

Article

# Discriminating among Alternative Dressing Solutions for Cereal Seed Treatment: Effect on Germination and Seedling Vigor of Durum Wheat

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**Abstract:** A prompt seed germination and emergence coupled with an excellent seedling vigor are highly desired features to ensure perfect crop establishment and subsequent vegetative growth. Seed dressing with pesticides represents the most common technology for enhancing seed performance after sowing, while little is known about biostimulant seed dressing. This practice could play a fundamental role in developing new sustainable starter fertilization for cereals. The enhancement of germination and seedling vigor of durum wheat seeds (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn) was the main target of this research. The experiment took place in a germination cabinet under controlled environmental conditions, settled at the constant temperature of 10 °C and under dark conditions for 8 days. The different seed dressings, sprayed on the seeds, were composed by a combination of a fungicide and different biostimulants. Coleoptile and root length, as well as biomass, were significantly increased by the different biostimulants, compared to the control. As for germination traits, seeds treated with *Codium fragile* and *Opuntia ficus-indica* extracts, containing phytohormones and different nutrients, showed a final germination (96%) significantly higher than the one obtained with the control treatment (86%). These results show that treating seeds with a suitable dressing solution can greatly improve the germination features and seedling vigor of durum wheat. This can help the crop to withstand future stresses, especially in early stages, and possibly increase the grain yield with a reduction in agrochemicals. However, the combination of the substances used in the present study rarely showed a synergistic effect on the tested variable.

**Keywords:** biostimulants; chelated zinc; *Opuntia* extract; seaweed extract; seed dressing



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## 1. Introduction

High physiological seed quality is a feature greatly requested by farmers for every crop. However, the lack of close cooperation between the seed industry and scientific community is slowing down the progress in obtaining high-performance seeds [1]. A rapid and uniform field establishment is the first target for all growers, and the degree to which this target is achieved determines the success and the profitability of crops [2–4]. Seeds and seedlings undergo various biotic and abiotic stresses that can hinder vigorous plant establishment and, consequently, future crop production [5–8]. Seed treatment is a crop-specific and objective-oriented method, developed to protect seeds from major stressors, thus allowing plant genotypes to entirely express their potential [9]. In the past years, dressing seeds with fungicides or insecticides became a fundamental tool to ensure high agronomic performances for all major crops. This technique is considered an environmentally friendly practice, because it can provide long-term protection of the plants by using a lower dose of pesticides per hectare as compared to conventional spraying [10,11]. Moreover, it has an economic benefit and ensures higher workplace safety for farmers [12,13]. Seed dressing is a valid solution not only to face most biotic stresses but also to reduce the negative

impact of abiotic stress, such as drought and salinity, through the application of nutrients, microorganisms and phytohormones [14–19].

In the Mediterranean area, wheat production is seriously threatened by severe abiotic stresses that usually result in a significant yield reduction even when the stress occurs during the initial growing stages of the crop [20–23]. Since durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn) is a fundamental crop in the Mediterranean area, methods and strategies aiming to enhance its resilience to abiotic stresses, without negatively affecting the environmental sustainability, will be the core subject to face food security in that peculiar geographic area [24].

