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Seed priming with iron and boron enhances germination and yield of dill (*Anethum graveolens*)

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Abstract: To study the effects of seed priming with solutions of Fe and B, each at concentrations of 0.5%, 1%, 1.5%, and 2%, and 1.5% Fe + 1% B, on the germination and yield of dill (*Anethum graveolens*), 2 separate laboratory and field experiments were conducted. The results of the laboratory experiments indicated that the effect of the studied treatments on the final germination percentage was significant. The seedling vigor index of dill was restricted when the Fe and B concentrations increased beyond 1.5% and 1%, respectively. The highest seed yield was recorded for the concentration of 1.5% Fe + 1% B in solution, which produced nearly 20% greater yield than the control. The essential oil concentration of the seeds ranged from 2.60% for 0.5% Fe to 2.81% for 1.5% B for the priming solutions. There was a positive response to seed priming with Fe and B regarding the essential oil yield. Priming dill seeds in the 1.5% Fe + 1% B solution resulted in a further increase in dill yield.

Key words: Dill, essential oil, priming, seedling vigor

Introduction

Dill (*Anethum graveolens* L.) is an annual herb of the family Apiaceae. It is native to southwestern Asia and southeastern Europe. It has been cultivated since ancient times (Bailer et al. 2001) as a vegetable, a carminative, an aromatic, and an antispasmodic plant (Hornok 1992; Sharma 2004). It is considered to be one of the most important medicinal plants in Iran, after saffron (*Crocus sativus* L.), cumin (*Cuminum cyminum* L.), and fennel (*Foeniculum vulgare* Mill.).

One of the world's major crop productivity constraints is the unavailability of crop nutrients, in both adequate amount and proper form, for crop plants (Hussain et al. 2006). The roles of both macro- and micronutrients in crop nutrition are undisputable; thus, they are quite important for achieving higher yields (Arif et al. 2006). However, they are limiting factors in most soils, and thus they must be supplemented through proper crop nutrient management (Hussain et al. 2006). Much research regarding the application of micronutrients has been done recently on the invigoration of seeds to improve the germination rate, hasten emergence time, and improve the uniformity of seedling growth in some field crops (Basra et al. 2003). Sivritepe and Dourado (1995) reported that priming is a physiological method that improves seed performance and provides faster, synchronized germination. In an

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experiment conducted by Kaymak et al. (2009) on radish (*Raphanus sativus* L.), the germination rate was higher in seeds treated with plant-growth-promoting rhizobacteria (PGPR) than in the control.

Micronutrients are required in very small quantities (Abd El-Wahab 2008). There are mainly 3 methods of micronutrient application in crops: application to soil, foliar sprays, and seed treatment (Johnson et al. 2005). Each method may affect plant growth differently. The use of micronutrient-enriched seeds (seed priming) has been reported to be a better strategy in overcoming micronutrient deficiencies (Musakhandov 1984; Harris et al. 1999). Seed priming has been shown to enhance the speed of germination (Deering and Young 2006), reduce the time between sowing and emergence, improve seedling vigor (Harris 1996) and stand establishment (Arif et al. 2005; Ali et al. 2007; Diniz et al. 2009), and increase yield (Rengel and Graham 1995a, 1995b; Yilmaz et al. 1998). There is evidence that sowing seeds enriched with micronutrients is also agronomically beneficial (Welch 1986; Rerkasem et al. 1990).

Multiple micronutrient deficiencies of Zn, Mn, Cu, B, Fe, and Mo occur in the soils of Iran, and they are becoming more prevalent as cropping intensity increases. As much as 49% of the soils in Iran have Fe and B deficiencies, but less than 16% of farmers use Fe and B fertilizers in their farms (Malakouti et al. 2009). Therefore, it was decided to determine the effects of priming dill seeds with Fe and B and to find their proper concentrations.

