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Magneto-Priming of Seeds Decreases the Saline Effect of Wastewater Irrigation on Broccoli Germination and Seedling Growth

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Abstract: Crop plant varieties exhibit diverse reactions when subjected to wastewater irrigation in terms of seed germination, seedling development, and overall productivity. Magneto-priming, which involves treating seeds with an appropriate magnetic field, is gaining popularity as the preferred technique due to its effectiveness and environmentally friendly characteristics for improving seed vigour, growth, and plant yield. In this study, magneto-primed and non-primed broccoli seeds were irrigated with distilled or wastewater and kept under observation for a 10-day period to record seedling growth. A laboratory study was conducted to evaluate the impact of magneto-priming on broccoli seeds with a homogeneous stationary magnetic-field strength of 5.9 mT for 1 h. They were irrigated with two types of water: distilled and wastewater. Another test was performed to evaluate the effect of 1-h and 2-h magneto-priming on seed germination when seeds were irrigated with wastewater. From the results, the broccoli seedlings irrigated with distilled water grew higher and heavier than the ones irrigated with wastewater, probably due to the significant amounts of salts in organic wastewater. Nonetheless, the saline effect of wastewater was ameliorated when seeds were previously magneto-primed. All the germination parameters of broccoli seeds irrigated with wastewater were significantly reduced when seeds were magneto-primed for both periods.

Keywords: magnetic treatment; salinity; sewage water; *Brassica oleracea*; sprout

Citation: Gutierrez, J.; Alonso, F.; Alvarez, J.; Carbonell, M.V.; Martinez, E.; Florez, M.; Delgado, M.d.M.; Franco, B.K.; Hernandez-Aguilar, C. Magneto-Priming of Seeds Decreases the Saline Effect of Wastewater Irrigation on Broccoli Germination and Seedling Growth. *Seeds* **2024**, *3*, 169–178. https://doi.org/10.3390/seeds3010013

Academic Editor: Łukasz Wojtyla

Received: 13 December 2023
Revised: 29 February 2024
Accepted: 9 March 2024
Published: 18 March 2024

1. Introduction

Water is one of the most precious resources and abundant compounds on Earth's surface. However, the expansion of urban populations and their use of domestic water supplies, recreational irrigation, and agriculture for food production has increased the freshwater demand [1]. To protect freshwater supplies, treated wastewater was introduced as an alternative; in addition, due to the scarcity of water for irrigation in recent years, attempts have been made to compensate for the mentioned scarcity by applying wastewater as a low-quality water resource for agriculture. In arid and semi-arid countries, since water use is often low, sewage tends to be very strong in comparison with that in water-abundant areas [2,3]. There are wastewater treatment plants, but the existing ones do not have sufficient capacity to treat such a large volume of wastewater [4]. Although wastewater reuse appears to be a water resource, its reuse for irrigation is not new; there is literature about wastewater used for irrigation in ancient Greek in the Minoan civilization [5,6]. In general, different crop plant species differ widely in response to wastewater irrigation with respect to seed germination, seedling growth, and productivity [7,8]. There are different resources of wastewater used for irrigation from local or industrial wastewater to mechanically purified or fully purified and biologically treated wastewater, and consequently, their quality is also different. Most studies in the literature deal with the different treatments of wastewater depending on the resources, but they agree that salinity is one of the most important parameters of wastewater, and its reuse in agriculture can cause substantial damage to crops.

Addressing the demands of the agricultural market has made it imperative to enhance seed quality, promote earlier germination, boost plant vigour, and increase overall crop yield [9]. The germination process is initiated with water absorption and culminates in the appearance of the embryo, typically with the radicle emerging first through the surrounding structure [10]. Ranal and Santana identified the reciprocal of mean germination time as a fundamental parameter for describing germination rate [11].

Seed priming, a widely employed practice by seed technologists on commercial seed lots, aims to improve seed vigour in terms of germination potential and stress tolerance [12]. Defined by the International Seed Testing Association (ISTA) as the sum of properties determining seed activity and performance during germination and seedling emergence, vigour is a crucial aspect [13]. Seed priming, involving controlled hydration of seeds before planting, has been utilized in various vegetables to enhance seed vigour, germination speed, total germination rate, and seedling uniformity, particularly under unfavourable environmental conditions, leading to improved crop standards and higher yields [14].

