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ORIGINAL ARTICLE

Boron seed treatments induce germination and seedling growth by reducing seed-borne pathogens in safflower (*Carthamus tinctorius* L.)

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Abstract

The objective of this study was to determine the effects of seed treatments with boron on germination, seedling growth, and disinfection of seed-borne pathogens in safflower. Safflower seeds of the Balcı cultivar were treated with solutions of 0, 5, 10, and 20 ppm boron for 6 hours or surface treated with powdery sodium borate ($Na_2B_8O_{13}.4H_2O$). The experiment was a completely randomized design with eight replications and 25 seeds per replicate. Treated or untreated seeds were germinated between papers and germination characteristics, emergence, seedling growth, and infected seed rate were investigated. The results showed that the peak germination percentage (96.5%) was achieved in seeds primed with 5 ppm B. The mean germination time was significantly reduced, and the germination index reached the maximum value (23.5) in seeds primed with 5 ppm boron. Emergence percentage improved from 76.5% in the control to 87.5% in the seed dressing. The root and shoot length of the primed seeds increased fourfold. Seedling fresh weight was positively affected by the boron treatments, and seeds primed with 20 ppm boron produced the heaviest fresh seedlings. All boron treatments significantly reduced the infection rate by about 73%, but the lowest infection rate (11.5%) was observed in seeds primed with 5 ppm boron. It was concluded that safflower seeds should be primed with 5-10 ppm boron or at least undergo seed dressing with boron to reduce the negative effects of seed-borne pathogens and promote germination and seedling growth.

Keywords: boron, *Carthamus tinctorius* L., germination, seed-borne pathogen, seed treatment

Introduction

Safflower (*Carthamus tinctorius* L.) is a valuable crop for vegetable oil production in Turkey because it thrives in areas with low agricultural input and different climatic conditions (Kaya *et al.* 2019). Safflower belongs to the Asteraceae family, and its seeds, called "achene", are surrounded by a protective pericarp, each containing a single seed. This structure is optimal for hiding disease spores in achenes (Ergin *et al.* 2021; Doolotkeldieva and Bobusheva 2022), resulting in a lack of germination and uneven plant density. Recently, several pathogens, including *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp., *Pseudomonas* spp., *Rhizoctonia* spp., and *Verticillium* spp. were found to have harmful effects on safflower seed germination and storage according to Abdullah and Al-Mosawi (2010) and Nečajeva *et al.* (2023). Therefore, obtaining healthy seed is essential for successful germination, emergence, and uniform plant density.

Seed treatments with pesticides can neutralize potentially harmful infections (Ergin *et al.* 2021). On the other hand, effective and sustainable alternative treatments need to be developed since the use of most chemical fungicides has been banned by the EU (Aguado *et al.* 2019). Pre-sowing seed treatment is an important alternative method that promotes rapid and uniform germination but prevents radicle protrusion from the seed coat (Sher *et al.* 2019). Furthermore, various priming agents including mannitol, polyethylene glycol, various salts, and plant growth regulators (gibberellic acids, IBA, BA, glycine betaine, etc.) have been employed to promote seed germination and vigor (Pallaoro *et al.* 2016; Waqas *et al.* 2019). In recent years, minor plant nutrients have been used as seed priming agents (nutripriming) to enhance the germination and seedling growth of wheat (Iqbal *et al.* 2017), maize (Muhammad *et al.* 2015; Rasool *et al.* 2019), rice (Farooq *et al.* 2018), bean (Majda *et al.* 2019), and rice (Ancy *et al.* 2022). One of the key micronutrients required for priming agents is boron (B), which plays a crucial role in the construction and stability of cell walls and has a positive impact on reducing disease severity (Picanço *et al.* 2021). Despite the uncertain role of B in disease resistance or tolerance, Machado *et al.* (2018) demonstrated that B reduced the severity of disorders induced by *Alternaria gradis* in potato. Similarly, the hazardous effects of *Botrytis cinerea* in grape (Qin *et al.* 2010), Asian soybean rust (*Phakopsora pachyrhizi*) in soybean (Picanço *et al.* 2021), and *Sclerotinia* stem rot (*Sclerotinia sclerotiorum*) in canola (Ni and Punja 2020) were successfully alleviated by B treatments. However, there is little information on the use of boron as a priming agent or seed dressing to eradicate seed-borne pathogens in safflower. In contrast, Ashagre *et al.* (2014) found that the toxicity level of boron to germination was 4 mg · l^{-1}. Therefore, the present study was conducted to investigate if the use of boron as a seed dressing and priming agent would affect the germination and seedling growth of safflower seed and to determine the effects on reducing the infection rate.

