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ORIGINAL ARTICLE

Alleviation of Boron Stress through Plant Derived Smoke Extracts in *Sorghum bicolor*

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Boron is an essential micronutrient necessary for plant growth at optimum concentration. However, at high concentrations boron affects plant growth and is toxic to cells. Aqueous extract of plant-derived smoke has been used as a growth regulator for the last two decades to improve seed germination and seedling vigor. It has been established that plant-derived smoke possesses some compounds that act like plant growth hormones. The present research was the first comprehensive attempt to investigate the alleviation of boron stress with plant-derived smoke aqueous extract on Sorghum (*Sorghum bicolor*) seed. Smoke extracts of five plants, i.e. *Cymbopogon jwarancusa*, *Eucalyptus camaldulensis*, *Peganum harmala*, *Datura alba* and *Melia azedarach* each with six dilutions (Concentrated, 1:100, 1:200, 1:300, 1:400 and 1:500) were used. While boron solutions at concentrations of 5, 10, 15, 20 and 25 ppm were used for stress. Among the dilutions of smoke, 1:500 of *E. camaldulensis* significantly increased germination percentage, root and shoot length, number of secondary roots and fresh weight of root and shoot while, boron stress reduced growth of Sorghum. It was observed that combined effect of boron solution and *E. camaldulensis* smoke extract overcome inhibition and significantly improved plant growth. Present research work investigated that the smoke solution has the potential to alleviate boron toxicity by reducing the uptake of boron by maintaining integrity of plant cell wall. The present investigation suggested that plant derived smoke has the potential to alleviate boron stress and can be used to overcome yield losses caused by boron stress to plants.

Key words: Sorghum, boron toxicity, alleviation, seed germination, plant-derived smoke

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Boron is an essential micronutrient required to maintain structural integrity of plant cell wall and metabolic pathways. The optimum B content for most of the plants is 20-100 ppm. Excess levels of Boron results in B toxicity as the B requirements and toxicity levels varies among plants. Boron availability in soil and in irrigation water is an important factor in agricultural production (Tanaka and Fujiwara, 2007). Boron occurs primarily as boric acid (H_3BO_3) in soil solution, which can be leached under high rainfall situations (Shorrocks, 1997; Yan *et al.*, 2006). Due to this, B becomes deficient for plants that grow there and plant growth is affected as highly leached soils are not able to retain boron. On the other hand, low rainfall conditions can not sufficiently leach B and therefore may accumulate to levels that are toxic to plant growth (Reid, 2007b).

Plant-derived smoke has the ability to break dormancy and stimulate germination of *Audouinia capitata*, a fynbos species that grows in a fire-prone habitat (De Lange and Boucher, 1990). Since this discovery originated an idea that aqueous smoke extracts can be used to improve seed germination in a broad range of plants related to many families, irrespective of their fire sensitivity (Dixon *et al.*, 1995; Roche *et al.*, 1997; Brown and Botha, 2004). This phenomenon is potentially applicable in seed technology and has been comprehensively discussed in a number of reviews (Brown and Van Staden, 1997; Van Staden *et al.*, 2000). Recently, a highly active and water soluble germination promoting compound, butenolide, 3-methyl-2H-furo [2, 3-c] pyran-2-one has been identified in smoke of burnt fynbos *Passerina vulgaris* and grass *Themeda triandra* L. (Van Staden *et al.*, 2004) as well as from the combustion of cellulose (Flematti *et al.*, 2004). This compound has the ability to stimulate germination at very low concentrations, i.e. 10^{-9} M (Flematti *et al.*, 2004; Van Staden *et al.*, 2004). The present research work is the first systematic attempt to investigate the alleviating effects of plant derived smoke extracts against toxicity caused by boron.

MATERIALS AND METHODS

Seeds Collection

Seeds of Sorghum (*Sorghum bicolor*), DS-2003 were obtained from NARC (National Agriculture Research Centre) Islamabad, Pakistan.

Preparation of Solutions

Plant materials of *M. azedarach, C. jwarancusa, P. harmala, D. alba* and *E. camaldulensis* were collected from Kohat region. Shade dried plant parts of these plants were used for preparation of concentrated aqueous smoke extracts by following methods of De Lange and Boucher (1990) as well as Dixon *et al.* (1995) with slight modifications. The smoke extracts were diluted up to 1:100, 1:200, 1:300, 1:400 and 1:500 by using distilled water.

Treatments

Initially the Sorghum seeds were treated with different boron concentrations, i.e. 5; 10; 15; 20 and 25 ppm respectively. Furthermore, smoke solutions with significant results (1:500) were mixed with boron solutions to prepare “B stress alleviating solutions” for assessment of alleviation potential of plant-derived smoke against B stress.