A possible solution to help reach this fundamental goal is the use of biostimulants. These products are garnering increasing attention among researchers and farmers due to recent market trends and their capabilities to enhance crop performance, especially under abiotic stresses [25]. Seed treatment with biostimulants and/or micronutrients appears to be a sustainable and economically valid option to ensure good crop establishment in different conditions. For this reason, scholars have started to investigate the effect of soaking and/or priming seeds with various substances on germination and early development of plants [26]. In the past years, seed treatment with zinc proved to be a good practice to enhance seed germination and vigor for different crops [27]. Instead, in recent years, the use of biostimulants like seaweed or plant extracts has spread as an efficient method for seed dressing. Seaweed extracts appear to be rich in phytohormones and other compounds able to trigger the expression of several genes, connected with defense and nutrient absorption mechanisms [28]. The plant extracts play a similar role in promoting the development of different crops, by providing nutrients, amino acids and other components directly to the plant or by triggering the pathways involved in phytohormones biosynthesis, photosynthesis regulation and nitrogen assimilation [29]. Despite the overall good results obtained by these kinds of compounds on wheat under different stress conditions, especially in terms of final germination percentage, fresh biomass of the seedlings and content of chlorophyll [30–36], the biostimulant seed treatment is still an uncommon practice for most farmers. That is probably because seed soaking, which is the most used method in germination studies, is not viable at an industrial scale, especially for the mechanical impediment of the soaked seeds to flow inside a seeder [37]. Foreseeing the great potential of this technique, several fertilizer companies have been developing new seed dressing solutions for years. However, the limited literature describing the industrial seed application of non-microbial biostimulants evidenced contrasting results, caused by the plethora of active compounds used and the interactions among them and with the different growing conditions and crop species [38–42]. To better understand the effect and the efficacy of the different seed coating products on durum wheat, especially in terms of germination rate, germination speed and seedling vigor, we carried out several germination trials using different plant biostimulants. The following hypotheses were tested: (i) when compared to untreated seeds, biostimulant seed dressing significantly improves germination rate and speed as well as the seedling vigor of durum wheat; (ii) the effect is improved by combining different substances.

## 2. Materials and Methods

### 2.1. Experimental Design

To have uniform experimental conditions, durum seeds (cultivar Odisseo) were visually chosen based on their dimensions. Subsequently, they were treated with eight different solutions containing the following components:

- A liquid fungicide, commercially known as VIBRANCE GOLD by SYNGENTA, at the dose of 200 mL per 100 kg seed, which contains 4.63% Sedaxane, 2.32% Fludioxonil, 2.32% Difenoconazole;
- A liquid NPK organic fertilizer containing 3% N, 4% P<sub>2</sub>O<sub>5</sub>, 3% K<sub>2</sub>O, free amino acids (e.g., phenylalanine, methionine, tyrosine, proline, etc.), humic and fulvic acids (hereinafter referred to as NPK);

- Zinc (hereinafter referred to as Zn), derived from a commercial product containing chelated Zinc with ethylenediamine tetra-acetic acid (EDTA, 9% Zn);
- An experimental product containing a mixture of aqueous extracts from the seaweed *Codium fragile* (10 g of dried alga per 1 L of distilled water) and the plant species *Opuntia ficus-barbarica* (50 g of fresh puree of prickly pear per 1 L of distilled water). Hereinafter referred to as SWEO;
- Distilled water.

The exact formulation of each solution is shown in Table 1.

**Table 1.** Active ingredients and formulations of the different dressing solutions.

Ingredient (g)	Control	NPK	Zn	SWEO	NPK + Zn	NPK + SWEO	SWEO + Zn	SWEO + NPK + Zn
Fungicide	2	2	2	2	2	2	2	2
Distilled Water	8	5	7	5	4	2	4	1
SWEO	0	0	0	3	0	3	3	3
NPK	0	3	0	0	3	3	0	3
Zinc	0	0	1	0	1	0	1	1

SWEO: a mixture of extracts from seaweed *Codium fragile* (Suringar) Hariot and plant *Opuntia ficus-barbarica* A. Berger; NPK: a liquid NPK organic fertilizer containing 3% N, 4% P<sub>2</sub>O<sub>5</sub>, 3% K<sub>2</sub>O, free amino acids, humic and fulvic acids.

Odisseo was chosen because it is a modern durum wheat cultivar extensively cultivated around the Mediterranean basin [43,44].

The eight treatments were applied at the rate of 1 L solution per 100 kg seed, to simulate the industrial dose. Seed treatment was applied by mixing 1 mL of dressing solution with 100 g of seeds and shaking them in a weighing bottle. This dosage was based on reference values found in the literature [45–47]. Four replicates (each consisting of 100 seeds) were used for each treatment. The treated seeds were placed into a Petri dish (120 mm diameter), with three layers of Whatman Grade 1 filter paper, soaked in distilled water, and left for 8 days in a seed germination cabinet, in dark conditions, at a constant temperature of  $10 \pm 1$  °C [48], to simulate a possible field temperature during sowing season. The Petri dishes were randomly allocated within the cabinet.