Materials and methods

Laboratory experiment

The laboratory seed priming tests of dill (*Anethum graveolens*) were performed in a completely randomized design, using solutions containing 4 concentrations of iron and boron. There were 3 replications of each priming solution, plus 3 replications of an unprimed control. Seeds were soaked in the required aqueous solutions of chemicals as $FeSO_4.7H_2O$ (Fe 26%, S 11.5%) or H_3BO_3 (B 17%). The priming solution concentrations chosen for the tests, as recommended by the Water and Soil Institute of Iran, were 0.5%, 1%, 1.5%, and 2% each of Fe and B, and a seed treatment with combined 1.5% Fe + 1% B. Each treatment involved weighing approximately 10 g of seeds into a plastic cup, adding 20 mL of the priming solution (sufficient to submerge the seeds), and allowing the seed-and-solution mixture to stay covered with a plastic cup for 12 h. Seeds, after soaking, were rinsed 3 times with distilled water to remove excess salts from the seed coat, as described by Johnson et al. (2005). A portion of the primed seeds were dried in an oven at 70 °C. The seeds were then ground to a powder in a Wiley micromill with stainless steel blades and sifted through a 40-mesh sieve. Seed subsamples were digested in a HNO_3-$HClO_3$ acid mixture (10 mL HNO_3 + 4 mL $HClO_3$), and then the Fe and B were determined at 248.3 and 420 nm wavelengths, respectively, using an AAnalyst-200 PerkinElmer atomic absorption spectrophotometer (Issac and Kerber 1971). The seed concentrations of Fe and B before and after priming are presented in Table 1.

Table 1. Effect of concentrations of Fe and B in the priming solution on their concentrations in primed dill seeds.

Micronutrients	Concentrations in		
	Priming solution (%)	Seeds before priming (mg kg^{-1})	Seeds after priming (mg kg^{-1})
Fe	0.5	5.83	46.00
	1		60.73
	1.5		70.11
	2		79.31
B	0.5	4.00	29.23
	1		38.93
	1.5		50.05
	2		69.31

For each replicate, 25 primed seeds were placed in a germinator at 25 ± 1 °C for a germination test in a petri dish containing Whatman filter paper No. 1 that had been thoroughly moistened with water. Germination was checked once a day for 10 days. The filter paper in the petri dishes was changed once after the first 24 h in case toxic levels of micronutrients leached from the seed coats, which might inhibit seedling growth after germination. The recorded data were final germination percentage, seedling biomass, and seedling vigor index.

The final germination percentage (GP) was calculated as the cumulative number of germinated seeds with normal radicles by using the equation below, as described by Larsen and Andreasen (2004):

$$GP = \Sigma n / N \times 100,$$

where n is number of germinated seeds at each counting and N is total seeds in each treatment.

The seedling vigor index (SVI) was calculated according to the method of Abdul-Baki and Anderson (1973) by using the equation below:

$$GP = SDW \times SVI,$$

where GP, SDW, and SVI are the final germination percentage, seedling dry weight, and seedling vigor index, respectively.

Field experiment

Field experiments were carried out at the Research Station of the Islamic Azad University, Tabriz Branch, northwestern Iran, during 2009. The climate of the research site is semiarid and cold with an average annual precipitation of 270 mm. The soil was sandy-loam with an electrical conductivity of 0.72 dS m^{-1}, pH of 7.9, total nitrogen of 0.09%, phosphorous and potassium contents of 70 mg kg^{-1} and 375 mg kg^{-1}, and Fe and B contents of 6 mg kg^{-1} and 1 mg kg^{-1} (Gupta 2008). The field was ploughed twice (October 2008 and March 2009), and the fertilizers applied, based on soil analysis, were 100 kg ha^{-1} each of urea and ammonium phosphate and 30 kg ha^{-1} of potassium sulfate. The field was then harrowed to prepare the final seed bed.

Plots were arranged in a randomized complete block design with 4 replications. Plots were 4 × 3 m in size with 4 rows of planting. Primed seeds were sown in beds on 26 April, at a depth of about 2 cm. The experimental area was hand-weeded as needed. Seeds were harvested at the half-yellow stage and the number of umbels per plant, thousand seed weight (g), seed yield (kg ha^{-1}), essential oil concentration (%), and essential oil yield (L ha^{-1}) were measured from a harvested area of 6 m^2. The essential oil was extracted by hydrodistillation for 3 h using Clevenger's apparatus based on the method of Guenther (1992).

