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Full Length Research Paper

Boron toxicity on seed germination and seedling growth of safflower (*Carthamus tinctorius* L.)

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To study the effect of boron on seed germination and seedling growth of safflower (*Carthamus tinctorius* L.), a laboratory experiment was undertaken at Department of Plant Science, Ambo University, Ethiopia. Seeds were sown in petri dishes with varying concentrations of boron (0, 0.25, 0.5, 1, 2, 4, 8, and 16 mg/l) at room temperature (24±2 ^{0}C) in completely randomized design with four replications. The result revealed that germination percentage and rate decreased beyond 2 mg/l and 1 mg/l, respectively. Shoot (≥1 mg/l) and root (>0.5 mg/l) lengths, shoot (>0.5 mg/l and >2 mg/l) and root (≥0.5mg/l and ≥1mg/l) fresh and dry weights, respectively decreased with increase in boron concentration. Root number, root - shoot ratio, and seedling vigor index were found to decrease beyond ≥0.25 mg/l, ≥2 mg/l, and ≥1 mg/l, respectively. In addition, phytotoxicity increased significantly (except 0.25 mg/l which promoted shoot and root) with increased in the concentration of boron. Safflower germination and seedling growth completely failed at ≥8mg/l of boron concentration.

Keywords: Boron, germination, phytotoxicity, safflower, seedling growth, tolerance, vigor

INTRODUCTION

Boron (B) toxicity may occur due to naturally high concentrations in the soil, the over use of B fertilizer or the continued use of irrigation waters high in soluble salts, including Boron. Boron is often found in high concentration in association with saline soils (Dhankhar and Dahiya, 1980). Irrigation water is the most important contributor to high levels of soil boron (Chauhan and Powar, 1978). Under low rainfall conditions, boron cannot be sufficiently leached and therefore may accumulate to levels that become toxic to plant growth (Reid, 2007). This occurs very often in arid and semi arid regions with high-boron groundwater, where the accumulation of boron in top soil due to evaporation of groundwater reaches toxic levels that can reduce crop yields (Tanaka and Fujiwara, 2007). In assessing the potential toxicity of B availability on irrigation water, the physical and chemical characteristics of the soil must be considered (Rauf et al., 2007). Boron can be regenerated through the mineralization of the soil organic matter, or through weathering processes of soil minerals (Peryea et al., 1985). Boron is widely used in industry for the production of commercial products such as insulation glass fibers, bleaches, borosilicate glass, chemical fertilizers, herbicides and insecticides (Schnurbusch, 2010).

Plants exposed to excess of boron have reduced vigor, retarded development, leaf burn (chlorotic and necrotic patches in older leaves), and decreased number, size, and weight of fruits (Nable et al., 1997). Boron toxicity is an important agricultural problem that limits crop productivity in different regions of the world, and can occur in B-rich soils or in soils exposed to B-rich irrigation waters, fertilizers, sewage sludge, or fly ash (Luis et al., 2012).

Safflower (*Carthamus tinctorius* L.) is a tap rooted annual crop which can tolerate environmental stresses including salinity and water stress (Lovelli et al., 2007). Safflower is produced on marginal lands that are relatively dry and deprived of the benefits of fertilizer inputs. Safflower is a candidate crop in dry land agro-ecosystems due to its potential for growth under water

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Table 1. Effect of boron on germination and seedling growth parameters of safflower

Boron Conc. (mg/L)	Germination (%)	Germination rate	Shoot length (cm)	Root length (cm)
0	100 a	7.49 (3.68) a	6.25 (3.50) a	7.25 (3.68) b
0.25	100 a	7.25 (3.64) a	6.40 (3.53) a	9.08 (4.01) a
0.5	100 a	8.75 (3.93) a	5.45 (3.33) a	6.45 (3.52) b
1	100 a	7.25 (3.64) a	3.28 (2.81) b	3.48 (2.85) c
2	92.5 a	3.60 (2.87) b	1.20 (1.95) c	0.43 (1.65) d
4	23.8 b	0.53 (1.36) bc	0.23 (1.24) d	0.08 (1.14) e
8	0 b	0 (1.00) c	0 (1.0) d	0.0 (1.0) e
16	0 b	0 (1.00) c	0 (1.0) d	0.0 (1.0) e
SEm(±)		0.50	0.29	0.22
CV (%)	26.1	19	0.09	9.5

Means with similar letters in each column are not significant at 5% level of probability. Data in parenthesis are square root transformed data

stress and the economical value in terms of both oil and seed (Kar et al., 2007). India, Mexico, USA, Ethiopia, Argentina and Australia together account for 99 and 87% of the world safflower area and production, respectively (Dwivedi et al.,2005). In Ethiopia, 500 thousand hectares of land devoted for safflower production in 2009/10 cropping season with an average yield of 11.93 qt/ha (CSA, 2011). It is a crop commonly grown in the lowland parts of the country as sole crop or being intercropped with *Eragrostis tef*.