Materials and Methods

A laboratory experiment was carried out at the Seed Science and Technology Laboratory of Eskişehir Osmangazi University. The seeds of safflower cultivar Balcı, which is extensively grown and registered by the Transitional Zone Agricultural Research Institute Eskişehir-Turkey, and sodium borate (20.9% $Na_2B_8O_{13} \cdot 4H_2O$, Etidot-67) were used as materials.

Seed treatments

For seed dressing, 200 seeds were weighed and placed in a falcon tube. Four grams of boron per kilogram of seed were poured over them and evenly distributed over the seed surface. For this purpose, the tube was sealed tightly with a lid and shaken for 5 minutes, first manually and then with a vortex. Excess boron was removed after treatment by sieving roughly.

For priming treatments, the seeds were soaked with different concentrations (0, 5, 10, and 20 ppm B) of sodium borate in an incubator at 10°C for 6 hours in the dark. After incubation, the water on the seed surface was gently removed with paper towels, and the seeds were directly dried to their initial seed weight with a hair dryer. They were left to cool overnight at room temperature.

Germination test

A germination test using 200 (8 × 25) seeds per treatment was carried out according to ISTA (2018) guidelines. Twenty-five seeds were placed in three layers of filter papers moistened with 21 ml of distilled water. To prevent water loss, the filter papers containing the seeds were rolled up and then placed in a plastic ziplock bag. The packages were incubated at 25°C in the dark, and checked every 24 hours for 14 days. The seeds with a 2 mm radicle were counted as germinated. At the end of the experiment (14th day), the infection rate (IR) was also calculated as follows:

IR = (Number of seeds on which infection appeared / Total number of seeds) × 100 (Arshad Javaid *et al.* 2006).

Mean germination time (MGT), as determined by ISTA (2018), was computed to assess the speed of germination. MGT is equal to $\Sigma(Dn)/\Sigma n$, where n is the seed number that germinated on day D and D is the number of days since the germination test started. The germination index (GI) was calculated with the formula: GI = Number of germinated seeds/days of first count + ... + Number of germinated seeds/days of final count (Salehzade *et al.* 2009). Ten seedlings from each treatment were selected randomly on day 14 to measure the root length (RL), shoot length (SL), seedling fresh weight (SFW), and seedling dry weight (SDW). After directly weighing fresh seedlings, they were transferred to an oven at 80°C for 24 h to determine the dry weight (Ergin *et al.* 2021). The vigor index was calculated by multiplying germination percentage (%) and seedling length (cm) (Sadeghi *et al.* 2011)

Experimental design and statistical analysis

The experiment was set up as a completely randomized design with eight replications, and all data collected were statistically analyzed using the JMP software. The percentage data were subjected to an arcsine transformation before an analysis of variance was performed.

The LSD test was used to assess the differences between the means (Düzgüneş *et al.* 1983). Pearson's correlation coefficient and significance levels ($p < 0.01$) between the characteristics studied were calculated using R software.

Results

Analysis of variance of boron treatments on safflower germination, seedling growth, and the infection rate is given in Table 1. Except for germination percentage and seedling dry weight, all the investigated characteristics were significant at $p < 0.01$.

