Journal of Agricultural Production (2023), *4*(1), 1-6 https://doi.org/10.56430/japro.1217929

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

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RESEARCH ARTICLE

Effects of Boron Forms and Doses on Germination in Sunflower, Soybean, and Opium Poppy Seeds

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ARTICLE INFO

Article History
Received: 12.12.2022
Accepted: 03.02.2023
First Published: 28.04.2023

Keywords
Boron
Germination
Glycine max
Helianthus annuus
Papaver somniferum

ABSTRACT

A laboratory experiment was planned to search the inhibitory effects of boron (B) concentrations constituted by two sources on seed germination and subsequent seedling growth of sunflower, soybean, and opium poppy. Seven levels (0, 2, 4, 8, 16, 30, 60, and 90 mg B L^{-1}) of boric acid (H_3BO_3) and sodium borate ($Na_2B_8O_{13}.4H_2O$) were used. Increasing B levels slightly influenced the germination percentage of sunflower, soybean, and opium poppy. Mean germination time in sunflower and opium poppy was shortened at 60 and 90 mg B L^{-1} by increasing B, while the germination index was promoted in sunflower. However, seedling growth of sunflower, soybean, and opium poppy was considerably prohibited by B doses. The shoot length of sunflower was decreased at 16 mg B L^{-1}, but low B levels enhanced root length, indicating that shoot growth showed more sensitivity to B doses than root length. The shoot and root length of soybean did not exhibit any significant trends against B levels. The seedling length of opium poppy was diminished at 60 mg B L^{-1} and above. There were no significant differences between boric acid and sodium borate except for shoot length in soybean, germination percentage and seedling length in opium poppy. It was concluded that there were no toxic effects of B levels up to 90 mg B L^{-1} on germination of sunflower, soybean, and opium poppy, while seedling growth of sunflower and opium poppy was restricted at 60 and 90 mg B L^{-1}, respectively.

Please cite this paper as follows:

Kaya, M. D., Ergin, N., & Harmancı, P. (2023). Effects of boron forms and doses on germination in sunflower, soybean, and opium poppy seeds. *Journal of Agricultural Production*, *4*(1), 1-6. https://doi.org/10.56430/japro.1217929

1. Introduction

Boron (B) is one of the most important micronutrients for crop plants affecting plant growth and yield. It is needed for many physiological processes such as protein formation, cell division, cell wall construction, cell membrane integrity, and root growth (Marschner, 1995; Gupta, 2016). It regulates the balance between sugar and starch, and pollination and seed development (Gupta et al., 1985; Goldbach et al., 2001). B plays a critical role in seed production because healthy flowers cannot be produced, and no seeds develop in case of deficiency in B (Mozafar, 1993). The arable soils contain between 1 and 467 mg kg^{-1} of B, on average between 9 and 85 mg kg^{-1}, and B availability ranges from 0.5 to 5 mg kg^{-1} (Gupta, 2016). In Türkiye, 46.2% of the agricultural soils suffer from B deficiencies while toxic concentrations of B have been 3.3% of the total area (Kıllıoğlu, 2022). Because irrigation water is another source of boron in the soils, it is very common in arid and semi-arid conditions with low rainfall, where B cannot be sufficiently leached and reaches the toxic levels to the plants (Reid, 2007; Tanaka & Fujiwara, 2008).

The responses of plants to boron levels vary within species, cultivars, and the plant growth stages. In literature, there are a lot of researches in relation to the effects of B on plant growth, morphological and physiological changes, and yield in crop

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plants while limited studies have been focused on germination and early seedling growth. For example, B doses up to 2 kg ha^{-1} had positive effects on seed yield (Jagadala et al., 2020) and foliar application between 28 and 1200 mM increased the total dry biomass of sunflower (Asad et al., 2003). Day (2016) reported depression in plant growth with increasing levels of B up to 128 mg kg^{-1} as H_3BO_3, Beyaz et al. (2018) determined the largest leaf areas in the seedlings irrigated with water containing 1 mg B L^{-1}. In soybean, Silva et al. (2020) found the beneficial effects of 0.95 kg B ha^{-1} on growth and seed yield. However, the toxic effect of B higher than 36 kg ha^{-1} was observed in plant growth and yield of opium poppy cultivars (Günlü & Öztürk, 2008). In this study, we concentrated on determining if there was toxic or promoter levels of boron on seed germination and early seedling growth of sunflower, soybean, and opium poppy.