Statistical analysis

All data were statistically analyzed based on a completely randomized design and randomized complete block design for laboratory and field experiments, respectively, using MSTAT-C software. The means of the treatments were compared using the least significant difference test at $P < 0.05$.

Results

Effects of seed priming on germination and seedling growth

The effect of studied treatments on the final germination percentage was significant. Seed priming with concentrations of 1% and 1.5% Fe and 1% B, solely or in combination, and 1.5% Fe + 1% B for 12 h increased the rate of germination of dill seeds, compared to an unprimed control. The mean of germination from the Fe-primed seeds was 85%, but from the B-primed seeds, the rate was the same as that of the unprimed seeds (Table 2). The final germination percentage after 10 days increased in all solutions as compared with the control, except for a 3% and 5% reduction in seed germination in the seeds primed with the 0.5% and 2% Fe solutions and a 4.5% and 5% reduction in germination in those seeds primed with the 1.5% and 2% B solutions, respectively.

In the laboratory experiments, the effect of seed treatments with Fe and B, solely or in combination, on seedling dry weight (SDW) was significant (Figure 1). The highest SDW (25 mg $plant^{-1}$) was noticed with 1.5% Fe + 1% B, followed by the 1.5% Fe and 1% B treatments (22 and 20 mg $plant^{-1}$, respectively). Lower values for SDW were recorded in the 2% B (10 mg $plant^{-1}$) and control treatments (12 mg $plant^{-1}$). In this experiment, the SDW from seeds treated with 0.5% Fe was found to be similar to that of the 2% Fe treatment. The difference between the control and seeds primed with 2% B was not significant for SDW.

Table 2. Some of the germination and growth parameters of dill as affected by priming treatments.

Priming treatments	Final germination percentage (%)	Seed yield (kg ha^{-1})	Essential oil concentration (%)	Essential oil yield (L ha^{-1})
Fe (0.5%)	80 ef	735 c	2.60 b	19.11 b
Fe (1%)	89 cd	762 bc	2.70 ab	20.57 b
Fe (1.5%)	93 b	801b	2.78 ab	22.27 ab
Fe (2%)	78 f	756 bc	2.71 ab	20.49 b
B (0.5%)	86 de	738 c	2.62 b	19.34 b
B (1%)	89.5 c	770 bc	2.80 ab	21.56 ab
B (1.5%)	78.5 f	779 bc	2.81 a	21.89 ab
B (2%)	78 f	710 c	2.79 ab	19.81 b
Fe (1.5%) + B (1%)	98 a	855 a	2.74 ab	23.43 a
Control	83 e	711 c	2.62 b	18.63 b
F-test	**	**	*	**
LSD	3.2	53	0.19	2.18

Values within each column followed by the same letter have no significant difference at the 0.05 probability level.

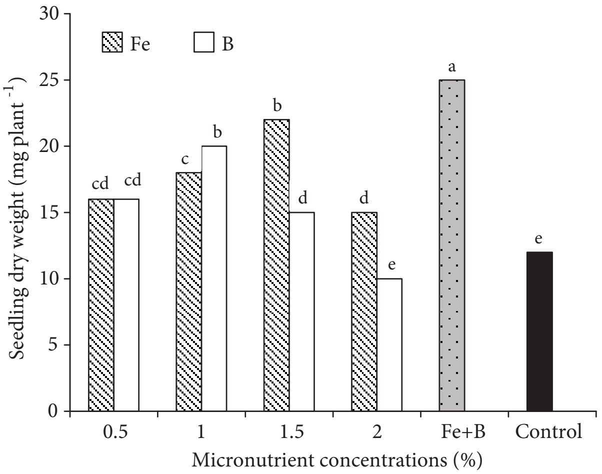

Figure 1. Seedling dry weight of dill seeds as affected by micronutrient seed priming. Columns with the same letter have no significant difference at the 0.05 probability level.

