416**; A × B: 877.892**					
DRW	B1	0.153	± 0.05	0.170	± 0.01	0.161 a	± 0.03
	B2	0.090	± 0.00	0.103	± 0.01	0.096 b	± 0.01
	B3	0.118	± 0.01	0.090	± 0.01	0.104 b	± 0.02
	B4	0.100	± 0.02	0.090	± 0.01	0.095 b	± 0.01
	B5	0.110	± 0.04	0.100	± 0.02	0.105 b	± 0.03
	Mean	0.114	± 0.03	0.111	± 0.03	0.112	± 0.03
	F Value	A: 0.262; B: 13.174**; A × B: 1.452					
DSW	B1	0.188	± 0.03	0.163	± 0.01	0.175 a	± 0.02
	B2	0.113	± 0.00	0.128	± 0.01	0.120 b	± 0.01
	B3	0.168	± 0.01	0.083	± 0.01	0.125 b	± 0.05
	B4	0.108	± 0.01	0.090	± 0.01	0.099 c	± 0.01
	B5	0.140	± 0.03	0.083	± 0.01	0.111 bc	± 0.04
	Mean	0.143 a	± 0.04	0.109 b	± 0.03	0.126	± 0.04
	F Value	A: 49.885**; B: 29.131**; A × B: 12.567**					

Continuation Table 3

RMC	B1	1.418	± 0.08	1.003	± 0.04	1.210 a	± 0.23
	B2	0.733	± 0.01	0.688	± 0.03	0.710 d	± 0.03
	B3	1.063	± 0.01	0.600	± 0.03	0.831 b	± 0.25
	B4	0.870	± 0.01	0.800	± 0.02	0.835 b	± 0.04
	B5	0.913	± 0.02	0.648	± 0.02	0.780 c	± 0.14
	Mean	0.999 a	± 0.24	0.748 b	± 0.15	0.873	± 0.24
	F Value	A: 590.682**; B: 283.842**; A × B: 68.647**					
SMC	B1	1.420	± 0.01	1.048	± 0.02	1.234 a	± 0.20
	B2	0.713	± 0.02	0.913	± 0.05	0.813 c	± 0.11
	B3	1.200	± 0.01	0.570	± 0.02	0.885 b	± 0.34
	B4	0.650	± 0.01	0.790	± 0.01	0.720 e	± 0.08
	B5	0.960	± 0.02	0.570	± 0.02	0.765 d	± 0.21
	Mean	0.989 a	± 0.30	0.778 b	± 0.19	0.883	± 0.27
	F Value	A: 908.928**; B: 691.251**; A × B: 539.287**					
EC	B1	0.808	± 0.01	0.673	± 0.01	0.740 c	± 0.07
	B2	0.853	± 0.01	0.640	± 0.01	0.746 c	± 0.11
	B3	0.793	± 0.01	0.810	± 0.01	0.801 a	± 0.01
	B4	0.930	± 0.01	0.657	± 0.00	0.794 a	± 0.15
	B5	0.813	± 0.01	0.703	± 0.01	0.758 b	± 0.06
	Mean	0.839 a	± 0.05	0.697 b	± 0.06	0.768	± 0.09
	F Value	A: 3045.176**; B: 93.774**; A × B: 364.363**					

* : $p < 0.05$ and ** : significant at $p < 0.01$ significance level; A: Chitosan; B: Aging

of root moisture of maize. Under the influence of the B factor, the values varied between 0.71 - 1.21 g and the highest value was recorded in B1 and the lowest value in the B2 application. Here, the Cht coating effect on the root moisture content was low. On the other hand, although there is no stable change in the aging factor which is dependent on old age, it was determined that the root moisture content of the control group (B1) plants was higher than the others.