Seed Germination

Ten seeds of Sorghum per petri plate were moistened and chilled in Petri dish covered with double layer filter papers and then kept at 25 ^{0}C for two days to obtain uniform germination. After germination the seeds with equal radical size were placed on a filter paper soaked with respective B stress alleviating solutions. The filter paper was rolled like a cigar and was placed in a growth chamber under controlled conditions at 25 ^{0}C for ten days. Seed germination percentage was recorded for ten days of incubation by following Chantachume *et al*. (1995) method. Root and shoot length as well as fresh and dry weight of seedlings were recorded after ten days of incubation.

Measurement of Boron contents

For determination of B level, oven dried roots and shoots of Sorghum plant were digested in sulfuric acid and hydrogen peroxide in 2:1 ratio and analyzed by spectrophotometer following Azomethine – H method reviewed by Basson *et al*. (1969).

Statistical analysis

Statistics 9 software was used to analyze the data. One-way ANOVA and LSD- tests were applied to determine the correlation among different variables. P-value less than 0.05 considered as significant value.

RESULTS

Present results revealed that smoke extracts collected from plant materials, improved seed germination (Fig. 1a). Significant increase in germination was observed by treating seeds with different smoke dilutions of *C. jwarancusa* (1:100 and 1:500), *D. alba* (1:300, 1:400 and 1:500), *M. azedarach* at dilutions (1:100 and 1:500), while all dilutions *E. camaldulensis* and *P. harmala* (1:100, 1:200 and 1:500) (Fig. 1b). Significant decrease in final germination of Sorghum with (1:200) dilution of both *C. jwarancusa* and *M. azedarach* was resulted. Similarly concentrated smoke extracts of all the plants significantly reduced seed germination percentage.

After 24 hours of germination, smoke treatment (1:500) of *E. camaldulensis* showed most significant results and increased germination percentage was observed with smoke treatment (Fig 2a), while inhibition resulted with B treatments. With the application of combined solutions of smoke and B, smoke alleviated the inhibitory effects of B on seed germination percentage (Fig. 2b).

Effects on Root and shoot length

Root length of Sorghum was significantly increased with application of *E. camaldulensis* smoke at dilutions (1:200 and 1:500) and *P. harmala* at dilution of 1:100 (Fig. 3a) while decreased by the application of *D. alba* dilutions (1:300, 1:400 and 1:500) and *M. azedarach* dilutions (1:100, 1:300 and 1:400). Similarly shoot length was significantly increased with the somoke dilutions (1:200 and 1:500) of *E. camaldulensis* and *P. harmala* (1:100 and 1:500) while dilutions of Melia and Datura as well as concentrated smoke extracts of plants decreased shoot length (Fig. 3b).

Effects on secondary roots of Sorghum

Number of secondary roots of Sorghum were significantly increased when treated with smoke dilutions (1:200 and 1:500) of *E. camaldulensis* while 1:400 dilution of *P. harmala* increased the number of secondary roots as compared to control. Similarly, 1:100 and 1:500 of *C. jwarancusa*, all dilutions of *D. alba* and *M. azedarach* as well as concentrated smoke extracts of all the plants significantly decreased the number of secondary roots (Fig. 4).

Effects on root fresh and dry weight

With application of plant-derived smoke, fresh and dry weight of plant roots were increased. Dilutions of *E. camaldulensis* (1:100, 1:200 and 1:500) and *P. harmala* (1:100) significantly increased root fresh weight, while *D. alba* and *M. azedarach* decreased root fresh weight at all dilutions. Similarly concentrated smoke extract of all the plants reduced fresh weight of roots. Similarly root dry weight was increased with treatment of *E. camaldulensis* and *P. harmala* at the same dilutions as for fresh weight (Table 1). However, smoke solutions of *D. alba* and *M. azedarach* as well as concentrated smoke of all the plants decreased root dry weight.

Effects on shoot fresh and dry weight

Fresh and dry weight of Sorghum shoot was increased with the smoke solutions of *E. camaldulensis and P. harmala*. *E. camaldulensis* smoke dilutions (1:200 and 1:500) and *P. harmala* dilution (1:500) showed promotory effects while decrease in fresh and dry weight was observed with *D. alba* and *M. azedarach* (1:300, 1:400 and 1:500). Concentrated smoke extracts of all the plants also resulted inhibition and significantly decreased shoot fresh and dry weights (Table 2).