2. Materials and Methods

Standard germination test was conducted at the Seed Science and Technology Laboratory, Eskişehir Osmangazi University in 2022 to assess the toxicity level of boron on germination and seedling growth of sunflower, soybean, and opium poppy. In the experiment, the seeds of sunflower (LG 59580), soybean (Arısoy), and opium poppy (Çelikoğlu) were germinated at seven boron levels (2, 4, 8, 16, 30, 60, and 90 mg B L^{-1}) prepared from boric acid (17% H_3BO_3) and sodium borate (20.9% $Na_2B_8O_{13}.4H_2O$). Distilled water was attained as a control.

Four replicates of 50 seeds were inserted into three-layer filter papers irrigated with 7 mL for each paper sheet of respective boron solutions. After the filter papers with seeds were rolled, they were placed into a sealed plastic bag to prevent moisture evaporation. The packages were incubated at 20 °C in opium poppy for 10 days and at 25 °C in sunflower for 10 days and in soybean for 8 days under the dark condition. The seed with 2 mm radicle protrusion was considered as the germination criterion and germinated seeds were counted every 24 h to assess the mean germination time (MGT) [ISTA, 2018]. Germination index (GI) was also calculated according to Salehzade et al. (2009) with the following formula.

$$MGT = \frac{\sum Dn}{\sum n} \qquad (1)$$

Where, n is the seed number germinated on day D, and D is the number of days from the beginning of the germination test.

$$GI = \frac{Number\ of\ germinated\ seeds}{Days\ of\ first\ count} + \cdots + \frac{Number\ of\ germinated\ seeds}{Days\ of\ the\ final\ count} \qquad (2)$$

On the final day, ten seedlings from each treatment were randomly sampled to measure root length, shoot length, and seedling fresh and dry weight. Dry weights were recorded after oven drying at 70 °C for 48 h.

The experiment was arranged at a two-factor factorial in a completely randomized design with four replications. The data were analyzed by ANOVA and the differences were compared by Tukey's test ($p<0.05$) via MSTAT-C computer software program (Michigan State University, v. 2.10).

3. Results

Analysis of variance showed that any significant effects of boron sources on germination and seedling growth of sunflower were determined, while boron levels significantly affected germination percentage, mean germination time, germination index, shoot length, and seedling fresh weight (Table 1). Two-way interaction between boron forms and levels was significant for germination percentage, germination index, shoot length, root length, and seedling dry weight. MGT was shortened and GI was increased under 60-90 mg B L^{-1} compared to control. Also, the shoot length was increased up to 8 mg L^{-1} and then it decreased. Seedling dry weight was influenced by increasing B levels, but heavier SDW was observed at 60 and 90 mg L^{-1}. The interaction showed that a prominent increase or decrease was observed except for shoot length. It was reduced by increasing B levels, but sodium borate affected more adversely it than boric acid. Shoot length was decreased from 9.78 to 7.19 mg L^{-1} in boric acid and from 9.78 to 6.66 mg L^{-1} in sodium borate.

In soybean, a significant variation for boron sources was determined only in shoot length. Also, boron levels did not affect germination percentage, germination index, and seedling dry weight. Two-way interaction was significant for MGT, SL, RL, and SFW; however, a prominent increase or decrease was observed for the investigated characteristics. In each B level, similar germination percentages were observed, and boron sources and levels did not affect it significantly (Table 2). Boric acid gave a longer shoot length than sodium borate, but the other parameters did not change by boron sources.

Table 1. Germination and seedling growth parameters of sunflower under different concentrations of boric acid and sodium borate.