Crop genotypic variation in susceptibility to B toxicity in crops has been reported (Torun et al., 2006). Paull et al., (1988) reported that wide range of intra-specific variation in response to B occurs in a number of crops. Germination and seedling growth parameters are the most viable criteria used for selecting stress tolerance in crop plants. Percentage of germination and seedling growth are important growth parameters to be studied for cultivar tolerance (Mordi and Zavareh, 2013). Hence, the current experiment was conducted to investigate the effect of boron on germination and seedling growth parameters of *Carthamus tinctorius* L.

MATERIALS AND METHODS

To investigate the effect of boron on germination and seedling growth of safflower, a laboratory experiment was conducted in October, 2013 at the Department of Plant Sciences, Ambo University. The experiment was arranged in completely randomized design with four replications. Local cultivar was treated with eight levels of boron (0, 0.25, 0.5, 1, 2, 4, 8, and 16 mg/l) for the experiment, deionized water was used for the control treatment. Boric acid (H_3BO_3) was used as a source of boron. Seeds were surface sterilized with 30% hydrogen peroxide solution for 5 min, and rinsed with deionized water. Twenty seeds were uniformly placed per petri dish (9.5 cm diameter) using a forceps after the petri dish were sterilized with 98% ethanol, and rinsed with deionized water. Filter papers were well soaked by adding 7 ml with the respective solutions (7 treatment solutions and the control) at an interval of 48 h as described by Naveed et al., (2001). All the petri dishes were covered with lids and kept at room temperature (24 ±2 ^oC). Germination continued for 10 days, and germinated seeds were counted daily. Germination was considered to have occurred when radicles attained a length of 2 mm. After 10 days, parameters such as percent germination and rate of germination were calculated according to ISTA (1999); and root and shoot lengths of seedling were measured using a scale. Root and shoot dry weights were recorded after oven drying for 72 h at 60 ^oC. The seedling vigor and tolerance indices were determined using the formula of Hosseein and Kasra (2011), and Iqbal and Rahmati (1992), respectively. The percentage of phytotoxicity on shoot and root of seedlings was calculated following the formula given by Chou and Lin (1976).

Statistical analysis of the data was performed using one-way ANOVA using SAS statistical software (Version 9). Based on the ANOVA results, mean separations were performed by Duncan's multiple range test at 5% level.

RESULTS AND DISCUSSION

Seed germination and seedling growth

The increased in boron concentration did not significantly influence germination percentage of safflower up to 2 mg/l. A significant decrease was observed on germination percentage as boron concentrations increased beyond 2 mg/l concentration. At 8 mg/l and 16 mg/l of boron concentrations, safflower seed failed to germinate, indicating that *Carthamus tinctorius* did not show good growth at high concentration of boron (Table 1). The consistent decrease in percentage and rate of

Table 2. Effect of boron on shoot, root fresh and dry weights

Boron Conc. (mg/L)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Seedling fresh weight (g)	Seedling dry weight (g)
0	0.22 (1.47) a	0.02 (1.12) a	0.043 (1.20) a	0.0030 (1.055) a	0.263(1.51) a	0.023 (1.135) a
0.25	0.20 (1.45) a	0.017 (1.13) a	0.035 (1.19) a	0.0023 (1.047) ab	0.235 (1.49) a	0.0193 (1.129) a
0.5	0.19 (1.44) a	0.015 (1.12) a	0.025 (1.16) b	0.0023 (1.047) ab	0.215 (1.47) a	0.0173 (1.138) a
1	0.10(1.29) b	0.011 (1.11) a	0.012 (1.11) c	0.0019(1.044) b	0.112(1.31) b	0.0129 (1.109) a
2	0.07 (1.26) b	0.010 (1.10) a	0.002 (1.04) d	0.0003(1.017) c	0.072(1.26) b	0.0103 (1.111) a
4	0.01 (1.05) c	0.002 (1.02) b	0.001(1.01) e	0.0003(1.008) cd	0.011 (1.05) c	0.0023 (1.024) b
8	0 .0(1.0) c	0.0 (1.0) b	0.0 (1.0) e	0.0 (1.0) d	0.0 (1.0) c	0.0 (1.0) b
16	0 .0 (1.0) c	0.0 (1.0) b	0.0 (1.0) e	0.0 (1.0) d	0.0 (1.0) c	0.0 (1.0) b
SEm(±)	0.06	0.02	0.01	0.01	0.05	0.02
CV (%)	4.5	1.95	1.3	0.6	4.2	1.9

Means with similar letters in each column are not significant at 5% level of probability. Data in parenthesis are square root transformed data

seed germination beyond 2 mg/l and 1 mg/l, respectively in the present study is in line with the findings of Yau and Saxena (1997), and Muhammad et al., (2013) who stated that high boron concentration reduced germination percentage of durum wheat and maize, respectively.