Dating back to the ancient Greek practices recommended by Theophrastus (372–287 BC), seed priming involves pre-soaking seeds in milk or water for earlier germination [15,16]. Various seed-priming methods have been successfully employed to invigorate seeds, ensuring uniformity, earlier and higher germination rates, and favourable impacts on plant growth, nutrient utilization, and stress tolerance [17,18].

Previous studies have indicated that magnetic treatment at 125 mT and 250 mT stimulates early growth stages and enhances the germination rate of rice, wheat, and barley seeds. Similarly, maize seed germination showed a reduced time requirement for each magnetic treatment compared to control values, resulting in a higher germination rate for treated seeds. Measurements of seedling growth at 7 and 10 days after sowing confirmed these effects, revealing significant differences in the length and weight of maize seedlings subjected to magnetic fields of 125 mT and 250 mT for varying durations compared to the control. Positive responses have also been observed in grasses; exposure to a magnetic field promotes earlier germination, increases the number of germinated seeds, reduces germination velocity, and enhances stem and root length of *Festuca arundinacea* Schreb. and *Lolium perenne* L. seeds. Additionally, studies on pea and lentil seeds exposed to 125 mT and 250 mT magnetic fields demonstrated increased growth parameters, including total and stem weight and total and stem length, measured on days 7 and 14, along with improved root development [19].

In summary, it is now generally accepted that magneto-priming of seeds and seedlings accelerates the germination and growth of various plant species. Furthermore, magnets have been progressively replaced by coils, providing greater flexibility and effectiveness with lower magnetic-field values.

Therefore, it is considered of great importance to continue research in a field that has been little-studied so far, which involves observing the effects of magneto-priming on seeds and seedlings subjected to extreme or challenging conditions for the species, such as high temperatures or very strong salinity conditions, to verify if these beneficial effects also occur under those circumstances.

One of the main problems for the use of wastewater in seed-production systems is that it has a saline condition, which is stressful for the plants. As mentioned by several authors, salinity stress reduces germination, which is why it is necessary to develop new methods that can help to make use of wastewater that has a saline condition. Physical methods such as magnetic-field treatment have been reported to have a beneficial effect on the salinity problem.

In this sense, the aim of the present work was to evaluate the effects of magnetic-field treatment on the germination of broccoli (*Brassica oleracea.* L) seeds that were irrigated with distilled water and wastewater.

2. Material and Methods

In this study, growth and germination tests were performed under laboratory conditions to evaluate whether the magneto-priming of broccoli seeds ameliorates the saline effect of irrigation with wastewater. In the growth test, magneto-primed and non-primed broccoli seeds were irrigated with distilled or wastewater and kept under observation for a 10-day period to record seedling growth. The test evaluated the impact of magneto-priming on broccoli seeds with a homogeneous stationary magnetic-field strength of 5.9 mT for 1 h on the growth of broccoli plants. They were irrigated with two types of water: distilled and wastewater. Comparisons with non-treated seeds were also carried out. The germination test was used for plotting the cumulative germination curves and obtaining the parameters most used to determine the germination rate. The laboratory study evaluated the impact of magneto-priming on broccoli seeds with the same stationary magnetic-field strength mentioned above for 1-h and 2-h exposure periods, respectively, in a chamber at 22 °C.

Seeds. Broccoli (*Brassica oleracea*. L) seeds belong to the cruciferous family Brassicaceae and are biennial vegetable crops found mostly in Europe. Broccoli is rich in proteins, minerals, vitamins, and antioxidants. Broccoli seeds of the variety Green Calabrese Natalino were provided by the brand "BATLLE", which ensured homogeneity and quality. The optimal temperature range recommended for their germination is 15–24 °C.