Factors	**GP (%)**	**MGT (day)**	**GI**	**SL (cm)**	**RL (cm)**	**SFW (mg plant^{-1})**	**SDW (mg plant^{-1})**
Boron forms (A)							
Boric acid (BA)	92.6	2.00	25.7	8.12	5.62	458	43.4
Sodium borate (SB)	93.7	1.97	26.2	8.08	6.32	460	44.5
Dose (B)							
Control	94.0^{a}	2.11^{ab}	24.8^{c}	9.78^{ab}	6.47	502^{ab}†	44.3
2 mg B L^{-1}	90.5^{b}	2.04^{b}	24.5^{c}	9.38^{b}	6.35	477^{bc}	45.1

Table 1. (continued)

Factors	**GP (%)**	**MGT (day)**	**GI**	**SL (cm)**	**RL (cm)**	**SFW (mg plant^{-1})**	**SDW (mg plant^{-1})**
Dose (B)							
4 mg B L^{-1}	95.0^{a}	1.91^{cd}	27.2^{b}	9.47^{b}	5.31	457^{c}	44.3
8 mg B L^{-1}	95.3^{a}	1.95^{c}	26.3^{b}	10.50^{a}	5.10	462^{c}	42.5
16 mg B L^{-1}	89.0^{b}	2.05^{ab}	24.7^{c}	6.41^{cd}	5.42	405^{d}	44.3
30 mg B L^{-1}	90.0^{b}	2.14^{a}	24.1^{c}	5.96^{d}	5.55	399^{d}	44.8
60 mg B L^{-1}	94.5^{a}	1.88^{cd}	26.8^{b}	6.36^{cd}	6.52	450^{c}	44.1
90 mg B L^{-1}	96.8^{a}	1.82^{d}	29.1^{a}	6.93^{c}	7.00	522^{a}	42.4
A×B							
BA × Control	92.0^{b-e}	2.20	23.3^{gh}	9.78^{ab}	5.56^{bc}	495	44.3^{ab}
BA × 2 mg B L^{-1}	90.5^{c-f}	2.10	23.9^{fgh}	8.96^{b}	5.96^{bc}	485	46.0^{a}
BA × 4 mg B L^{-1}	96.0^{ab}	1.92	27.3^{bc}	8.79^{b}	3.89^{c}	434	44.8^{ab}
BA × 8 mg B L^{-1}	94.5^{abc}	1.94	25.9^{b-f}	10.60^{a}	3.91^{c}	431	39.3^{c}
BA × 16 mg B L^{-1}	89.5^{def}	2.09	25.3^{c-g}	6.04^{de}	5.23^{bc}	410	42.8^{abc}
BA × 30 mg B L^{-1}	86.5^{f}	2.11	22.9^{h}	6.34^{cde}	5.85^{bc}	429	44.0^{ab}
BA × 60 mg B L^{-1}	93.5^{bcd}	1.90	26.1^{b-e}	7.27^{c}	5.86^{bc}	451	45.0^{ab}
BA × 90 mg B L^{-1}	98.0^{a}	1.78	30.7^{a}	7.19^{c}	8.66^{a}	533	41.3^{bc}
SB × Control	96.0^{ab}	2.02	26.2^{b-e}	9.78^{ab}	7.39^{ab}	510	44.3^{ab}
SB × 2 mg B L^{-1}	90.5^{c-f}	1.98	25.1^{d-h}	9.81^{ab}	6.74^{ab}	470	44.3^{ab}
SB × 4 mg B L^{-1}	94.0^{abc}	1.91	27.0^{bcd}	10.20^{a}	6.74^{ab}	481	43.8^{ab}
SB × 8 mg B L^{-1}	96.0^{ab}	1.95	26.8^{bcd}	10.40^{a}	6.30^{b}	494	45.8^{a}
SB × 16 mg B L^{-1}	88.5^{ef}	2.01	24.2^{e-h}	6.78^{cd}	5.62^{bc}	400	45.8^{a}
SB × 30 mg B L^{-1}	93.5^{bcd}	2.17	25.2^{c-g}	5.59^{e}	5.26^{bc}	370	45.5^{a}
SB × 60 mg B L^{-1}	95.5^{ab}	1.86	27.5^{b}	5.45^{e}	7.17^{ab}	449	43.3^{ab}
SB × 90 mg B L^{-1}	95.5^{ab}	1.87	27.5^{b}	6.66^{cd}	5.35^{bc}	511	43.5^{ab}
Analysis of Variance							
A	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
B	**	**	**	**	*ns*	**	*ns*
A×B	*	*ns*	**	**	**	*ns*	*