The shoot and root lengths, shoot and root fresh and dry weights, and seedling dry weight decreased significantly with increase in boron concentration. However, the highest shoot length (6.40 cm) and root length (9.08 cm) obtained at 0.25 mg/l boron concentration; and the lowest shoot and root lengths were with 8 mg/l and 16 gm/l concentrations that caused a complete failure of germination. Boron inhibits root growth primarily through limiting cell elongation rather than cell division (Brown et al., 2002). Nable et al., (1997) and Habtamu et al., (2014) reported that shoot and root growth reduced when exposed to high B levels.

Shoot and root fresh and dry weights, and seedling fresh and dry weights decreased significantly with increase in boron concentration beyond 0.5 mg/l. Fresh weight and dry matter yield of the plants decreased significantly with increasing levels of applied boron beyond 0.25 mg/l (Habtamu et al., 2014; Ayvaz et al., 2012). Muhammad et al., (2013) and Turan et al., (2009) reported that shoot and root fresh and dry weights of maize and wheat decreased with the increase in concentrations of boron, respectively. The highest shoot and root fresh and dry weights, and seedling fresh and dry weights obtained on the control treatment. The significant increase at low concentration of boron could be due to its involvement in cell elongation or cell division and meristematic growth (Khan et al. 2006). Farr (2010) reported that low concentrations of exogenous boric acid stimulate the seed germination and seedling growth, while high concentrations showed an inhibitive effect on these parameters (Ölçer and Kocaçalışkan, 2007). (Table 2)

Root number and root- to-shoot ratio

The analysis of variance indicated that increase in boron concentration significantly reduced root number and root-to-shoot ratio. Nevertheless, there were no significant difference observed for root-to-shoot ratio among control treatment, 0.5 mg/l, and 1 mg/l of boron concentrations (Figure1). Cokkizgin (2013) and Habtamu et al., (2014) reported similar finding on bean and wheat, respectively, and the increase in B concentration inhibited the secondary root emergence (Rehman et al., 2012).

Seedling vigour, tolerance and phytotoxicity

The increase in boron concentrations above 0.5 mg/l have a significant effect on seedling vigour index, shoot and root vigour indices (Figure 2). The highest value for each trait was noticed on control treatment and up to 0.5 mg/l. An increase in concentration of boron beyond 0.5 mg/l decreased vigour indices significantly. Mirshekari (2012) and Cokkizgin (2013) reported similar finding on seedling vigor index of wheat, dill, and *Phaseolus vulgaris* at high level of boron concentrations, respectively.

Boron have a significant effect ($p<0.05$) on shoot and root phytotoxicity (Figure 3). Phytotoxicity of shoot and root increased with an increase in boron concentration. Lesser shoot and root toxicity was recorded with control treatment and lower concentration (0.25 mg/l), while it increased at higher concentration. Maximum phytotoxicity