With increase in B concentration, significant reduction in root and shoot length was found (Fig. 5 - 7). However, plant derived smoke significantly alleviated the stress of B on root length (Fig. 5a and 7). Though an improvement was found in shoot length with application of alleviated solutions as compared to B stress but it was not significant (Fig. 5b and 7).

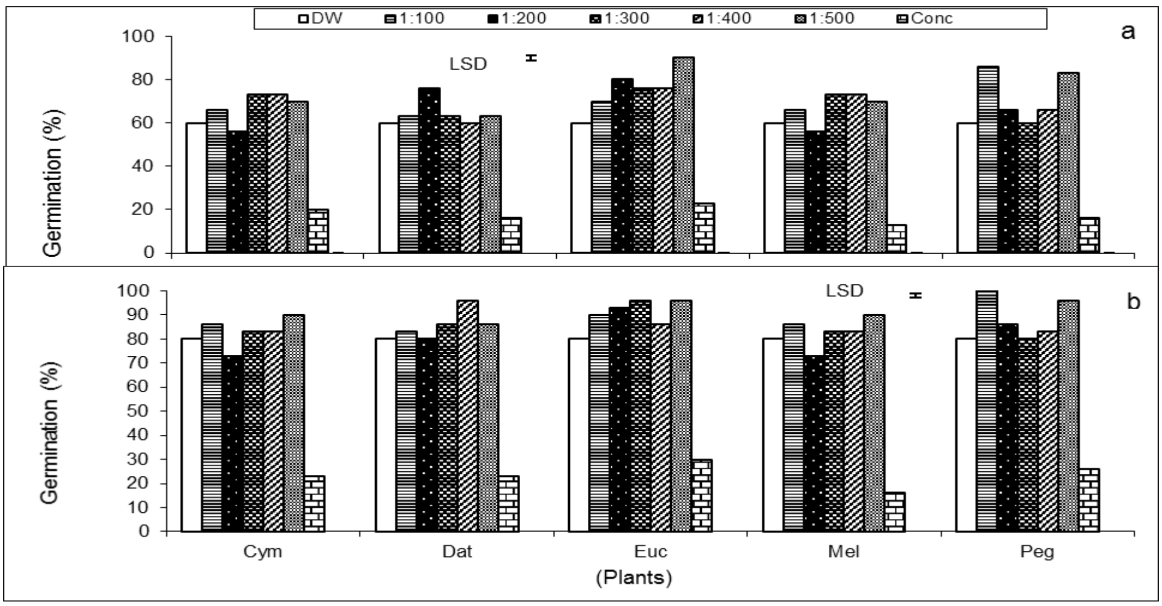

Figure 1. Effect of *C. jwarancusa* (Cym), *D. alba* (Dat), *E. camaldulensis* (Euc), *M. azedarach* (Mel) and *P. harmala* (Peg) derived smoke on the germination (%) of Sorghum after 24 (a) and 48 (b) hours. ($P < 0.05$ S).