Wastewater was provided by a wastewater treatment plant, EDAR Viveros (Madrid), and its physicochemical characteristics were pH 7.66, conductivity 4.49 dS/m, COD 43.30 mg O_2/L, BOD_5 15.66 mg O_2/L, TOC 37.5 mg C/L, TSS 6.67 mg/L. NH_3 48 g/L, N 65.67 mg/L, and $p < 0.08$ mg/L. The vegetable substratum used in the experiment has the following compounds: sand, Puzolana, vegetal compost, coconut fibre, and perlite.

Magneto-priming. Seed magneto-priming was set up by placing seeds in the axis of a pair of Helmholtz coils with a radius R = 15 cm, switched in parallel and separated by the same distance of the radius R, and plugged into a power supply. Control seeds were placed in an analogous structure as described above, but coils were not plugged. The number of turns of each coil was 124, the selected current intensity was I = 8 A, and the magnetic-field intensity was B = 5.9 mT (Figure 1). Superposition of these two magnetic fields guaranteed a largely uniform magnetic field in the axis of the two Helmholtz coils and depends on the circulating current I according to the Equation (1):

$$B = \left(\frac{4}{5}\right)^{\frac{3}{2}} \frac{\mu_o I n}{R} \quad (1)$$

where $\mu_0 = 4\pi \cdot 10^{-7}$ N/A^2 is the permeability constant, n is the number of turn, and R is the radius of coils.

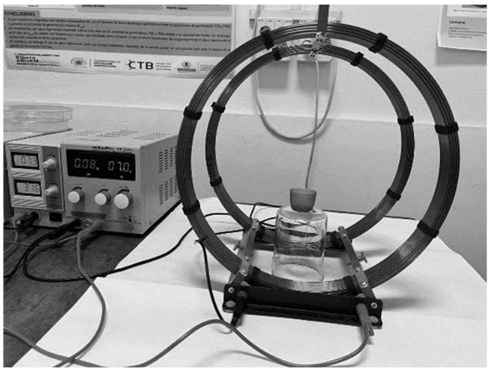
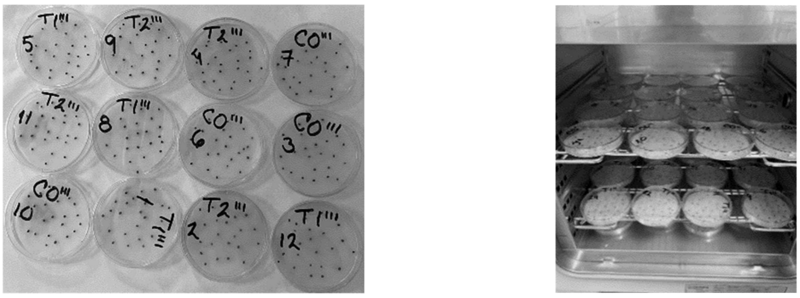


Figure 1. Plastic box containing seeds in the axis of a pair of Helmholtz coils.

Experimental design of germination test. Seeds were soaked in distilled water for 2 h, and after that, groups of 100 seeds were magnetically primed for 1 h or 2 h, as explained in the magneto-priming section, and another group was used as control. Groups of 100 seeds (four repetitions for the control and another for each exposure time) of 25 seeds each were placed in 9 cm Petri dishes on filter papers soaked with 12 mL of wastewater and were randomly placed into the chamber, as shown in Figure 2, to ensure a homogeneous temperature during the germinating process. The conditions in the chamber were 22 °C in darkness, except when counting the seeds outside the chamber, which was at 500 lux. As for humidity, it ranged between 70 and 80%. The Petri dishes were labelled according to the time of magnetic treatment (Table 1).



(**a**) (**b**)

Figure 2. (**a**) Labelled Petri dishes; (**b**) Petri dishes randomly placed into the chamber.

Table 1. Petri dishes labels according to the applied treatment.

Exposure Time	Label
0	CO/CO$'''$
2 h	T1/T1$'''$
1 h	T2/T2$'''$

Control (C); T1 (exposure time 2 h); T2 (exposure time 1 h). $'''$ indicates irrigated with wastewater; absence indicates irrigated with distilled water.

The number of germinated seeds at any time, t, was determined by removing the Petri dishes from the chamber and scoring as germinated when radicle length sprouted with more than 2 mm. Counts were performed at two-hour intervals, and as soon as the record was finalized, the germinated seeds were removed. Germination was considered completed when no further change was perceived during two successive counts.