†: Letter(s) connected with the means show significance levels at $p<0.05$. *, **: significant at $p<0.05$ and 0.01; ns: not significant. GP: Germination percentage, MGT: Mean germination time, GI: Germination index, SL: Shoot length, RL: Root length, SFW: Seedling fresh weight, SDW: Seedling dry weight.

Table 2. Germination and seedling growth parameters of soybean under different concentrations of boric acid and sodium borate.

Factors	**GP (%)**	**MGT (day)**	**GI**	**SL (cm)**	**RL (cm)**	**SFW (mg plant^{-1})**	**SDW (mg plant^{-1})**
Boron (A)							
Boric acid (BA)	93.0	2.05	22.8	10.2^{a}	9.30	759	108
Sodium borate (SB)	92.8	2.07	23.2	9.6^{b}	9.45	768	110
Dose (B)							
Control	91.5	2.05^{bc}	23.3	10.1^{bc}	8.99^{d}	765^{bc}†	106
2 mg B L^{-1}	92.5	2.10^{a}	22.7	11.8^{a}	10.70^{a}	847^{a}	112
4 mg B L^{-1}	94.5	2.10^{a}	20.9	9.8^{bcd}	9.21^{cd}	795^{b}	107
8 mg B L^{-1}	94.0	2.13^{a}	22.9	8.8^{f}	7.93^{e}	712^{d}	112
16 mg B L^{-1}	95.5	2.09^{ab}	23.7	10.4^{b}	9.81^{bc}	781^{bc}	111
30 mg B L^{-1}	91.0	1.98^{d}	23.6	9.1^{ef}	9.29^{cd}	743^{cd}	109
60 mg B L^{-1}	91.8	1.99^{cd}	23.6	9.5^{cde}	10.50^{ab}	750^{cd}	110
90 mg B L^{-1}	92.8	2.03^{cd}	23.2	9.4^{def}	8.97^{d}	715^{d}	105
A×B							
BA × Control	92.0	2.02^{d-g}	23.4	9.2^{fgh}	8.62^{def}	721^{c-f}	105
BA × 2 mg B L^{-1}	95.0	2.07^{b-f}	23.6	13.9^{a}	12.70^{a}	928^{a}	112
BA × 4 mg B L^{-1}	92.5	2.15^{ab}	17.7	10.3^{bcd}	10.20^{c}	812^{b}	107
BA × 8 mg B L^{-1}	93.5	2.09^{bcd}	23.1	8.6^{hıi}	7.96^{f}	694^{f}	113
BA × 16 mg B L^{-1}	96.5	2.05^{def}	24.4	10.6^{bcd}	9.41^{cd}	769^{bcd}	108
BA × 30 mg B L^{-1}	90.8	1.96^{g}	24.2	7.9^{i}	6.59^{g}	710^{ef}	113
BA × 60 mg B L^{-1}	91.3	1.99^{fg}	23.1	10.7^{bc}	11.40^{b}	790^{b}	103
BA × 90 mg B L^{-1}	92.8	2.04^{d-g}	22.9	10.0^{c-f}	8.76^{def}	716^{def}	103
SB × Control	91.0	2.07^{b-e}	23.3	10.9^{b}	9.37^{cd}	809^{b}	108

Table 2. (continued)