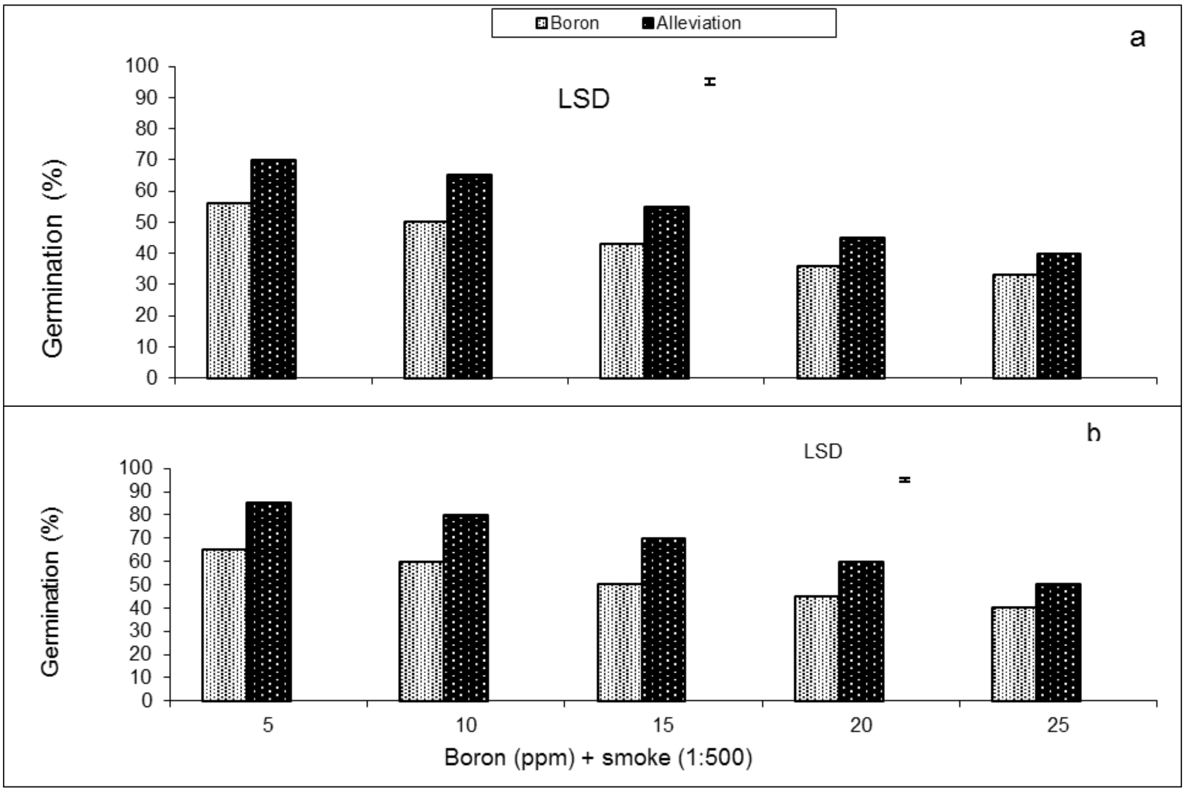

Figure 2. Germination (%) of Sorghum treated with boron and alleviating solutions (*E. camaldulesis* + B) after 24 (a) and 48 (b) hours. Vertical bar shows LSD at P < 0.05 S.

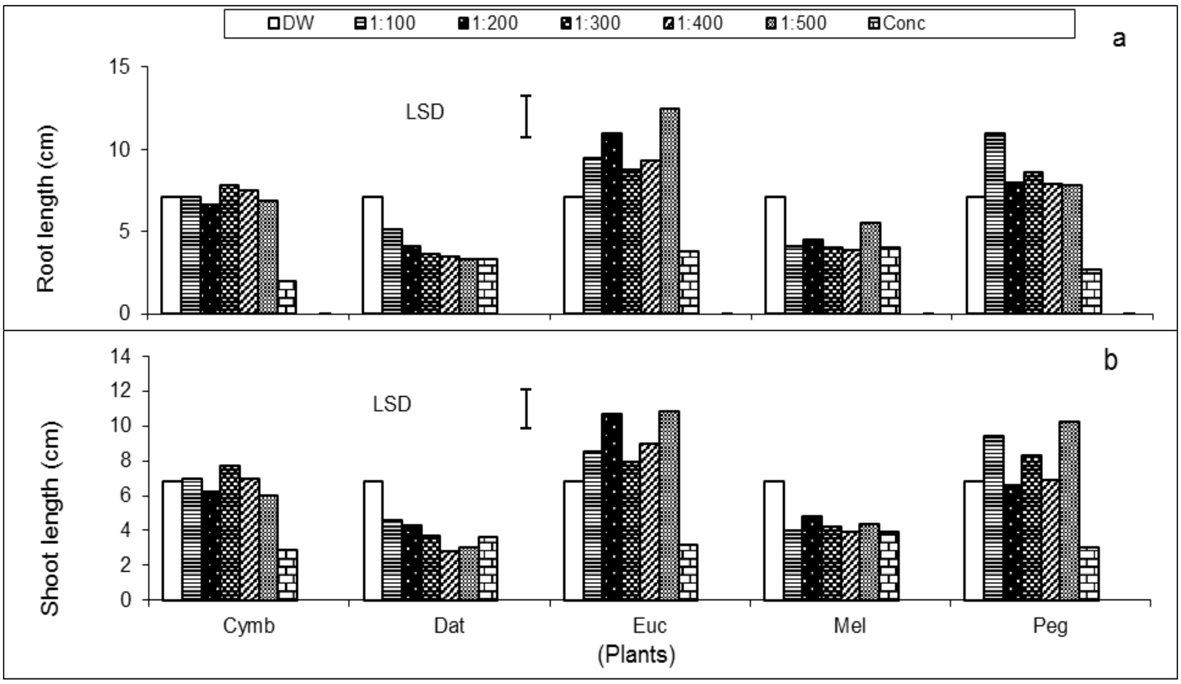

Figure 3. Effects of *C. jwarancusa* (Cym), *D. alba* (Dat), *E. camaldulensis* (Euc), *M. azedarach* (Mel), *P. harmala* (Peg) and their concentrated (Con) smoke on root (a) and shoot length (b) of Sorghum (P < 0.05 S).