The effects of A, B and A x B interaction in shoot moisture content caused significant ($p < 0.01$). While the SMC values changed between 0.78 (A2) - 0.99 (A1) under the influence of the A factor, they changed between 0.72 (B4) - 1.23 (B1) under the influence of the B factor. As in the root, although the highest value in the shoot was obtained from the B1 application, the lowest value was obtained from the B4 application. Here, it was observed that there was a relative consistency between the aging levels compared to the root part, and the moisture content decreased with the increase in the aging effect.

Finally, it was determined that the EC parameter was significantly affected by the interaction of A, B and A × B. The values in the A factor varied between 0.70 and 0.84, and it was noted that this value was lower in the coating application with Cht. In the B factor, the values varied between 0.74 (B1) -0.80 (B4). Here, although there are fluctuations between the aging times, it was observed that the EC value increased with the increase in the aging time.

The values of the parameters examined on the 60 th day of seedling development are given in Table 4. In terms of root length value, the difference in 60 th-day values was insignificant in terms of A, B and A × B interactions. While the RL value varied between 10.24 - 44.68 cm under the A factor effect, it changed between 36.66 - 53.50 cm under the B factor effect. Here, it is thought that the environmental effects on the 60 th-day root length decrease and the plant recovers itself.

While the effect of factor A did not cause differences in SL values, the effect of B and A × B interaction was significant ($p < 0.05$). While the SL value in the A factor was 14.43 - 14.65 cm, it was between 12.55 - 16.33 cm in the B factor. Here, the lowest value was obtained from the control group plants, and the highest values were determined in the B2 and B3 group plants.

In the FRW feature, A factor of 5%, B and A × B interaction created significant differences at 1% significance level. Here, a higher FRW value was obtained in the plants without Cht than the treated plants. Under the efficiency of the B factor, the values varied between 3.36 and 4.41 g, while the highest value was determined in the B3 group and the lowest in the B1 group.

When the DRW feature was examined, it was noted that while the A and A × B interaction effect created significant ($p < 0.01$) differences in the values, the B factor did not affect the DRW values. Here, it was found that the Cht application (0.36 g) decreased the DRW value compared to the control group (0.47 g).

While FSW value was affected by the B factor and A × B interaction ($p < 0.01$), it was not affected by the A factor, in factor A, FSW values ranged between 8.10 - 8.34 g, while in factor B, it varied between 5.46 - 11.16 g, the lowest value in the B factor effect was observed in the B1 (control) group, while the highest value was observed in the B2 and B3 group plants.

Again, DSW values were not affected by the A factor in FSW but were significantly affected by the B and A × B interaction. While the values in the B factor varied between 0.61 - 1.20 g, the lowest value was B1; the highest values were determined from B2 and B3 group plants.

On the other hand, it was noted that factor A did not significantly affect both RMC and SMC properties, and the effect of B and A x B interaction was significant at a $p < 0.01$ significance level. Under the efficiency of the B factor, RMC values ranged between 2.91 - 3.95 g, while the highest value was B3; It was observed that the lowest value was obtained from the B1 application. SMC values varied between 4.85 - 9.96 g; the lowest value was

Table 4 - 60th day of the seedlings of the applications, root length (RL); shoot length (SL); fresh root weight (FRW); fresh shoot weight (FSW); dry root weight (DRW); dry shoot weight (DSW); root moisture content (RMC); body moisture content (SMC) and electrical conductivity (EC) values