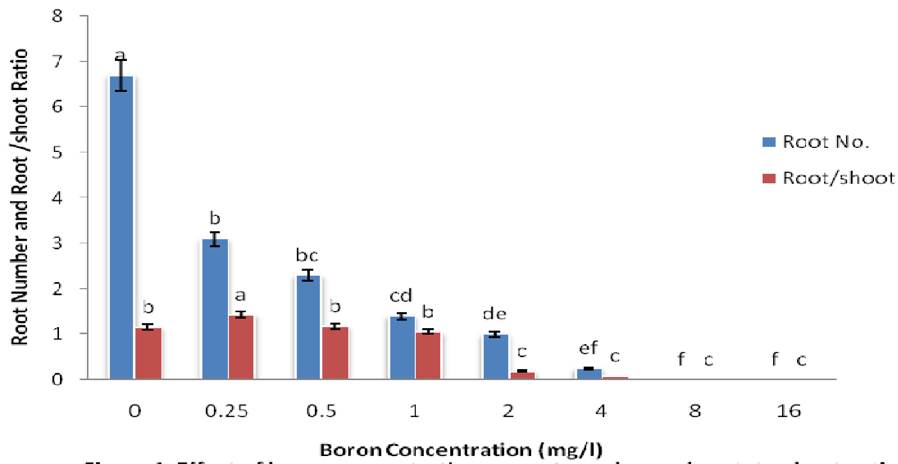

Figure 1. Effect of boron concentration on root number and root -to-shoot ratio

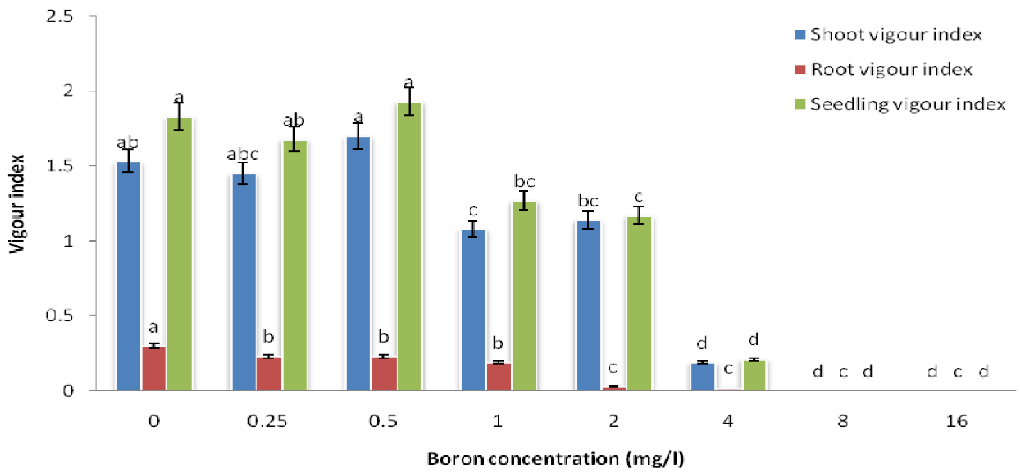

Figure2. Effect of boron on vigour indices of safflower

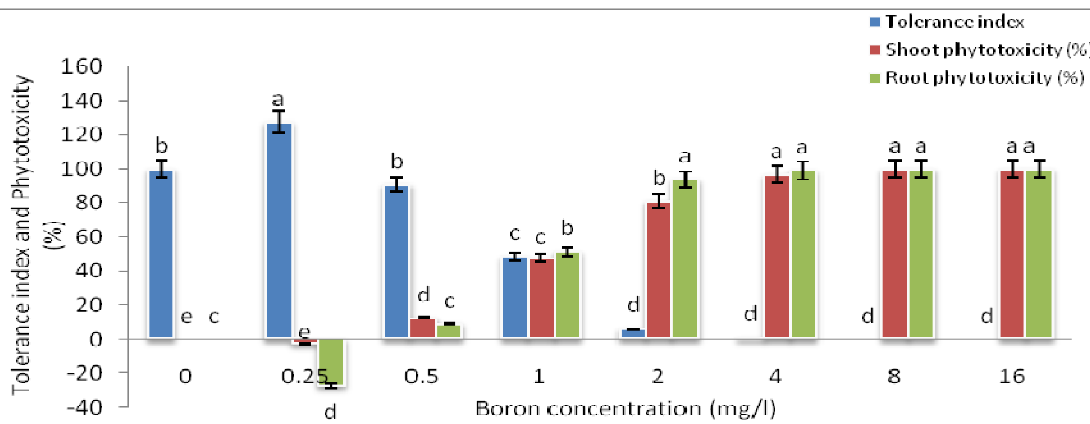

Figure 3. Effect of boron on tolerance index and phytotoxity of safflower

of boron on shoot and root were observed at ≥4 mg/l and ≥2 mg/l, respectively. The finding of our study is in agreement with the recent reports of Shaikh et al., (2013) who reported that micronutrient toxicity of shoot and root decreased at lower concentration, and increased at higher concentrations.

The tolerance index of safflower seed declined significantly with increase in boron concentration beyond 0.5 mg/l. Maximum tolerance index was obtained on 0.25 mg/l boron followed by control and 0.5 mg/l treatments. 0.25 mg/l boron concentration promoted seedling growth of safflower. However, it showed zero tolerance for boron concentrations beyond 2mg/l. This result is in agreement with the recent findings of Shaikh et al., (2013), and Habtamu et al., (2013); who reported increasing micronutrient concentrations decreased wheat and tomato tolerance index, respectively. However, the tolerance index for boron toxicity varied with species, and varieties of crops.

CONCLUSION

Germination percentage and rate decreased significantly beyond 2 mg/l and 1 mg/l boron concentrations, respectively. While shoot and root lengths, shoot and root fresh and dry weights, vigor and tolerance indices decreased at high level of boron concentrations. Safflower shoot and root, and seedling toxicity increased at high concentration of boron (≥2 mg/l). Boron concentration up to 0.5 mg/l have no significant effect on shoot fresh weight, shoot and root dry weights, seedling dry weight, and low toxicity on shoot length. Even though boron concentrations up to 1 mg/l have no effect on germination of safflower, beyond ≥ 0.5 mg/l had influential effect on seedling growths. Boron concentrations ≥ 8 mg/l caused deleterious effect on safflower.

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