## 2.2. Measured Traits

Germination was determined by radicle emergence. The germinated seeds of each replicate were counted every day to calculate

- Final germination percentage: (germinated seeds/total seeds)  $\times$  100, after 8 days;
- T50: time to achieve 50% of final germination calculated according to the following formula [49,50]:

$$T50 = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{(n_j - n_i)} \quad (1)$$

where N is the final number of germinating seeds and  $n_j$  and  $n_i$  are the cumulative number of seeds germinated by adjacent counts at times  $t_j$  and  $t_i$ , respectively, when  $n_i < N/2 < n_j$ ;

- Germination curves.

After 8 days, all germinated seeds were analyzed, and the following traits were measured for each replicate:

- Main root's length (cm), measured by a ruler;
- Length of the coleoptile (cm), measured by a ruler;
- Mean dry biomass of the roots (weight, mg). For each replicate, the roots were separated from the seeds, dried for 48 h at 60 °C and then the total biomass was divided by the number of germinated seeds;

- Mean dry biomass of the coleoptile (weight, mg). For each replicate, the coleoptiles were separated from the seeds, dried for 48 h at 60 °C and then the total biomass was divided by the number of germinated seeds.

### 2.3. Statistical Analysis:

In order to allow a better explanation of germination experiments, the data were analyzed as time-to-event data [51]. To estimate fundamental traits (e.g., T50 and final germination) and their corresponding standard errors, an event-time approach has been used by fitting a three-parameter log-logistic model to data from each germination curve separately [52]. The model equation used in this study was

$$F(T) = \frac{d}{1 + \exp[b\{\log(T) - \log(T50)\}]} \quad (2)$$

where  $d$  denotes the proportion of germinated seeds (upper limit or final maximum germination), and T50 is the time (number of days) to reach 50% of final germination (median germination time). The parameter  $b$  is proportional to the slope of  $F$  at time  $T$  equal to the parameter T50.

Quantitative data indicating the seedling vigor, obtained by the analysis of germinated seeds, were evaluated with the analysis of variance (ANOVA) to test the efficacy of the different seed dressing solutions. Two means were considered different at the 95% probability level by Fisher's protected least significant difference.

Statistical analysis was performed with the open-source environment R [53] using the add-on packages 'drcte 1.0.30', for event-time models, which allowed us to analyze the germination data, with a time to event curve fitting procedure, considering only three fundamental indicators for seed germination. This approach was enough to ensure a clear description of the whole-time course of events, for a simple experiment scheme [54].

## 3. Results

### 3.1. Final Germination Percentage and Median Germination Time (T50)

As reported in Table 2 and Figure 1, all treatments strongly influenced the germination of durum wheat seeds. The application of biostimulants enhanced both the final germination percentage and the median germination time (T50). SWEO was the best treatment, significantly raising the germination percentage by 11% compared to the control. The other formulations showed a germination rate that ranged from 90.5% (NPK) to 92.5% (SWEO + Zn), all significantly higher than control (86%). All treated seeds showed a significantly higher germination speed compared to control. The dressing solutions containing SWEO alone displayed the fastest germination (T50 = 1.71 days), followed by NPK and Zn (both with T50 = 1.84 days). The seed dressing solutions containing a mixture of different ingredients showed a germination speed significantly higher than that obtained with the single ones (except for SWEO + Zn) but still lower than that of the control treatment, which had a median germination time of 2.26 days.