		A1		A2		Mean	
RL	B1	45.930	± 18.34	46.043	± 19.61	45.986	± 17.58
	B2	40.755	± 8.09	66.243	± 17.60	53.499	± 18.61
	B3	38.183	± 16.03	39.620	± 17.54	38.901	± 15.57
	B4	36.253	± 5.89	37.058	± 18.85	36.655	± 12.93
	B5	40.093	± 19.91	34.458	± 14.51	37.275	± 16.41
	Mean	40.243	± 13.53	44.684	± 19.63	42.463	± 16.80
	F Value	A: 0.742; B: 1.562; A × B: 1.101					
SL	B1	13.263	± 2.53	11.843	± 1.83	12.553 b	± 2.18
	B2	14.213	± 1.13	18.442	± 2.77	16.328 a	± 2.99
	B3	14.073	± 3.19	17.468	± 1.20	15.770 a	± 2.88
	B4	15.943	± 2.42	12.578	± 1.60	14.260 ab	± 2.62
	B5	14.678	± 3.62	12.900	± 2.15	13.789 ab	± 2.91
	Mean	14.434	± 2.57	14.646	± 3.32	14.540	± 2.93
	F Value	A: 0.080; B: 3.295*; A × B: 4.046*					
FRW	B1	4.213	± 0.20	2.508	± 0.08	3.360 d	± 0.92
	B2	3.973	± 0.58	4.145	± 0.53	4.059 ab	± 0.52
	B3	2.908	± 0.15	5.903	± 0.48	4.405 a	± 1.63
	B4	4.383	± 0.76	3.318	± 0.32	3.850 bc	± 0.79
	B5	4.430	± 0.27	2.595	± 0.27	3.513 cd	± 1.01
	Mean	3.981 a	± 0.70	3.694 b	± 1.33	3.837	± 1.06
	F Value	A: 4.718*; B: 8.043**; A × B: 45.629**					
DRW	B1	0.553	± 0.02	0.343	± 0.02	0.448	± 0.11
	B2	0.423	± 0.14	0.420	± 0.10	0.421	± 0.11
	B3	0.368	± 0.07	0.540	± 0.09	0.454	± 0.12
	B4	0.533	± 0.14	0.255	± 0.03	0.394	± 0.18
	B5	0.475	± 0.04	0.248	± 0.11	0.361	± 0.14
	Mean	0.470 a	± 0.11	0.361 b	± 0.13	0.416	± 0.13
	F Value	A: 15.494**; B: 1.548; A × B: 9.338**					

Continuation Table 4

FSW	B1	6.608	± 0.92	4.308	± 0.19	5.458 c	± 1.37
	B2	9.815	± 2.14	12.503	± 0.37	11.159 a	± 2.02
	B3	4.983	± 1.06	16.273	± 0.19	10.628 a	± 6.08
	B4	10.818	± 0.69	3.413	± 0.28	7.115 b	± 3.99
	B5	9.460	± 0.34	4.010	± 0.55	6.735 b	± 2.94
	Mean	8.337	± 2.48	8.101	± 5.42	8.219	± 4.16
	F Value	A: 0.720; B: 66.192**; A × B: 145.574**					
DSW	B1	0.775	± 0.07	0.440	± 0.02	0.608 b	± 0.18
	B2	1.188	± 0.50	1.215	± 0.12	1.201 a	± 0.34
	B3	0.550	± 0.14	1.543	± 0.09	1.046 a	± 0.54
	B4	1.033	± 0.13	0.338	± 0.14	0.685 b	± 0.39
	B5	1.028	± 0.11	0.465	± 0.11	0.746 b	± 0.32
	Mean	0.915	± 0.32	0.800	± 0.51	0.857	± 0.42
	F Value	A:3.607; B: 14.219**; A × B: 25.199**					
RMC	B1	3.663	± 0.19	2.163	± 0.05	2.913 d	± 0.81
	B2	3.530	± 0.45	3.723	± 0.45	3.626 ab	± 0.43
	B3	2.540	± 0.12	5.358	± 0.41	3.949 a	± 1.53
	B4	3.850	± 0.62	3.060	± 0.30	3.455 bc	± 0.62
	B5	3.955	± 0.24	2.348	± 0.28	3.151 cd	± 0.89
	Mean	3.508	± 0.62	3.330	± 1.22	3.419	± 0.96
	F Value	A: 2.550; B: 10.573**; A × B: 53.730**					
SMC	B1	5.833	± 0.85	3.870	± 0.18	4.851 c	± 1.19
	B2	8.630	± 1.63	11.288	± 0.29	9.959 a	± 1.79
	B3	4.438	± 0.95	14.728	± 0.11	9.583 a	± 5.54
	B4	9.783	± 0.62	3.075	± 0.15	6.429 b	± 3.61
	B5	8.430	± 0.23	3.548	± 0.48	5.989 b	± 2.63
	Mean	7.423	± 2.20	7.302	± 4.92	7.362	± 3.76
	F Value	A: 0.287; B: 81.177**; A × B: 181.615**					
EC	B1	0.612	± 0.01	0.607	± 0.01	0.610 a	± 0.01
	B2	0.641	± 0.00	0.582	± 0.01	0.612 a	± 0.03
	B3	0.497	± 0.00	0.471	± 0.01	0.484 c	± 0.02
	B4	0.230	± 0.00	0.440	± 0.01	0.335 d	± 0.11
	B5	0.524	± 0.02	0.529	± 0.00	0.527 b	± 0.01
	Mean	0.501 b	± 0.15	0.526 a	± 0.07	0.513	± 0.11
	F Value	A: 66.531**; B: 1085.536**; A × B: 238.307**					