Factors	**GP (%)**	**MGT (day)**	**GI**	**SL (cm)**	**RL (cm)**	**SFW (mg plant^{-1})**	**SDW (mg plant^{-1})**
A×B							
SB × 2 mg B L^{-1}	90.0	2.14^{abc}	21.7	$9.7^{d\text{-}g}$	8.74^{def}	$767^{b\text{-}e}$	112
SB × 4 mg B L^{-1}	96.5	$2.06^{c\text{-}f}$	24.1	$9.3^{e\text{-}h}$	8.27^{ef}	779^{bc}	108
SB × 8 mg B L^{-1}	94.5	2.18^{a}	22.7	$9.1^{\text{ghı}}$	7.90^{f}	$729^{c\text{-}f}$	112
SB × 16 mg B L^{-1}	94.5	2.13^{abc}	23.0	10.3^{bcd}	10.20^{c}	792^{b}	115
SB × 30 mg B L^{-1}	91.3	2.00^{efg}	23.0	$10.2^{b\text{-}e}$	11.90^{ab}	776^{bc}	104
SB × 60 mg B L^{-1}	92.3	1.99^{fg}	24.0	$8.3^{\text{ıi}}$	9.47^{cd}	709^{ef}	116
SB × 90 mg B L^{-1}	92.8	2.01^{efg}	23.6	$8.7^{\text{hıi}}$	9.18^{cde}	714^{def}	107
Analysis of Variance							
A	*ns*	*ns*	*ns*	**	*ns*	*ns*	*ns*
B	*ns*	**	*ns*	**	**	**	*ns*
A×B	*ns*	*	*ns*	**	**	**	*ns*

†: Letter(s) connected with the means show significance levels at $p<0.05$. *, **: significant at $p<0.05$ and 0.01; ns: not significant. GP: Germination percentage, MGT: Mean germination time, GI: Germination index, SL: Shoot length, RL: Root length, SFW: Seedling fresh weight, SDW: Seedling dry weight.

Table 3. Germination characteristics of poppy seeds under different concentrations of boric acid and sodium borate.

Factors	**GP (%)**	**MGT (day)**	**GI**	**SL (cm)**
Boron (A)				
Boric acid (BA)	96.1^{a}	2.07	23.5	4.93^{a}†
Sodium borate (SB)	94.6^{b}	2.06	23.3	4.47^{b}
Dose (B)				
Control	97.8^{a}	2.11^{b}	23.5	4.69^{bc}
2 mg B L^{-1}	96.3^{ab}	2.11^{b}	23.2	4.74^{abc}
4 mg B L^{-1}	96.5^{ab}	2.16^{a}	22.9	4.85^{ab}
8 mg B L^{-1}	96.5^{ab}	2.09^{b}	23.4	4.85^{ab}
16 mg B L^{-1}	92.5^{c}	2.00^{d}	23.3	4.80^{ab}
30 mg B L^{-1}	95.3^{abc}	2.04^{c}	23.6	4.95^{a}
60 mg B L^{-1}	94.0^{bc}	1.99^{d}	23.8	4.51^{c}
90 mg B L^{-1}	94.0^{bc}	1.99^{d}	23.8	4.20^{d}
A×B				
BA × Control	98.5	2.13^{bc}	23.5	$4.67^{d\text{-}g}$
BA × 2 mg B L^{-1}	97.5	2.14^{b}	23.3	$5.01^{a\text{-}d}$
BA × 4 mg B L^{-1}	97.0	2.11^{bcd}	23.3	4.57^{efg}
BA × 8 mg B L^{-1}	97.5	2.11^{bcd}	23.5	$4.87^{b\text{-}e}$
BA × 16 mg B L^{-1}	94.0	2.02^{fg}	23.5	5.20^{ab}
BA × 30 mg B L^{-1}	96.0	2.06^{ef}	23.5	5.33^{a}
BA × 60 mg B L^{-1}	94.0	1.99^{g}	23.8	$4.90^{b\text{-}e}$
BA × 90 mg B L^{-1}	94.5	1.99^{g}	23.8	$4.89^{b\text{-}e}$
SB × Control	97.0	2.09^{cde}	23.5	$4.72^{d\text{-}g}$
SB × 2 mg B L^{-1}	95.0	2.08^{de}	23.1	4.47^{fgh}
SB × 4 mg B L^{-1}	96.0	2.21^{a}	22.4	5.13^{abc}
SB × 8 mg B L^{-1}	95.5	2.08^{de}	23.3	$4.83^{c\text{-}f}$
SB × 16 mg B L^{-1}	91.0	1.99^{g}	23.0	4.41^{gh}
SB × 30 mg B L^{-1}	94.5	2.03^{fg}	23.6	4.58^{efg}
SB × 60 mg B L^{-1}	94.0	2.01^{g}	23.9	4.12^{h}
SB × 90 mg B L^{-1}	93.5	1.99^{g}	23.8	$3.50^{\text{ı}}$
Analysis of Variance				
A	*	*ns*	*ns*	**
B	*	**	*ns*	**
A×B	*ns*	**	*ns*	**