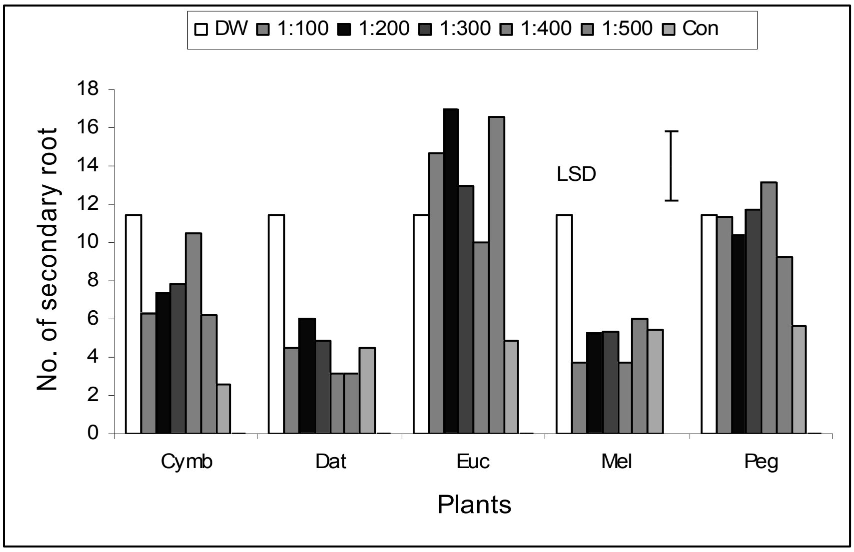

Figure 4. Effects of *C. jwarancusa* (Cym), *D. alba* (Dat), *E. camaldulensis* (Euc), *M. azedarach* (Mel), *P. harmala* (Peg) and their concentrated (Con) smoke on secondary root of Sorghum (P < 0.05 S).

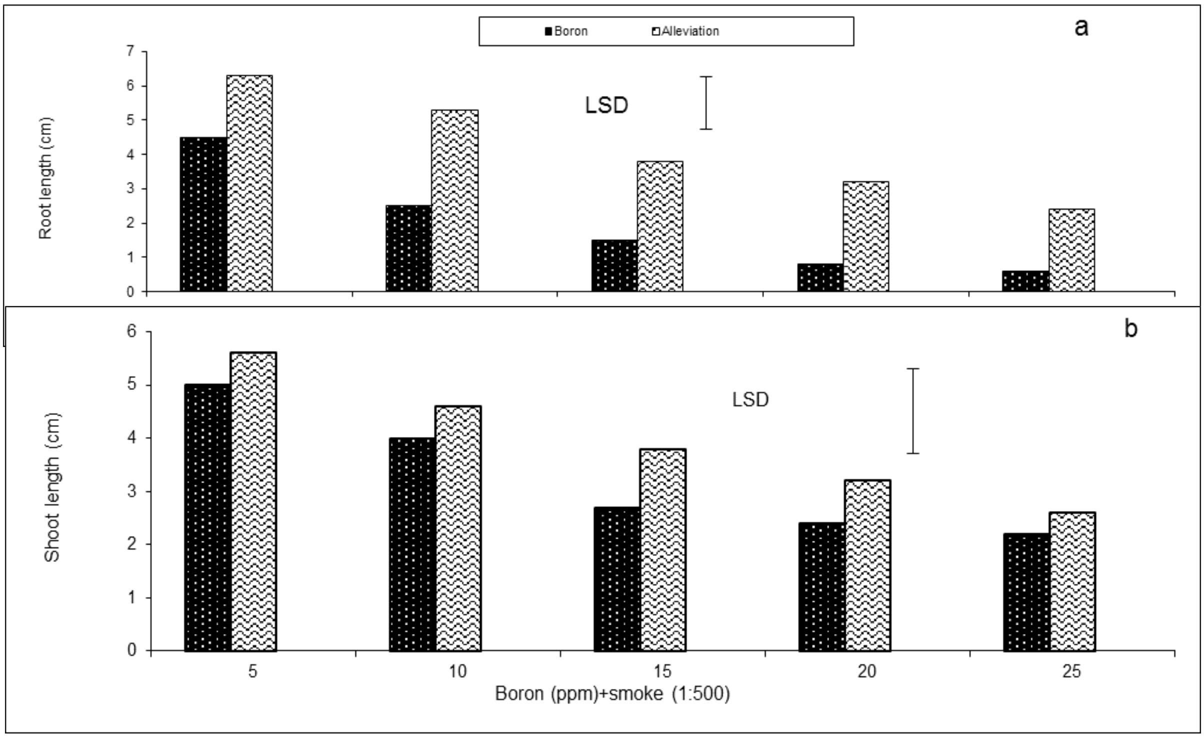

Figure 5. Effect of B and alleviating solutions on root (a) and shoot length (b) of Sorghum. Vertical bar shows LSD at P < 0.05. Each data point shows mean of three replicates.