* : $p < 0.05$ and ** : significant at $p < 0.01$ significance level; A: Chitosan; B: Aging

obtained from the B1 group, while the highest values were obtained from the B2 and B3 applications.

Finally, when the EC parameter was examined, it was seen that all applications caused significant differences. The values in the A factor varied between 0.50 - 0.53, and it was seen that the Cht coating increased the EC value. In the B factor, the values were found to vary between 0.34 and 0.61. While the highest values in terms of EC values were observed in B1 and B2 applications, it was observed that the EC value decreased with increasing B doses even though it was unstable.

In this study, where the seedling emergence test was applied, the grown plants were evaluated in terms of many characteristics on the 14 th, 30 th and 60 th days after planting. Among the examined features, while the root length values increased on the 14 th and 30 th days in the Cht application, no significant change was observed in the 60 th-day data. However, it was noted that the root lengths decreased only on the 30 th day in the aging process. The results obtained on the 60 th day determined that the root lengths were not different in any of the applications. This situation shows that depending on the seedling development, the effects of Cht and aging, that is, the differences between these applications disappear.

On the other hand, studies have reported that the effects of chitosan vary and depend on the type of product used, product properties, molecular size, method of use, concentrations, plant species and growing conditions (KANAWI; AL HAYDAR; RADHI, 2021; RUIZ-DE-LA-CRUZ *et al*., 2017). Kanawi, Al Haydar and Radhi (2021) and Parfenova, Lasareva and Azovtseva (2020) also reported that chitosan significantly increased the shoot length, but no significant change in shoot length was observed at any stage of this study when seed coating with Cht was examined. However, the increase in the aging period decreased the SL value on the 14 th day, increased it at the 30 th and 60 th days, and then decreased it afterwards. In addition, as the seedling development time increased, the differences in SL values between applications decreased.

In the data related to fresh root weight, chitosan had a positive effect in the first 14-day period, while root weight decreased in seedlings of Cht-coated seeds on the 30 th and 60 th days. The Cht application did not affect the 14 th and 30 th days on the root dry weight, while a significant decrease was observed on the 60 th day. In the first 14-day period, the root fresh weight of chitosan increased the water uptake but did not affect the root dry weight, while reducing the fresh weight in 30 days did not affect the dry weight. In the subsequent measurement, it caused a decrease in both wet and dry weights. In aging applications, increasing aging time on the 14 th and 30 th days decreased the fresh root weight, and on the 60 th day, it first increased and then decreased compared to the increasing doses. While significant decreases were observed in dry root weight on 14 th and 30 th days, no change was observed in the 60 th-day dry weight. Zayed *et al.* (2017) found that chitosan significantly increased fresh root and dry weights; Odat *et al.* (2021) stated that even with the increase of NaCl salinity concentration, the seeds were covered with different levels of Cht (2, 4 and 8 g l^{-1}) and reported that it improved growth parameters and alleviated salt stress.