The application of boron resulted in significant improvements in the germination index and mean germination time. However, there was no change in germination percentage (Table 1). The germination percentage of the control seeds was 89.5%, while the seeds primed with 5 ppm B germinated at 96.5%, which was the highest germination percentage but the increase in germination percentage was not found to be significant (Fig. 1A). Furthermore, all seeds treated with different boron levels germinated faster than the control seeds. Although the priming applications were in the same statistical group, the seeds primed with 5 and 10 ppm boron had the shortest mean germination time of 1.04 days (Fig. 1B). The germination index was higher in the boron-treated seeds than in the control, with the seeds primed with 5 ppm boron having the highest index value (23.5) (Fig. 1C). Boron treatments enhanced the percentage of emergence compared to control, and seed dressing (87.5%) and seed priming with 5 ppm B (84.0%) gave a higher emergence percentage than the others (Fig. 1D).

The growth of safflower seedlings was enhanced by all boron treatments. However, boron-primed seeds produced the highest root and shoot lengths (Fig. 1E, F). Compared to the control and seed dressing, priming applications promoted the elongation of both roots and shoots. Root length increased significantly from 1.90 cm in the control to 8.85 cm in the seeds primed with 20 ppm boron. Priming had a stronger effect on root growth than on shoot growth. The seedlings grown from seeds primed with 10 ppm boron had the highest shoot length of 5.17 cm (Fig. 1F). Depending on the root and shoot length, seeds primed with different amounts of B appeared to produce heavier seedling fresh weights than control seeds. However, no significant changes were observed in the dry weight of seedlings (Fig. 1G, H).

The seed vigor index increased significantly with boron treatments. In the boron priming treatments, it was found that seeds treated with 10 ppm B had a vigor index about four times higher than the control. (Fig. 2A). The highest infection rate during the germination test was observed in untreated safflower seeds at 43.0%, but all boron treatments reduced the infection rate more than the control (Fig. 2B). The lowest infection rate was observed in primed seeds at 5 ppm B (11.5%) and 10 ppm B (12.0%), respectively. The rate of infected seeds was reduced by 73.3% at 5 ppm B, 72% at 10 ppm B, 71% at seed dressing, 57% at 20 ppm, and 51.2% at 0 ppm B.

The correlation coefficients among the investigated characteristics and their significance levels are given in Figure 3. The highest positive and significant correlation was found between germination index and root length ($r = 0.88^{**}$), while a negative significant correlation ($r = -0.93^{**}$) was calculated between germination index and mean germination time. The infection rate was significantly correlated with the mean germination time with a coefficient of $r = 0.54^{**}$.

Discussion

Seeds have high nutrient content, which makes them vulnerable to contamination by various microorganisms. Since oilseeds are rich in oil, they are particularly susceptible to damage by fungi, from development on growing plants to consumption (Kakde and Chavan 2011). Previous studies have shown that *Alternaria* toxins are found primarily in field-dried grains or when the harvest is delayed due to rain, excessive moisture, or early frost (Bianchini and Bullerman

Table 1. Variance analysis of the examined characters in safflower with boron treatments

V.S.	*df*	Mean Square								
		GP	MGT	GI	EP	RL	SL	SFW	SDW	IR
Treatment	5	45	1.27*	124.0*	441*	73.0*	12.3*	10874*	8.9	177*
Error	42	30	0.01	1.7	38	1.1	0.5	44	4.8	11

*show significance level at $p < 0.01$. V.S. – variation source, *df* – degrees of freedom, GP – germination percentage, MGT – mean germination time, GI – germination index, EP – emergence percentage, RL – root length, SL – shoot length, SFW – seedling fresh weight, SDW – seedling dry weight, IR – infected seed rate