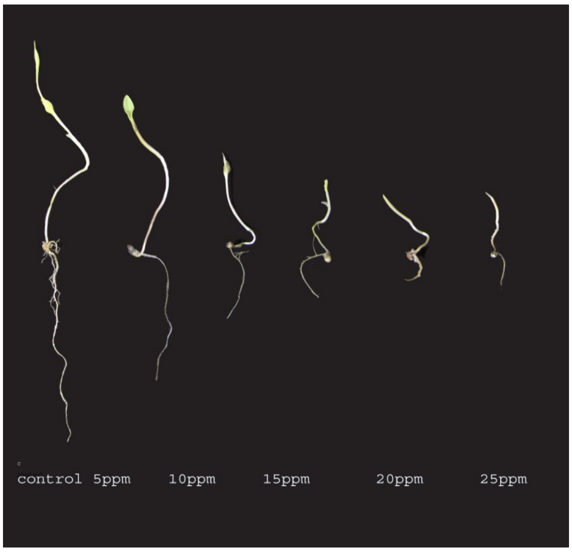

Figure 6. Effect of B on root and shoot length of Sorghum

Figure 7. Effect of alleviating solutions on root and shoot length of Sorghum.

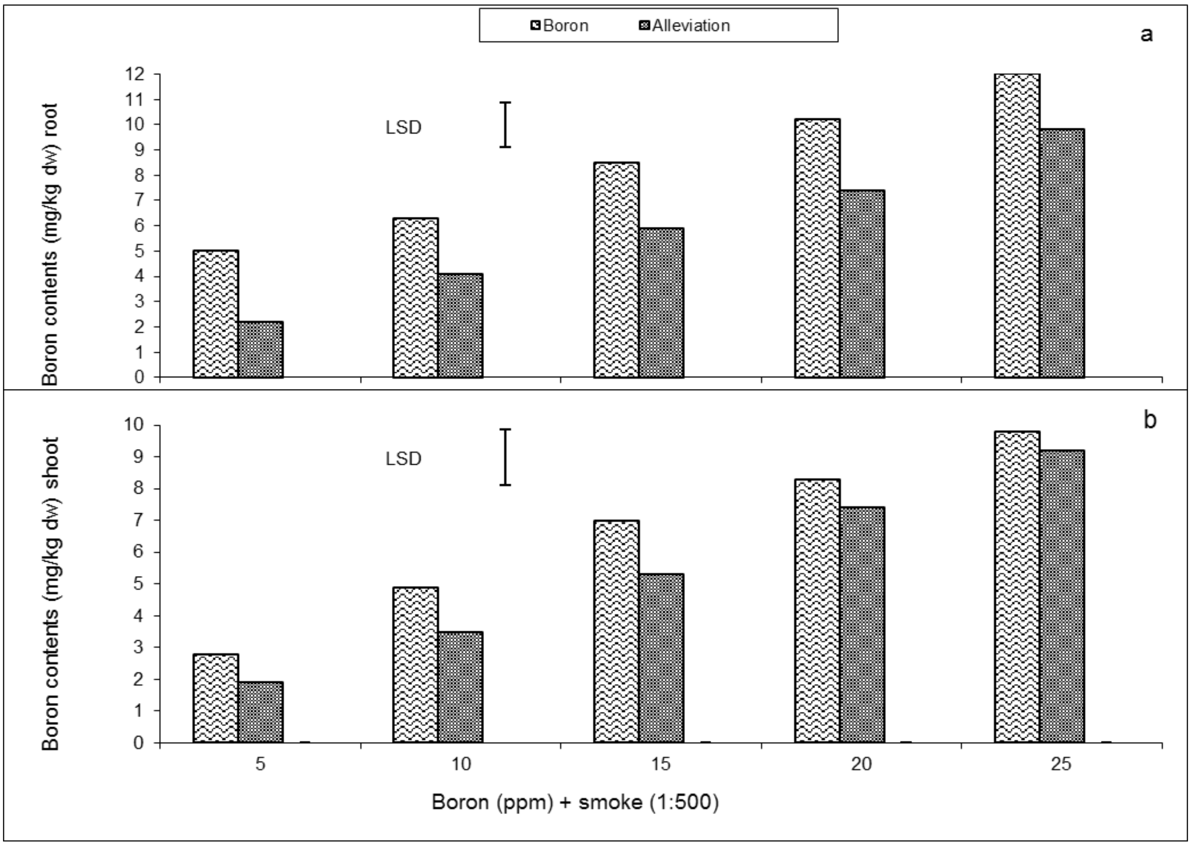

Figure 8. B contents of Sorghum root (a) and shoot (b). Each data point represents the mean of three replicates. The vertical bar represents LSD at $p < 0.05$.