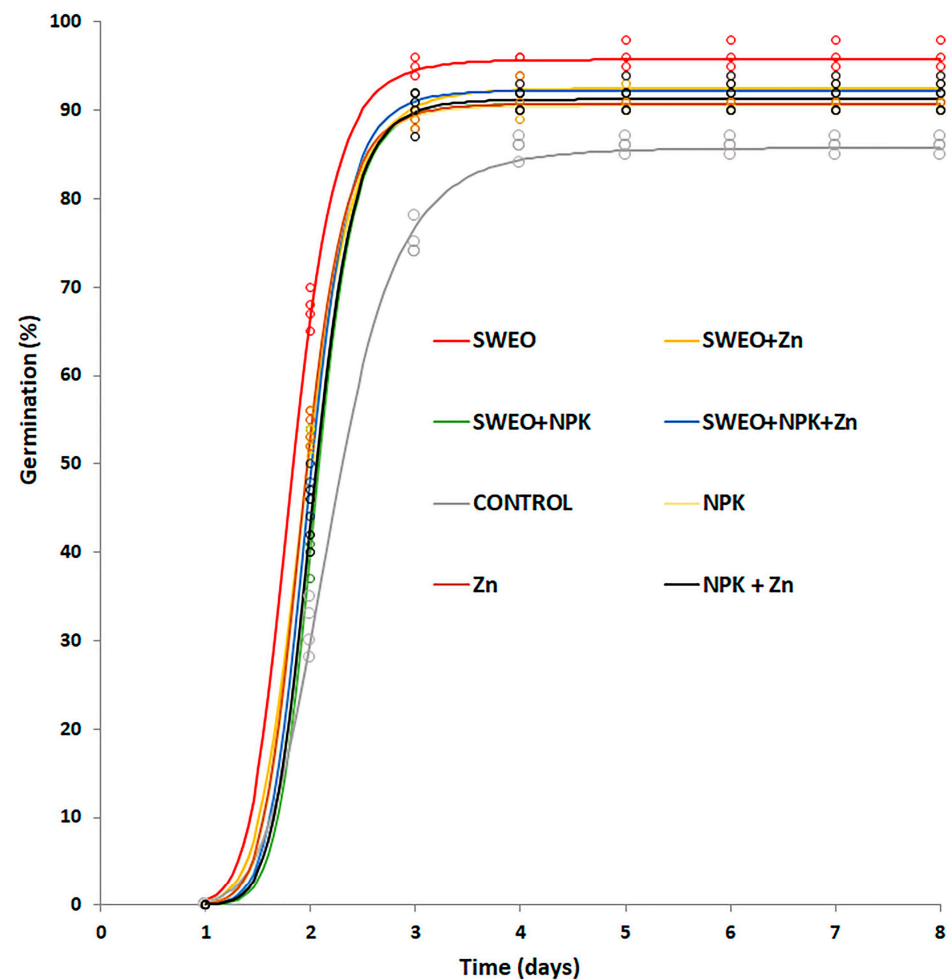
### 3.2. Length of the Main Root

As shown in Figure 2, the mean length of the main roots was significantly enhanced by the biostimulant seed dressing as compared to the control treatment ( $p < 0.001$ ). The best result was obtained by the application of SWEO (11.4 cm), which led to a 32% increase in root length as compared with the control (8.6 cm). All the treatments containing SWEO showed a length of the main root significantly higher than that of the other treatments (except for SWEO + NPK + Zn, with 10.3 cm). The application of zinc, NPK and their mixture had a similar effect on the root length of durum wheat seedlings (9.86 cm, 9.56 cm and 9.94 cm, respectively).

**Table 2.** Germination percentage at 8 days and median germination time (T50) for each seed treatment  $\pm$  standard errors.