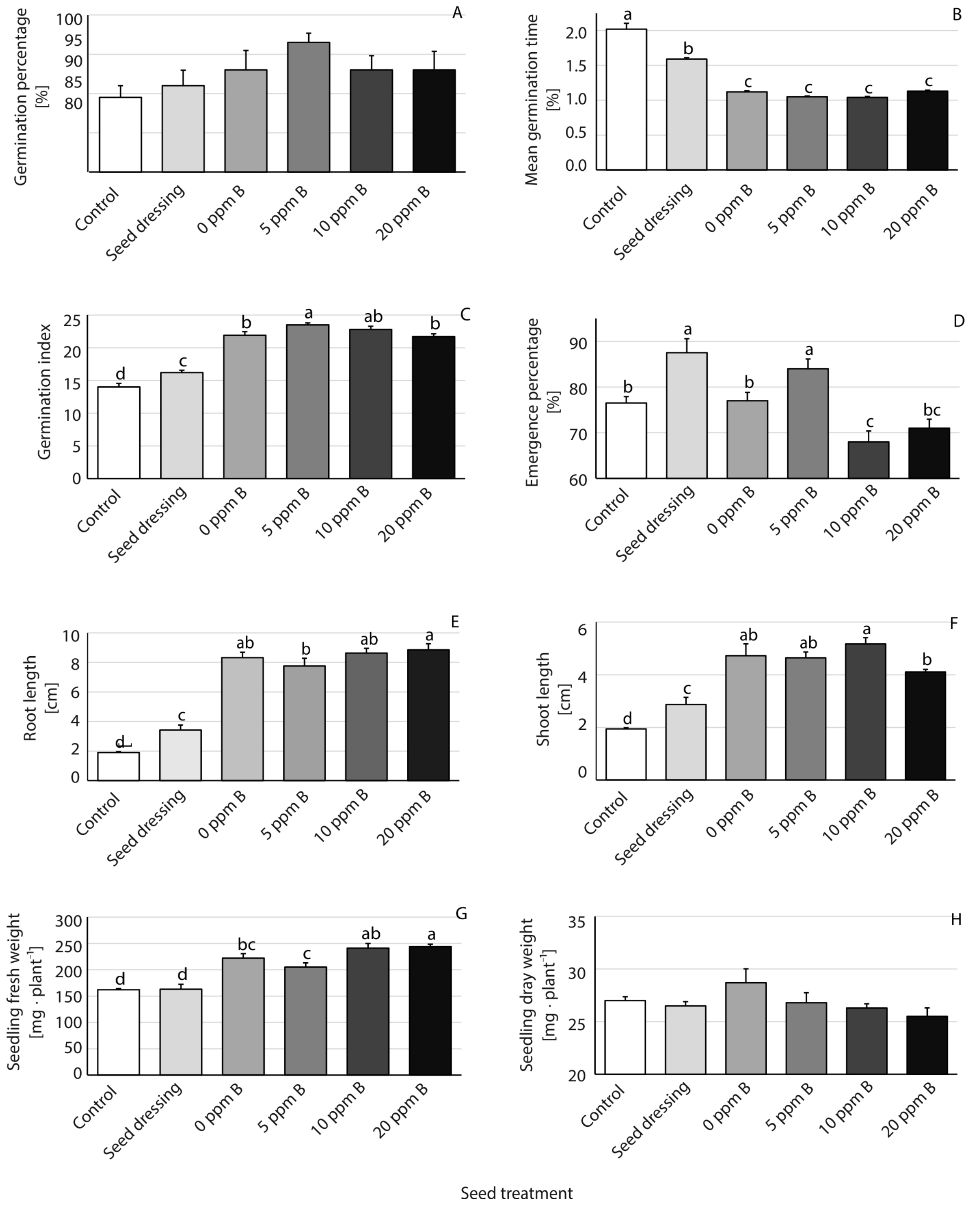


Fig. 1. Effects of seed boron treatments on germination percentage – A, mean germination time – B, germination index – C, emergence percentage – D, root length – E, shoot length – F, seedling fresh weight – G and seedling dry weight – H. Bars and letters on each column show standard error and significance levels at $p < 0.05$, respectively

2014). Singh *et al.* (2007) and Vankudoth *et al.* (2015) reported that the genera *Penicillium*, *Aspergillus*, and *Fusarium* associated with agricultural crops produce toxic compounds that lead to a variety of diseases in plants, animals, and humans.