Table 1. Effect of smoke dilution on root fresh and dry weight of sorghum

Smoke dilutions	Root fresh weight					Root dry weight				
	Cym	Euc	Peg	Dat	Mel	Cym	Euc	Peg	Dat	Mel
DW	0.164b	0.164d	0.164b	0.164a	0.164a	0.028bc	0.028c	0.028bc	0.028a	0.028a
1:100	0.160b	0.288bc	0.318a	0.101b	0.090b	0.053ab	0.082ab	0.103a	0.011b	0.012b
1:200	0.218ab	0.345ab	0.184ab	0.061bc	0.092b	0.054ab	0.085ab	0.059b	0.011b	0.013bc
1:300	0.192ab	0.241cd	0.183ab	0.054bc	0.073b	0.049ab	0.056bc	0.038bc	0.010b	0.010b
1:400	0.161b	0.236cd	0.168b	0.081bc	0.066bc	0.041abc	0.054bc	0.030bc	0.010b	0.010b
1:500	0.237ab	0.390a	0.165b	0.078b	0.088b	0.064ab	0.088a	0.034bc	0.011b	0.011b
Conc.	0.011c	0.015e	0.017c	0.018c	0.016c	0.007d	0.006c	0.004d	0.003c	0.002b
LSD	0.0993	0.0969	0.1454	0.0542	0.051	0.0395	0.0250	0.0364	0.0151	0.0154

All the data points shown are mean of three replicates with ten seeds in each Petri dish.

Table 2. Effect of smoke on shoot fresh weight and dry weight of Sorghum

Smoke dilutions	Shoot fresh weight					Shoot dry weight				
	Cym	Euc	Peg	Dat	Mel	Cym	Euc	Peg	Dat	Mel
DW	0.372a	0.372bc	0.372bc	0.372a	.372a	0.090a	0.090bc	0.090bc	0.090a	0.090a
1:100	0.473a	0.559ab	0.750ab	0.271abc	0.247abc	0.106a	0.192b	0.244ab	0.025ab	0.025ab
1:200	0.413a	0.837a	0.383bc	0.318ab	0.329ab	0.109a	0.328a	0.099bc	0.035ab	0.022ab
1:300	0.310a	0.381bc	0.506ab	0.074c	0.086bc	0.087a	0.181b	0.147bc	0.0167b	0.015b
1:400	0.350a	0.580ab	0.386bc	0.100bc	0.097bc	0.117a	0.190b	0.127bc	0.021b	0.011b
1:500	0.400a	0.928a	0.876a	0.105b	0.125bc	0.065a	0.415a	0.386a	0.017b	0.019b
Conc.	0.023b	0.012d	0.029d	0.041b	0.0361c	0.009b	0.009d	0.004d	0.004c	0.006b
LSD	0.2540	0.3988	0.4085	0.2372	0.2447	0.0690	0.1337	0.1637	0.0684	0.0713

All the data points shown in the table 1 are mean of three replicates with ten seeds in each Petri dish.

Table 3. Effect of boron on root and shoot fresh and dry weight of Sorghum

Treatments B (ppm)	Root fresh weight	Root dry weight	Shoot fresh weight	Shoot dry weight
DW	0.164a	0.028a	0.372a	0.090a
5	0.123ab	0.031a	0.355ab	0.034ab
10	0.062bc	0.013b	0.205abc	0.032ab
15	0.056bc	0.012b	0.262ab	0.025ab
20	0.058bc	0.010b	0.101c	0.012b
25	0.052c	0.011b	0.108bc	0.008b
LSD	0.0679	0.0127	0.2535	0.0731

All the data points shows mean of three replicates and each replica contain ten seeds.

Table 4. Effect of alleviating solution of boron and smoke on root and shoot fresh and dry weight of Sorghum.