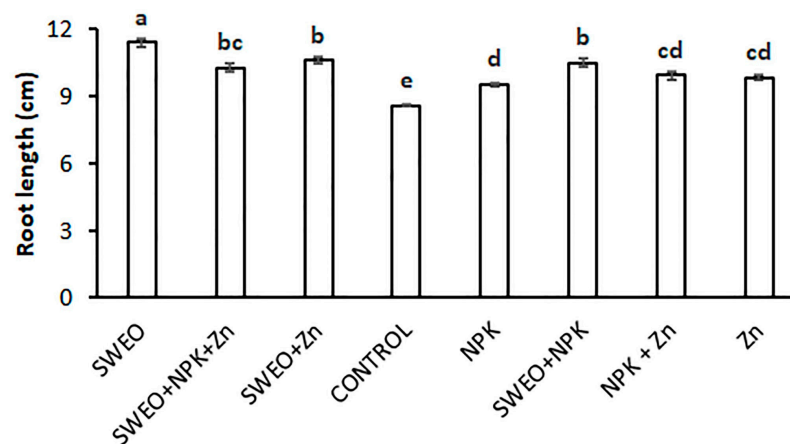
Seed Treatment	Final Germination (%)	T50 (Days)
Control	86.0 $\pm$ 0.4 <sup>c</sup>	2.26 $\pm$ 0.03 <sup>e</sup>
NPK	90.5 $\pm$ 0.29 <sup>b</sup>	1.84 $\pm$ 0.01 <sup>b</sup>
Zn	90.8 $\pm$ 0.48 <sup>b</sup>	1.84 $\pm$ 0.02 <sup>b</sup>
SWEO	95.8 $\pm$ 0.85 <sup>a</sup>	1.71 $\pm$ 0.01 <sup>a</sup>
NPK + Zn	91.3 $\pm$ 0.75 <sup>b</sup>	2.04 $\pm$ 0.03 <sup>d</sup>
NPK + SWEO	90.8 $\pm$ 0.48 <sup>b</sup>	2.1 $\pm$ 0.02 <sup>d</sup>
SWEO + Zn	92.5 $\pm$ 0.65 <sup>b</sup>	1.87 $\pm$ 0.02 <sup>b</sup>
SWEO + NPK + Zn	92.3 $\pm$ 0.63 <sup>b</sup>	1.94 $\pm$ 0.04 <sup>c</sup>

Means sharing letters are not significantly different at  $p < 0.05$ .

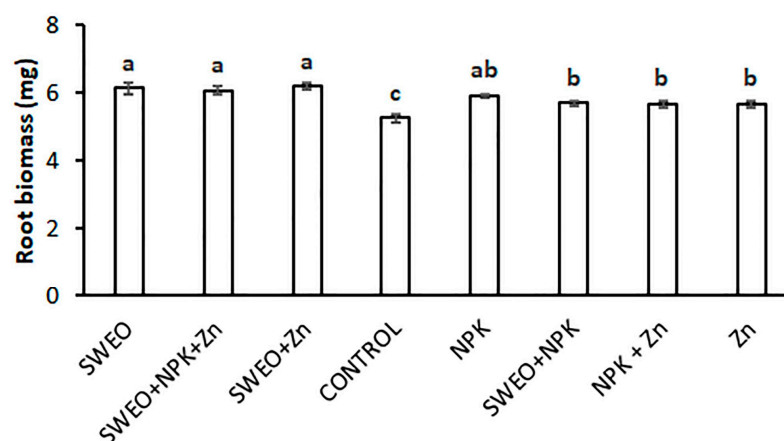
**Figure 1.** Germination curves of seeds treated with different biostimulants. Curves were generated by fitting the three-parameter log-logistic model (2).

### 3.3. Root Biomass

The mean root biomass of the single seedling was significantly influenced by the different seed treatments ( $p < 0.001$ ). SWEO and SWEO + Zn were the treatments showing the highest value (6.2 mg), not significantly different to the treatment containing all the biostimulants (6.1 mg) and NPK alone (5.9 mg). As shown in Figure 3, all the other formulations produced a root biomass significantly greater, with an average increase of 8%, in comparison with the control treatment (5.3 mg).



**Figure 2.** Main root's length affected by the different seed dressing solutions. The vertical bars show the standard error of the mean. Letters above the histograms are the ranking of Fisher's protected test at  $p < 0.05$ .



**Figure 3.** Mean dry biomass of the roots as influenced by the different seed dressing solutions. The vertical bars show the standard error of the mean. Letters above the histograms are the ranking of Fisher's protected test at  $p < 0.05$ .