Experimental design of growth test. After the magnetic priming of seeds, a growth test was performed under laboratory conditions to evaluate the length and weight of magneto-primed and non-primed broccoli seeds irrigated with distilled or wastewater for 10 days. Thirty primed and another thirty non-primed seeds were cultured in alternated rows containing 15 seeds each and in plastic containers (32.5 cm × 21.5 cm × 4.5 cm), irrigated with 500 mL of distilled water; an identical container with primed and non-primed seeds was irrigated with wastewater, and both were saved to evaluate growth parameters on the seventh day after seeding. An identical design was simultaneously set up and saved until the tenth day to evaluate the growth of seedings. On the seventh and tenth day after seeding, the seedling growth was evaluated in terms of stem and root length and total fresh weight. Plants were removed from the soil, carefully washed, and dried with filter paper, and their length and weight were measured.

Statistical analysis. StatGraphics v. 19.5.01 and Germinator Software package v.1.29 were used for statistical analysis of growth and germination data, respectively. Growth data were statistically analysed with StatGraphics Software with the Bonferroni test ANOVA, where the mean of a numerical outcome can be compared between two or more independent groups. Bonferroni test of multiple comparisons was used to demonstrate statistical significances. The Germinator Software Package plots the cumulative germination curves for all magnetic doses and also provides the most used parameters to determine the rate of germination: the mean germination time (MGT), the time required to germinate 1, 10, 25, 50, 75, and 90 percent of seeds (parameters t_1, t_{10}, t_{25}, t_{50}, t_{75}, and t_{90}); the uniformity (U8416, time interval between 16% and 84% germination of viable seeds); and the maximum germination percentage G_{max}.

3. Results

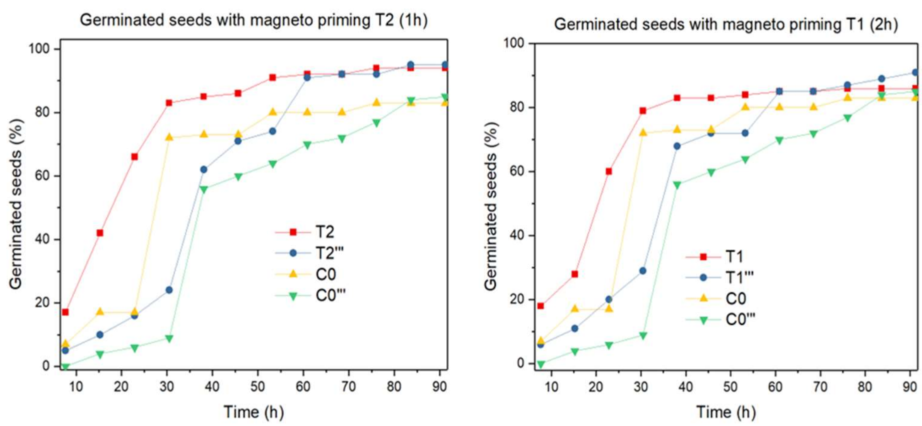
3.1. Germination Test

The cumulative germination curves were plotted for all magnetic doses and control by using the Germinator Software Package, which also provides the most used parameters to determine the rate of germination, as explained in the Material and Methods section. The results are summarized in Table 2, and the cumulative germination curves are plotted in Figure 3.

Table 2. Summary of the most important parameters of broccoli seed germination using wastewater.

Label	t_{10} (h)	t_{50} (h)	t_{75} (h)	MGT (h)	G_{max} (%)
C0‴	26.5 ± 0.3 ^{b}	38.7 ± 2.0 ^{b}	64.2 ± 0.9 ^{b}	39.2 ± 1.2 ^{b}	85 ± 3 ^{a}
T1‴	20.5 ± 1.6 ^{a}	32.8 ± 1.3 ^{a}	49.7 ± 4.2 ^{a}	34.2 ± 1.2 ^{a}	91 ± 4 ^{ab}
T2‴	23.2 ± 2.3 ^{ab}	36.0 ± 2.5 ^{ab}	47.9 ± 3.7 ^{ab}	37.2 ± 2.4 ^{ab}	95 ± 3 ^{b}

Control (C); T1‴ (exposure time 2 h), T2‴ (exposure time 1 h). Different letters indicate significant differences; $p < 0.01$.