†: Letter(s) connected with the means show significance levels at $p<0.05$. *, **: significant at $p<0.05$ and 0.01; ns: not significant. GP: Germination percentage, MGT: Mean germination time, GI: Germination index, SL: Seedling length.

Seed germination of opium poppy was higher in boric acid than sodium borate, such increases were attained in shoot length (Table 3). In addition, mean germination time and seedling length were reduced at 60 and 90 mg B L^{-1}. Germination index was not changed by B applications. Two-way interaction in MGT and SL exhibited that differences between control and 90 mg B L^{-1} were similar to each other, but the seedling length was reduced by increasing levels of sodium borate.

4. Discussion

This study exhibited the germination and early seedling growth parameters of sunflower, soybean, and opium poppy under various levels of boron sources. Considering germination parameters, soybean was not affected by B levels whose effects were found uneven. Although there were statistical differences among B levels in sunflower and opium poppy, which did not exactly reflect any trends upward or downward. Similar results were also observed in sunflower and opium poppy. It could be easily said that the germination of these species was not influenced by boron doses up to 90 mg B L^{-1} only if a 2 mm of radicle hook from the seeds was considered the germination criterion. Our results showed similarity with the findings of Patil et al. (2012) in soybean, Lima (1998) in pea, and Jadhav and Bhamburdekar (2014) in sorghum, who determined that no significant reduction in germination percentage due to increasing B. In contrast, Ashagre et al. (2014) reported the seeds of safflower did not germinate at 8 and 16 ppm B levels and Bonilla et al. (2004) found a reduction in germination percentage in pea. This may result from that they considered the appearance of radicle and hypocotyl from the seeds as the germination criterion. In sunflower, mean germination time shortened slightly and the germination index increased at 60 and 90 mg B L^{-1}. However, any clear tendency was observed in soybean and opium poppy.

Seedling growth of sunflower, soybean, and opium poppy was more sensitive to B levels than germination. The shoot length of the sunflower declined significantly at 60 and 90 mg B L^{-1}, while a precise B level decreasing root and shoot length in soybean could not be detected. Our results disagree with the findings reported by Ashagre et al. (2014) showed that root and shoot growth of safflower were linearly dropped by increasing B levels and higher levels than 4 ppm B were toxic. Jadhav and Bhamburdekar (2014) found the promoter effect of 5 and 10 ppm B, while higher levels led to decreasing in root and shoot length of sorghum. Culpan et al. (2019) reported a significant reduction in seedling growth of safflower when B doses were increased. In the previous researches, there have been controversial results regarding boron's effects on germination and seedling growth of various plants, which were detected positive neutral, and toxic. Although this may be explained by species and cultivar differences, here we suggest that the seed vigor should be considered for germination performance under boron stress.

5. Conclusion

In conclusion, our results showed that there was no remarkable adverse effect of boron on germination parameters of sunflower, soybean, and opium poppy seeds. Although the shoot length of sunflower and seedling length of opium poppy were diminished by increasing boron levels, the beneficial effects of boron doses between 2 and 8 mg B L^{-1} were found for seedling growth of sunflower, soybean, and opium poppy. It was concluded that the inhibitory effects of boron for sunflower and opium poppy started at 60 mg L^{-1}, while soybean did not show a clear sensitivity to boron levels.

Conflict of Interest

The authors declare that they have no conflict of interest.

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