Boron seed treatments promoted germination percentage and the highest germination was obtained in seeds primed with 5 ppm B. Similar findings were reported by Chakraborty and Dwivedi (2022), who recorded a 5% improvement in wheat germination with boron priming. Also, Moharana *et al.* (2023) reported an increase in the germination percentage of groundnut seeds primed with 1 g · l^{-1} borax. Additionally, the mean time for germination was shortened by boron

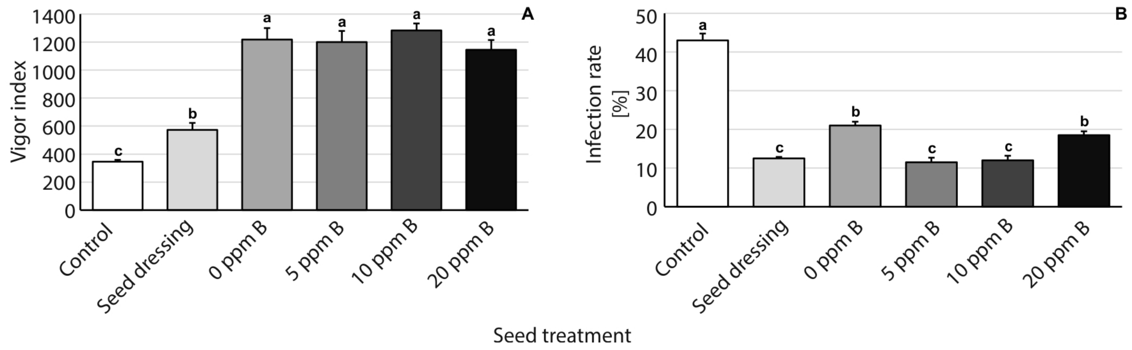
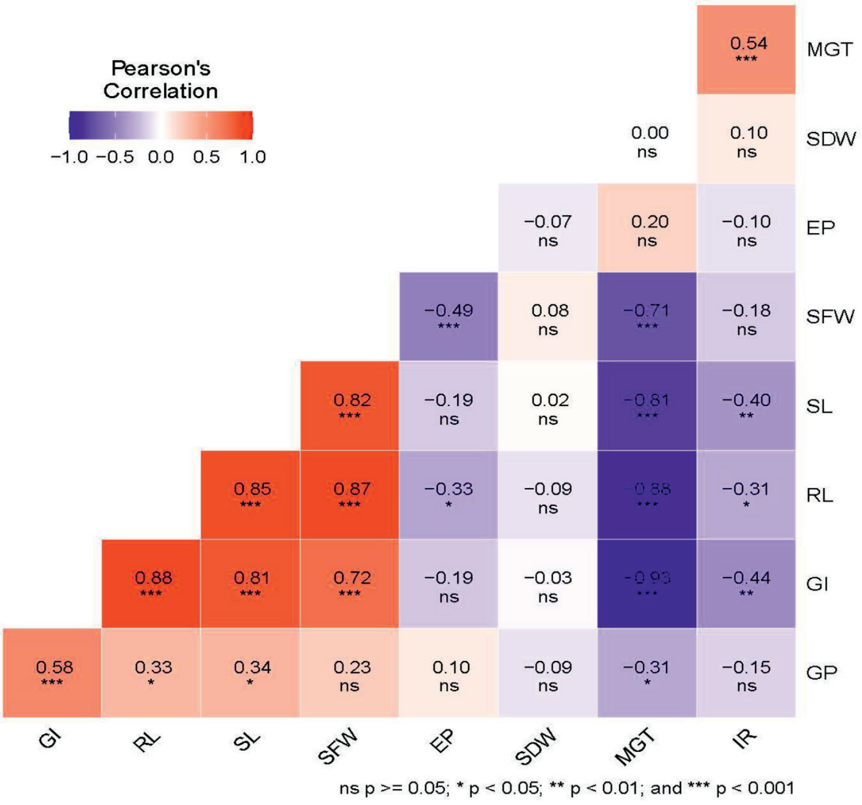


Fig. 2. Effects of seed boron treatments on vigor index and infection rate of safflower seeds. Bars and letters on each column show standard error and significance levels at $p < 0.05$, respectively