(**a**) (**b**)

Figure 3. Cumulative germination curves of broccoli seeds magneto-primed for (**a**) T2 (exposure time in magnetic field 1 h) and (**b**) T1 (exposure time 2 h). T1‴, T2‴, and C0‴ (irrigation with wastewater); control T1, T2, and C0 (irrigation with distilled water).

From the results, it was shown that the salinity of wastewater affects the germination rate of broccoli seeds; all germination parameters were reduced when seeds were irrigated with wastewater and magnetically treated, although the most significant differences were observed after a 2-h priming process. The onset of the germination can be characterized by the t_{10} parameter; seeds magneto-primed for 2 h sprouted 6 h earlier than control seeds. The time required to see 75% of control seeds germinated was 64.2 ± 0.9 h, while seeds magneto-primed for 1 h required 47.9 ± 3.7 h.

Figure 3 shows the germination percentage of seeds that were pre-treated with a magnetic field generated by a Helmholtz coil array: (a) T1 for 2 h and (b) T2 for 1 h. When comparing the seeds treated with magnetic field and the control samples, there were significant statistical differences. In relation to the comparison of the magnetic treatment T1 and the control seeds (C0: irrigated with distilled water), it can be observed that from the time of 7.5 h, the tendency of improvement in the quantity of germinated seed of the seed treated with magnetic field began, with the maximum difference existing at 22.8 h. After that, the test established a percentage difference of 43%. Similarly, the seed treated with magnetic field showed improvement when compared to the control seed (both treated with wastewater water); in this case, the maximum percentage difference (20%) between both seed conditions was presented 30 h after the germination test was established. It should be noted that seed germination is affected by the type of irrigation used. In this research, the amount of germinated seed was found to be more greatly affected in the samples that were treated with reclaimed water (Figure 3a).

A similar pattern of behaviour can be observed for the samples treated with a magnetic field for 1 h. In the case of seeds irrigated with distilled water, the maximum difference with respect to the control samples was found at 22.8 h, with an increase of 49%. In the case of seeds treated with a magnetic field and irrigated with reclaimed water, an increase of 15% was found at 30 h. In the same way as in the magnetic treatment with an exposure time of 2 h, the seeds improved in germination with the magnetic treatment, expressing the greatest difference in the initial stages of germination. Thus, seed conditioning by means of a magnetic field applied for 1 or 2 h improves germination, with a slight tendency to a greater increase in the number of germinated seed by means of the magnetic field applied for 1 h with an applied field strength of 5.9 mT.

3.2. Growth Test

Table 3 shows the growth parameters on the seventh day after seeding. The stem length of primed (D-P) and non-primed (D-C) seeds irrigated with distilled water was significantly higher than the stem length of seeds irrigated with wastewater (W-C and W-P).

Table 3. Growth parameters of broccoli plants measured on the seventh day after seeding.

Water	**Label**	**Stem Length (cm)** $p < 0.01$	**Root Length (cm)** $p < 0.01$	**Fresh Weight (mg)** $p < 0.01$
Distilled water	D-C	4.50 ± 0.15 ^{b}	2.66 ± 0.19 ^{ab}	53.20 ± 1.98 ^{b}
	D-P	5.65 ± 0.15 ^{c}	4.21 ± 0.18 ^{c}	76.93 ± 1.97 ^{c}
Wastewater	W-C	3.47 ± 0.15 ^{a}	1.92 ± 0.18 ^{a}	43.66 ± 1.96 ^{a}
	W-P	4.05 ± 0.14 ^{ab}	3.03 ± 0.16 ^{b}	50.04 ± 2.00 ^{ab}

D (distilled); W (wastewater); P (primed); C (control, non-primed). Different letters indicate significant differences; $p < 0.01$.