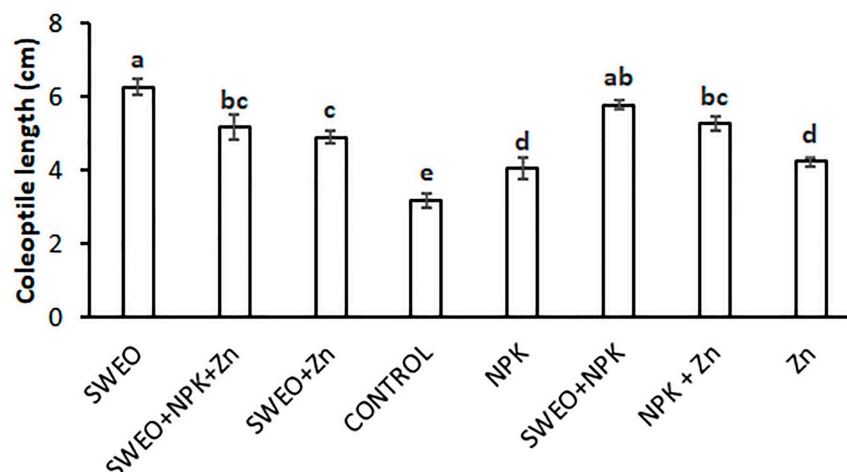
### 3.4. Coleoptile Length

As with the root system, the coleoptile development was significantly influenced by the different seed dressing solutions ( $p < 0.001$ ). The application of biostimulants always produced a coleoptile significantly longer than that of the control treatment (Figure 4). As compared to the control, SWEO doubled the coleoptile length (6.3 cm vs. 3.2 cm), while the other treatments produced an increase ranging from 26% (NPK, 4 cm) to 81% (SWEO + NPK, 5.8 cm). Formulations containing Zn or NPK alone showed a result significantly lower than that obtained by applying their mixture (4.0 cm vs. 5.3 cm).

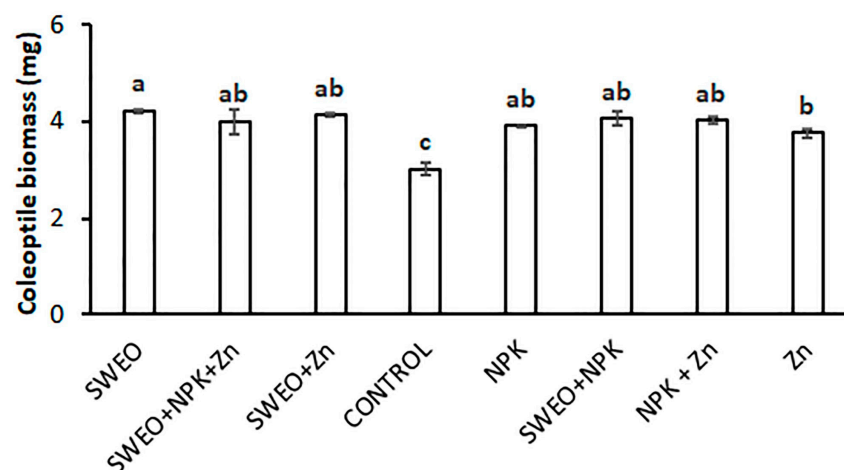
### 3.5. Dry Biomass of the Coleoptile

Different seed dressings significantly influenced the biomass of the coleoptile of durum wheat seedlings ( $p < 0.001$ ). Again, the SWEO treatment produced the highest biomass (4.2 mg), but the effect was less evident than that obtained for coleoptile length (Figure 5). The application of mixtures did not produce an effect significantly different from that of the single components. The Zn treatment resulted in a coleoptile biomass significantly lower than the SWEO treatment did, with a decrease of about 10%. The Zn seed dressing, however, still produced a 23% enhancement as compared to the control (3.0 mg).





**Figure 4.** Coleoptile length as influenced by the different seed dressing solutions. The vertical bars show the standard error of the mean. Letters above the histograms are the ranking of the Fisher's protected test at  $p < 0.05$ .



**Figure 5.** Mean dry biomass of the coleoptile as influenced by the different seed dressing solutions. The vertical bars show the standard error of the mean. Letters above the histograms are the ranking of Fisher's protected test at  $p < 0.05$ .

#### 4. Discussion

Since the different treatments markedly improved seed germination traits and the seedling vigor of the durum wheat cultivar Odisseo, our first hypothesis was verified. Conversely, considering our findings, the second hypothesis must be rejected. Indeed, as compared to the application of single components, the combination of different biostimulants in the same seed dressing solution did not significantly enhance the performance of durum wheat, except for coleoptile length. This phenomenon, especially in the case of SWEO and NPK, could be explained by the negative effect that excessive doses of nutrients and phytohormones can have on early plant development. Similar results were found by other studies in which high doses of biostimulants or their combinations reduced the germination percentage and the seedling performance [14,55,56]. As for treatments containing Zn, the negative effect could be probably due to EDTA and its competition with other nutrients [57,58].