Treaments B (ppm)	Root Fresh weight	Root dry weight	Shoot fresh weight	Shoot dry weight
D. W	0.164a	0.028ab	0.505a	0.053a
5+1:500	0.227a	0.043a	0.451ab	0.037ab
10+1:50	0.192a	0.040a	0.383ab	0.032ab
15+1:500	0.014b	0.011c	0.285bc	0.027b
20+1:500	0.013b	0.011c	0.276bc	0.022b
25+1:500	0.044b	0.010c	0.187c	0.017b
LSD	0.0674	0.0159	0.1776	0.0241

All the data point shows mean of three replicates and each replica contain ten seeds.

Effect of B stress and alleviating solutions on root and shoot fresh and dry weight

In case of root fresh and dry weight, there was found no effect at lower concentrations of B but higher concentrations showed a significant reduction in both the parameters (Table 3). Present results also revealed that combined solutions of B and smoke (alleviating solutions) significantly alleviated the toxic effects of B and improved fresh and dry weight of roots and shoots (Table 4).

Analysis of B in Sorghum roots and shoots

Present investigation showed that smoke solution potentially alleviated boron stress probably by reducing the uptake of B. However B uptake was reduced significantly in roots as compared to shoot (Fig. 6a and b). Though smoke solution reduced the uptake of B in the shoot, but the reduction was not so significant.

DISCUSSION

This study highlights the effect of *C. jwarancusa*, *E. camaldulensisa*, *P. harmala*, *D. alba* and *M. azedarach* extracted smoke on the germination and seedling vigor of Sorghum in order to alleviate boron stress. Smoke collected from these plants enhanced seed germination significantly (Fig.1a). In smoke extracts of the plants, concentrated solutions showed inhibition against all the parameters studied. Present results are in accordance with the investigations of Brown and Van Staden (1997) who reported that smoke extracts at lower concentrations improve seed germination. Improvement in germination at lower concentrations of smoke could be attributed to the removal of germination inhibitors such as ABA and phenolics (Da Cuhna and Casali, 1989; Hilhorst and Karssen, 1992). Similarly in case of root and shoot length, plant derived smoke significantly overcame the inhibitory effects caused by excessive amount of B and co-relates with the results of Sparge *et al*. (2006). Present investigations also indicated that, smoke solutions resulted an increase in seedling fresh and dry weights (Table 1 and 2). These results are in agreement with that of Kulkarni *et al*. (2006). Similarly number of secondary roots were significantly increased by eucalyptus at the dilution of 1:200 and 1:500 (Fig. 4).

Excess concentration of B in soil may interrupt growth and development of wheat and other higher plants. The most frequent symptoms of boron toxicity in cereals crops include chlorosis and necrosis (Cartwright *et al*., 1984), delay in development, reduced shoot growth and plant height (Paull *et al*., 1988) and root growth suppression (Huang and Graham, 1990). Significant yield losses due to excess boron have been observed in cereals (Cartwright *et al*., 1984; Paull *et al*., 1992). Present work Indicates that treatment of plants with B solutions inhibited both root and shoot growth (Fig. 5 and 6). On the other hand plant derived smoke extracts has the potential to overcome the inhibitory effects of B stress through alleviating solutions. Present investigations revealed that Sorghum has lower B contents in shoots as compared to root (Fig. 8). The selection of smoke dilution i.e. 1:500 of *E. camaldulensis* for alleviating solution was done on the basis of strong vigour among all the dilutions. The germination (%) and the root and shoot length were significantly increased by smoke as well as alleviating solutions overcome the toxicity of boron stress considerably (Fig. 2 and 5).

A significant relationship was found between diverse factors that could manage B tolerance in Sorghum while treating with alleviating solutions. There was a significant interaction between B stress, alleviating solutions and boron contents of seed and seedlings. In addition, Sorghum seeds treated with boron gave lower seed germination and low root and shoot length. Present results suggested that higher dose of boron retard the growth of Sorghum. Similarly, alleviating effects of smoke extracts against B toxicity is just because smoke has the potential to reduce the uptake of boron which ultimately leads to tolerance of Sorghum to B. However the mechanism by which plant-derived smoke solutions reduce Boron uptake is still a mystery to be unveiled.

The present study investigated that, smoke has the potential to alleviate phytotoxicity caused by B stress and improved seed germination and seedling vigour in Sorghum under B stress condition. Investigations also revealed that, B toxicity has direct relation to B contents of root and shoot, i.e. more B contents, resulted lower growth of root and shoot while, alleviating solutions of smoke and B, smoke reduced the uptake of B resulted an increase in root and shoot length. Hence, the tolerance to B toxicity could be attributed to lower B uptake. Present work also suggested that, smoke is involved in physiological and metabolic activities that improves plant growth under B toxic condition. It is predicted that this study will provide a basic strategy for reducing the risks of B toxicity and maintaining sustainable plant production.

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