Similar behaviour was observed for root length. Roots of primed (D-P) and non-primed seeds (D-C) irrigated with distilled water were longer than the ones irrigated with wastewater (W-P and W-C), but only significant differences were obtained for primed seeds. Significant differences were found in total fresh weight of primed and non-primed seeds irrigated with distilled water (D-C and D-P) in comparison to the seeds irrigated with wastewater (W-C and W-P).

On one hand, all the growth parameters of primed seeds (D-P) were significantly greater than those obtained for non-primed seeds (D-C) when irrigated with distilled water. A similar effect was obtained for seeds irrigated with wastewater, but only significant differences were found for root length (W-P and W-C). In summary, the seventh-day broccoli seedlings irrigated with distilled water grew higher and heavier than the ones irrigated with wastewater, probably due to the notable amounts of salts in organic wastewater. But the saline effect of wastewater was ameliorated when seeds were previously magneto-primed. Table 4 shows the growth parameter of broccoli plants measured on the tenth day after seeding. The stem and root lengths of both primed (D-C and D-P) and non-primed seeds were longer when irrigated with distilled water than with wastewater (W-C and W-P), but only significant differences were observed in the first case, and identical behaviour was observed in total fresh weight.

Table 4. Growth parameters of broccoli plants measured the tenth day after seeding.

Water	Label	Stem Length (cm) $p < 0.01$	Root Length (cm) $p < 0.01$	Fresh Weight (mg) $p < 0.01$
Distilled water	D-C	8.55 ± 0.21^{b}	3.64 ± 0.20^{b}	73.18 ± 2.36^{b}
	D-P	7.62 ± 0.19^{a}	3.00 ± 0.16^{b}	69.16 ± 2.02^{ab}
Wastewater	W-C	7.00 ± 0.21^{a}	2.09 ± 0.19^{a}	62.09 ± 2.21^{a}
	W-P	7.40 ± 0.21^{a}	3.18 ± 0.18^{b}	66.32 ± 2.18^{ab}

D (distilled); W (wastewater); P (primed); C (control, non-primed). Different letters indicate significant differences $p < 0.01$.

On the other hand, the growth parameters of non-primed seeds (D-C) were lower than those obtained for primed seeds (D-P) when irrigated with distilled water, but no significant differences were obtained for most parameters.

4. Discussion

In recent years, a comparative study of several cereals subjected to different stationary magnetic fields was carried out; the results revealed that magneto-priming may be an effective technique for enhancing germination across all species studied [20]. Research conducted with triticale (X *Triticosecale Wittmack*) showed that 125 mT or 250 mT magnetic treatment favoured and advanced germination, and plants of greater length and weight were obtained; the most significant differences were obtained for exposure times greater than 1 h [21]. A magnetic time model for triticale seed germination was developed in an investigation into the impact of a stationary and homogeneous magnetic field within the range of 2.23–3.72 mT on triticale germination; it was observed that the mean germination time decreased with an increase in magnetic-field induction. Similar trends were noted for most of the germination parameters studied [22]. A subsequent examination focused on the germination and water absorption of triticale seeds previously subjected to magnetic treatment. The seeds underwent a magnetic treatment for 10 h under 3.71 mT, and the resulting hydration data were modelled using Peleg's equation. A noteworthy observation was a nearly 50% increase in the water-uptake rate of treated seeds compared to untreated seeds, likely attributed to the applied magnetic field. This increase in water-uptake rate might explain the observed enhancement in the germination rate of treated seeds. The adjustment of water absorption by seeds to the Peleg's model obtained at least an R^2 of 0.97 [23].

Therefore, the adverse effect of salinity and drought on water absorption can be circumvented by magneto-priming seeds with a stationary magnetic-field induction of 3.71 mT during 10 h. The water uptake of seeds under stress conditions generated by PEG and NaCl was improved by implementing magnetic treatment of seeds prior to imbibition [24].