While the effect of chitosan was positive on the fresh shoot weight values, on the 14 th-day data, no effect was observed on the dry weight. During this period, it was understood that the water uptake rate of the plants increased, but it was not effective on the dry weight. On the 30 th day, which is the following period, the presence of chitosan resulted in a decrease in both wet weight and dry weight. However, by the 60 th day, it was noted that it had no effect on both parameters.

Chitin and Cht are known as stimulators of photosynthetic reaction and (C, O, N) act as a biostimulator to increase plant growth through minerals containing and positively affect fresh-dry shoot weight values (AMINE *et al.*, 2020; KHAPTSEV *et al.*, 2021). However, the effects of chitosan vary according to the plant, application method and dose (KANAWI; AL HAYDAR; RADHI, 2021; RUIZ-DE-LA-CRUZ, 2017). Regarding this, Tovar *et al*. (2020) presented an increase in dry leaf weight of plants growing under the influence of chitosan. These values mean that the leaf invests less biomass per unit area and improves water transport from the root (MARTÍNEZ-FERNÁNDEZ; KOMÁREK, 2016). This effect of chitosan has also been reported on metal nanoparticles encapsulated with this polysaccharide as an effective growth promoter in maize crops (CHOUDHARY *et al*., 2019). Behboud, Moradi and Farajee (2020) reported that seed treatment with 0.5% Cht could reduce the detrimental effects of osmotic potential on some germination, biochemical properties and improve seedling growth in sweet corn seedlings.

It was noted that root and stem moisture content values increased significantly with the effect of Cht coating on the 14 th day, decreased on the 30 th day, and the effect was insignificant on the 60 th day. However, the aging application caused a decrease in the RMC values on the 14 th and 30 th days depending on the increasing dose, and increased before the 60 th day and then decreased. It was determined that the aging application caused an increase and then a decrease in SMC values on the 14 th and 60 th days. In addition, it was noted that SMC values decreased on the 30 th day depending on the increasing doses in the aging application.

Another established methodology for assessing the vigorous seedling potential of seed lots is electrical conductivity testing. Intact seeds have lower conductivity values due to their robust cell systems. The lower the membrane integrity, the higher the electrical conductivity values in the test; in practice, this affects the initial development of seedlings during germination (DUTRA; MEDEIROS FILHO; TEÓFILO, 2006).

In the analysis of the EC value, it was noted that the effect of chitosan on the 30th day was positive; that is, it decreased the EC value. However, it was determined that the EC value was high in the seedlings of the seeds treated with Cht on the 60 th day. On the other hand, it was observed that the aging application increased the EC value on the 30 th day and decreased it on the 60 th day. Accordingly, it was understood that the adverse effects of the aging application on the 30 th day were higher.

CONCLUSIONS

1. This study investigated the effects of Cht coating of maize seeds on seed viability and seedling growth. In this study, the intervals of applied aging periods did not cause a distinctive difference in germination parameters in the maize plant. In order to investigate the effect of chitosan on seed aging, it is recommended to be aged for more extended periods. On the other hand, according to the plant development stages, the application's effect mechanism has changed causing some changes in the investigated parameters. While chitosan caused an increase in all parameters examined in the first 14-day period, it caused significant decreases in the 30 th day. It was ineffective in almost all parameters at day 60 with increased seedling development. In the aging application, adverse effects were observed in all parameters until the first 30 days, but it was determined that this negative effect fluctuated on the 60 th day and increased first depending on the aging period, but adverse effects were observed in further aging;
2. In other studies, because chitosan has different effects depending on its chemical content, molecular weight, application method, application time, and plant species and varieties, more studies should be required in this area to determine the method and dose that will positively affect germination and seedling growth in the corn plant.

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