The seedling vigor index (SVI) responded positively and significantly to seed priming with Fe, B, and Fe + B as compared to that of unprimed seeds. The data show that the SVI can be increased by seed priming with Fe and B up to 1.58 and 1.21, respectively, against the control. In the present study, seeds primed with 1.5% Fe had a higher SVI (2.11), but a further increase in Fe concentration in the solution did not increase the SVI. Similarly, when the B concentration increased beyond 1%, the SVI in B-primed seeds was restricted (Figure 2).

Effects of seed priming on yield and yield components

Results indicated that the umbel number per plant (Figure 3) was significant due to the effect of seed treatment by Fe and B. The mean number of umbels

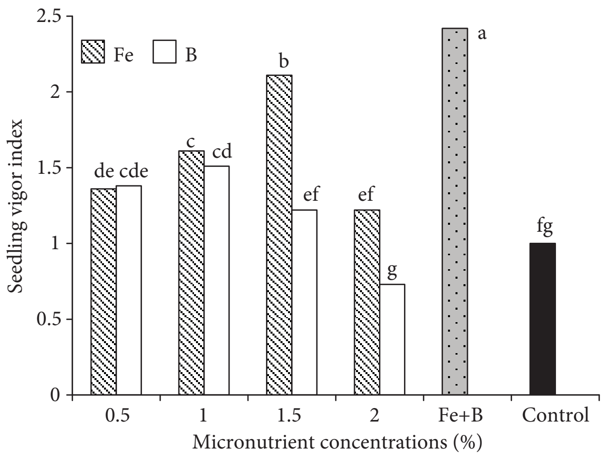

Figure 2. Seedling vigor index of dill seeds as affected by micronutrient seed priming. Columns with the same letter have no significant difference at the 0.05 probability level.

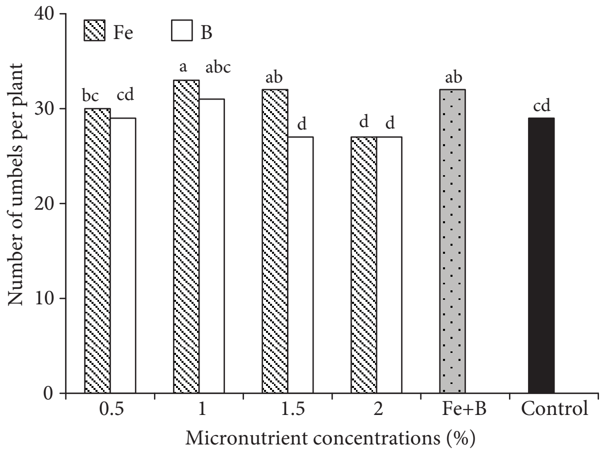

Figure 3. Number of umbels per plant of dill as affected by micronutrient seed priming. Columns with the same letter have no significant difference at the 0.05 probability level.

from seeds primed with 1% and 1.5% Fe before sowing was 32.5 per plant, approximately 12% higher than in the control. The umbel number per dill plant decreased by 7% when the seeds were enriched with the highest concentration of Fe, in comparison with that of the unprimed control. The highest increase in the thousand seed weight (TSW), nearly 33%, was obtained when seeds were primed with 1.5% Fe and 1% B, as compared to control plots (Figure 4).

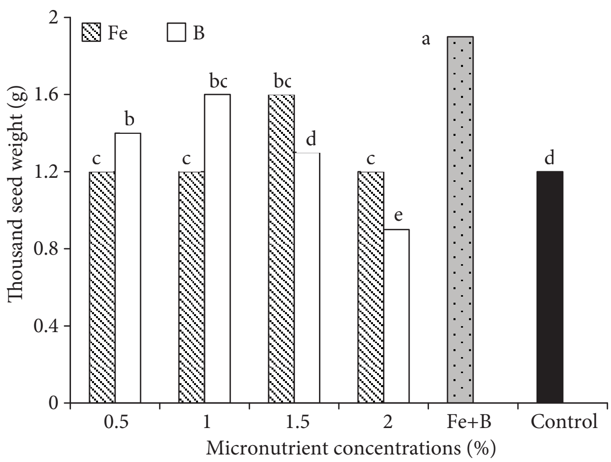

Figure 4. Thousand seed weight of dill as affected by micronutrient seed priming. Columns with the same letter have no significant difference at the 0.05 probability level.