One of the most important parameters of wastewater is salinity, and many studies have been carried out to evaluate the effects of wastewater on the germination and growth of plants. The results depend on the source and treatment of the wastewater. Although the nutrient contents of wastewater are beneficial for plant growth, in some cases, plant toxicity occurs [25,26]. Other studies were also performed under salinity stress conditions, and germination percentage and germination index decreased significantly. There was also a decrease in chlorophyll and carotenoid content in maize [27]. The saline effect of wastewater irrigation can be ameliorated by using magneto-priming and can be observed in both seed germination and the growth of plants during the first stages of development. Enhanced percentage and early germination of both maize and soybean under different salinity levels indicated that magneto-priming was more effective in alleviating salinity stress [28] and increasing α-amylase and protease activities.

The number of germinated seeds was increased, and there were significant differences when seeds were magneto-primed for 1 h. From the germination test outcomes, it may be concluded that the percentage of seeds irrigated with wastewater can be increased by magneto-priming, and the germination rate can also be increased by using this eco-friendly technique. The benefits of magnetic treatment on seed germination in different vegetal species have been widely reported; exposure of cereal and beans prior to sowing led to an improvement of germination [29–31], and the same effect was noted on groundnut, onion, rice seeds [32], chickpea, and sunflower [33,34], among others.

Wastewater's high salinity usually consists of several concentrated ions such as Na^+, Ca^{2+}, and Al^{3+} that are generated through industrial processes including reverse osmosis or food processing, which account for as much as 5% of the wastewater requiring treatment worldwide [35,36]. Salinity exerts an impact on the seedling growth of plants, manifesting in the deceleration of reserve food mobilization, suspension of cell division and enlargement, and injury to hypocotyls [37]. Osmo-priming treatments with varying salinity levels were found to influence the mean germination parameters of wheat seeds [38].

In addition, all the growth parameters of magneto-primed seeds (W-P) were higher than the ones obtained for non-primed seeds (C-W) when irrigated with wastewater, although only significant differences were obtained for root length. According to other authors, magneto-primed seeds have the potential to minimize the adverse effects of drought and diseases on crop productivity [27].

5. Conclusions

1. On the seventh day, the stem and root lengths of primed and non-primed seeds irrigated with distilled water were significantly higher than those of seeds irrigated with wastewater. Roots of primed and non-primed seeds irrigated with distilled water were longer than those of seeds irrigated with wastewater, but only significant differences were obtained for primed seeds. Significant differences were found in total fresh weight of primed and non-primed seeds irrigated with distilled water in comparison to the seeds irrigated with wastewater;
2. On the tenth day, the stem and root lengths of both primed and non-primed seeds were longer when irrigated with distilled water than with wastewater, but only significant differences were observed in the first case, and identical behaviour was observed on total fresh weight. The growth parameters of non-primed seeds were lower than those obtained for primed seeds when irrigated with distilled water, but non-significant differences were obtained for most parameters. In addition, all the growth parameters of magneto-primed seeds were higher than those obtained for non-primed seeds when irrigated with wastewater, although only significant differences were obtained for root length;
3. Broccoli seedings irrigated with distilled water grew higher and heavier than the ones irrigated with wastewater, probably due to the significant amounts of salts in organic wastewater. Nonetheless, the magneto-priming of seeds decreases the saline effect of wastewater irrigation on broccoli plant growth;
4. All the germination parameters were enhanced when seeds were irrigated with wastewater and magnetically treated; in comparison with those that had not been

treated, the most significant differences were observed in seeds that were magneto-primed for 2 h;

5. The onset of the germination, characterized by the t_{10} parameter, was reduced by 6 h versus the control when seeds were magneto-primed for 2 h;
6. The percentage of germinated seeds irrigated with wastewater was 85 ± 3 h, while the magneto-primed seeds achieved 91 and 95%, respectively.

Author Contributions: Conceptualization, M.F., M.d.M.D., B.K.F., C.H.-A. and F.A.; methodology, M.F., M.V.C., M.d.M.D. and F.A.; software: J.G., B.K.F. and F.A.; validation: M.V.C., C.H.-A. and M.F.; formal analysis: investigation, E.M. and J.A.; resources, F.A. and M.d.M.D.; data curation, J.G. and B.K.F.; writing—original draft preparation, J.A. and E.M.; writing—review and editing, M.V.C., E.M., J.G., J.A. and C.H.-A.; visualization: supervision, M.V.C., E.M., J.G. and J.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

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