The *Codium* and *Opuntia* extracts showed the best results in all the analyzed traits. The effectiveness of seaweed extracts in promoting growth and the physiological traits of wheat has been reported by several studies [59,60]. Extracts from *Codium fragile* were recently found to enhance the germination performance and seedling vigor of durum wheat both

under normal and salt stress conditions [61]. *Codium* spp., like all macroalgae, contain phytohormones (e.g., cytokinin, gibberellins and auxins), as well as essential nutrients and amino acids [61–66]. All these substances are positively involved in seed germination and seedling development [67–69]. Additionally, *Opuntia* spp. contain carbohydrates, proteins, vitamins, fatty acids and minerals (e.g., magnesium, potassium, calcium and zinc), as well as osmoprotective compounds, especially phenylalanine and proline [70–75]. Phenylalanine plays a fundamental role in early seedling growth and development [76,77], while proline is involved in the protective mechanism against several abiotic stresses, like water and salt stress [78]. The extremely effective action of *Opuntia* extracts in promoting seed germination and seedling vigor is also reported by another study, in which the final germination of tomato seeds increased from 64% with the untreated seeds to 100% with the seeds treated with the plant extracts; similar results were obtained also in terms of the fresh weight of the tomato plants [79].

The positive effect the NPK treatment had on germination traits as well as on root and coleoptile development was due to the its formulation of macronutrients as well as humic and fulvic acids. Other studies found that fulvic acids increased the germination rate and growth speed of spring cereals [80], while humic substances improved the seedling vigor of wheat [81]. As for macronutrients, the key role that phosphate fertilizer plays in promoting early growth of different crops is well referenced [82–84]. Similarly, nitrogen and potassium can boost seedling vigor, as reported by a study conducted on tomatoes [85]. The efficacy of NPK fertilizer in promoting wheat development was reported by another study, in which seeds of different bread wheat cultivars, soaked in different NPK solutions, showed an increase in plant height ranging from 2% to 11% [86]. In our study, seeds treated with chelated zinc performed better than those of the control for all the analyzed traits. This is consistent with other findings, in which seed priming with zinc strongly enhanced the germination percentage of wheat compared to the untreated seeds (from 22% to 38%); the dry weight of the seedlings was enhanced as well [87]. Seed dressing applied as zinc sulphate or zinc oxide was reported to be one of the most common and effective ways to increase the germination percentage and the seedling vigor of many crops [88–90]. While the use of chelated zinc is still little explored for seed coating, its use could be very effective in alkaline or calcareous soil, where Zn availability is limited [91,92].

## 5. Conclusions

Biostimulant seed dressing is a promising technique that deserves a deeper understanding to help the industry in obtaining an increasingly high-performance seed. Our study clearly demonstrated that different biostimulants delivered together with a fungicide seed dressing were great enhancers of the germination and vigor of durum wheat seeds. The application of extracts from *Codium fragile* and *Opuntia ficus-barbarica* constantly was the most effective treatment. Additionally, it never showed a synergistic action when used in a mixture with other substances. A fast and uniform emergence would result in a more resilient crop, especially in the earliest growth stages, thus leading to an important reduction in the use of agrochemicals. For these reasons, farmers should pay more attention to seed quality and to proper seed dressing. Our study, despite clearly showing the effect of different seed dressings in promoting germination and seedling vigor of durum wheat, still has strong limitations, including i) the controlled environmental conditions and ii) the relatively short duration of the experiment. Further studies are needed to reveal the mechanism of action of the different substances, especially their interactions, and to evaluate their effectiveness on diverse crops in longer studies under field conditions. Having a more complete understanding of these processes could help farmers to better face seeding uncertainties with the right seed dressing.

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tion, F.R.; supervision, R.R. and F.R. All authors have read and agreed to the published version of the manuscript.

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