	GI	RL	SL	SFW	EP	SDW	MGT	IR
MGT								0.54 ***
SDW							0.00 ns	0.10 ns
EP						−0.07 ns	0.20 ns	−0.10 ns
SFW					−0.49 ***	0.08 ns	−0.71 ***	−0.18 ns
SL				0.82 ***	−0.19 ns	0.02 ns	−0.81 ***	−0.40 **
RL			0.85 ***	0.87 ***	−0.33 *	−0.09 ns	−0.88 ***	−0.31 *
GI		0.88 ***	0.81 ***	0.72 ***	−0.19 ns	−0.03 ns	−0.93 ***	−0.44 **
GP	0.58 ***	0.33 *	0.34 *	0.23 ns	0.10 ns	−0.09 ns	−0.31 *	−0.15 ns

ns p >= 0.05; * p < 0.05; ** p < 0.01; and *** p < 0.001

Fig. 3. Correlation coefficients between investigated characteristics. MGT – mean germination time, GP – germination percentage, GI – germination index, RL – root length, SL – shoot length, SFW – seedling fresh weight, EP – emergence percentage, SDW – seedling dry weight, IR – infection rate

treatments, and the minimum time was recorded in 5 and 10 ppm B priming. This finding confirms the results of Shahverdi *et al.* (2017), who reported that the application of boric acid shortened the mean time to germination of *Stevia* seeds. A higher germination index results from vigorous or healthy seeds. Our results showed that the germination index reached the highest value in seeds primed with 5 ppm B. Similarly, Farooq *et al.* (2011) found that rice seeds primed with an aerated solution containing 0.001% B for 24 hours had the highest germination index. Depending on the achievement in germination performance of safflower, the emergence percentage was increased by priming with 5 ppm B and seed dressing. This result is consistent with the findings of Rehman *et al.* (2013), who observed great enhancement in seedling emergence in rice seed primed with 0.001% B. Chakraborty and Dwivedi (2022) also observed rapid emergence and stimulated growth of wheat seedlings when primed with 2 mM borax compared to unprimed and hydro-primed seeds. Our results are in agreement with those of Iqbal *et al.* (2021), who found that wheat seeds coated with 0.25 and 0.5 g B per kilogram of seed improved the final percentage of emergence. Moreover, Shahverdi *et al.* (2017) reported that priming with boric acid (2.0%) increased the viability index of *Stevia* seeds.

The improvement in germination performance of safflower with boron seed treatments could be mainly due to a reduction in infection rate, as boron-treated seed had a lower rate of infected seed than the control. All germination parameters except MGT were negatively correlated with the infection rate. A lower infection rate resulted in shorter mean germination time, since a positive and significant correlation was calculated. Boron-treated seed consistently had lower infection rates than control seed, consistent with previous observations by Ni and Punja (2020) in canola, who reported a 44-66% reduction in *Sclerotinia sclerotiorum* stem rot symptoms. Li *et al.* (2012) reported the inhibitory effect of potassium tetraborate and borax at a concentration of 20 g · l^{-1} on *Fusarium sulphureum* in potato tubers. Estevez-Fregosa *et al.* (2021) also reported that boron reduced infections caused by *Penicillium expansum*, *P. chrysogenium*, and *Cladosporium herbarum*.

Conclusion

Seed-borne pathogens play a critical role in safflower germination. Safflower seeds are difficult to disinfect because they consist of kernel and hull parts. Our objective was to improve safflower germination and seedling growth by reducing seed-borne pathogens using boron treatments (as a seed surface and priming agent). The results showed that the application of boron on the seed surface and the use of priming agents significantly improved the growth of safflower seedlings and reduced the negative effects of pathogens. It was concluded that safflower seed should be primed with 5–10 ppm B for 6 hours at 10°C or at least a seed dressing with boron should be applied to reduce seedborne pathogens and improve germination, emergence, and growth of safflower seedlings. Seed dressing may be more practical, reasonable, easier to apply in farm conditions and may reduce the risk of infection and boron deficiency better than priming.

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