Seed yield was also influenced by Fe and B treatments. The highest seed yield was recorded for seeds soaked for about 12 h in solutions with concentrations of 1.5% Fe + 1% B and 1.5% Fe, which had a nearly 20% and 13% greater yield than the control, respectively. The essential oil concentration of seeds ranged from 2.60% for the 0.5% Fe to 2.81% for the 1.5% B solutions. In the seeds treated with 1.5% Fe + 1% B, the essential oil yield increased significantly as compared with the control (Table 2).

Discussion

Seed treatment with iron and boron, either solely or in combination, gave beneficial effects for seed germination under laboratory conditions. The beneficial effect of seed priming on seedling emergence is consistent with farmers' perceptions of its effects on some other medicinal plants, such as cumin and marigold (*Calendula officinalis* L.) (Tabrizian and Osareh 2007). Seed priming with Fe + B in this study resulted in a total germination rate of 98%. In on-station trials of priming, Harris et al. (1999) reported that soaking seeds in priming solution for 12 h decreased the time of emergence by about 50%.

Concentrations exceeding 1.5% Fe and 1% B in the priming solutions affected germination negatively. Ajouri et al. (2004), similarly studying the effect of seed priming on germination, showed that concentrations exceeding 0.04 M boric acid significantly reduced the germination rate in barley. A reduced germination percentage was also registered for treatment of sweet pepper (*Capsicum annum* L.) when higher dosages of micronutrients were used for seed priming (Diniz et al. 2009).

The importance of seedling vigor on the rapid stand establishment and early growth of medicinal plants to compete for water, light, and nutrients has been stressed by Tabrizian and Osareh (2007). In the present study, it was observed that priming seeds with $FeSO_4$ and H_3BO_3 resulted in a higher SVI of dill. Radpoor and Rimaz (2007), in their study on priming fennel seeds with iron, molybdenum, and boron solutions, came to the same conclusion. Furthermore, Louzada and Vieira (2005) verified that the application of very high doses of micronutrients to bean seeds, due to their toxic effects, caused an increase of abnormal and dead seedlings. Significant differences have been observed in the dry matter accumulation and the SVI of red periwinkle (*Catharanthus roseus* L.) between primed and unprimed seeds (Karthikeyan et al. 2007).

The roles of Fe, Cu, Mn, Zn, Mo, and B on yield and yield components of cumin were studied by Mirshekari et al. (2010). They reported that coating seeds with microelements increased the TSW and number of umbels per plant. An increased grain yield in dill is associated with a higher SDW under nutrient priming. The results of this study indicate that seeds enriched with micronutrients could increase the growth and yield of dill. This is in agreement with the results reported by Arshad Ullah et al. (2002) on peela raya (*Brassica carianata* L.), Mirshekari et al. (2010) on cumin, and Johnson et al. (2005) on some cereals and legumes. However, higher doses of Fe and B in soil solutions may slow seedling establishment.

Priming the seeds with micronutrients makes them able to rapidly imbibe water and revive metabolism and germination. This then results in a higher germination rate (Rowse 1995), improved stand establishment, increased drought and pest tolerance, and ultimately higher yields (Harris et al. 1999). A significant increase in essential oil yield due to seed priming, in comparison to those seeds not primed, was expected because the germination rate, uniformity in stand, seed yield, and oil content in primed seeds were high. Thus, all of these factors contribute to a higher essential oil yield. However, priming may increase yield indirectly through its effect on even stand establishment, because uniformity in the stand results in higher yields (Wade and Meinke 1994).

Conclusion

It can be concluded that priming dill seeds in a determined Fe + B solution results in a further increase in seed and essential oil yields as compared to the control.

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