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Syzygium Aromaticum Aqueous Extract Alleviates Boron Toxicity in Rice Seedlings Better than *Terminalia Arjuna* by Preventing Boron Uptake and Reducing Oxidative Stress in the Seedlings

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Abstract **– The buds of *Syzygium aromaticum* (Sa) and the bark of *Terminalia arjuna* (Ta) contains a range of natural antioxidants and have been used to protect animal cells against oxidative stress. In the present study, we have compared alleviating effects of Sa bud and Ta bark aqueous extracts against boron (B) toxicity in rice (*Oryza sativa* L.) seedlings. When rice seedlings were raised for 8 days in hydroponics in Yoshida nutrient medium containing 1.5mM H_3BO_3, a decline in height, reduction in biomass, increase in B uptake, increase in the level of $O2^{\cdot-}$,, H_2O_2 and, increased lipid peroxidation, decline in the level of photosynthetic pigments, increase in the activity of the antioxidative enzymes superoxide dismutase and catalase and alteration in the ultrastructural changes in the guard cells were observed. Scanning electron microscopy (SEM) showed damage to guard cells marked by distortion in the outer covering under B toxicity. Exogenously adding Sa bud extract (0.5 mg/ml) and Ta bark extract (3.2 mg/ml) to the growth medium considerably alleviated B toxicity in the seedlings by reducing B uptake, suppressing generation of reactive oxygen species, reducing lipid peroxidation, restoring level of photosynthesis pigments and protecting the ultrastructure of guard cells, and restoring the activity of antioxidative enzymes. Results suggest that both extracts considerably alleviates B toxicity in the seedlings by preventing B uptake and reducing oxidative stress in the seedlings. Further Sa bud extract appears to alleviate B toxicity in rice seedlings better than Ta extract.**

Keywords **– *Syzygium Aromaticum*, *Terminalia Arjuna*, Alleviation, Antioxidant, Rice, Boron Toxicity, Oxidative Stress.**

I. INTRODUCTION

Boron (B) is an essential element for growth and development of plants due to its role in the formation of crosslinks between dimers of the pectin rhamnogalacturonan II (a low-molecular-mass pectic polysaccharide) in the cell walls (O'Neill *et al*. 2004). However, the physiological role of B in plants is still poorly understood. B has a limited range of suitable concentrations between its deficiency and toxicity. The amount of B required varies among plants species and among genotypes of the same species. Earlier studies suggest large genetic variations in the tolerance of crops to B toxicity (Torun *et al*. 2006).

Rice (*Oryza sativa*) is a staple food crop in the humid tropics and subtropics and is sensitive to excess B (Ochai *et al*. 2011). Similar to other crop species, irrigation with water containing high level of B, which occurs both naturally and anthropologically through contamination by industrial wastewater, is the major cause of B toxicity in rice fields (Imaizumi and Okimura 1981, Cayton 1985). Boron toxicity causes decrease in rice grain yield from 30% to 83% in comparison to control on exposure with 10 mg B L^{-1} in the irrigation water (Ochiai *et al*. 2008). Excess B has a greater effect on plant height in rice, and a drop-off in the number of panicles and has been regarded as the main cause of the decreased grain yield (Imaizumi and Okimura, 1981, Ochiai *et al*. 2008). There may be a specific developmental stage that is particularly sensitive to excess B. B toxicity is a serious threat to agriculture particularly in arid and semiarid regions. B toxicity is more prevalent in saline soils and also in inland desert areas where native soils are rich in B and coexist with sparse rainfall and irrigation with high-B groundwater (Gupta 1979). High B levels in the soil inhibit seed germination, cell wall expansion, cause decreased synthesis of photosynthetic pigments and reduce lignin and suberin contents (Noble *et al*. 1997, Reid 2007). Similar to other abiotic stressors such as salinity, heavy metals, drought, cold and heat, excess B also induces the formation of reactive oxygen species (ROS) such as superoxide ($O^{\bullet-}$) and hydroxyl ($OH^{\bullet}$) radicals, which are strong oxidizers of lipids, proteins, and nucleic acids (Ardic *et al*. 2009, Cervilla*et al*. 2007) and lead to destabilization of cellular homeostasis (Tombuloglu *et al*. 2011) and cause oxidative damage. To combat oxidative damage, plants possess an antioxidative defense system comprising of non-enzymic antioxidants like ascorbate, glutathione and enzymic antioxidants such as superoxide dismutase, catalase, peroxidase, etc.

Plant products have been widely used for alleviation of metal toxicity in animals due to their antioxidant properties. *Syzygium aromaticum* (synonym: *Eugenia cariophylata*) commonly known as clove, is a medium size evergreen tree (8-12 m) belonging to the family Myrtaceae, an inhabitant of the Maluku islands in east Indonesia. For centuries the trade of clove and the search of this precious spice stirred the economic development of the Asiatic region (Kamatou *et al*. 2012). Flower buds of *S. aromaticum* are collected in the maturation phase before flowering. Clove is in use for centuries as a spice, food preservative, an ayurvedic medicine and has potent antioxidant (Weerachat, *et al.* 2016) and antimicrobial activities (Anna et al 2017).

Terminalia arjuna (English name: *Arjunamyrobalan*) from Combretaceae family is a large tree which is found throughout the South Asian region. It is usually found growing on river banks or near dry river beds in Bangladesh, West Bengal and south and central India (Tarun *et al*. 2011). It is one of the most resourceful medicinal plants having a wide spectrum of biological activity. *T. arjuna* is an important cardiotonic plant described in the Ayurveda (Dwivedi. S. 2007). The bark of *T. arjuna* contains phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrates (Ghani 2003). *T. arjuna* plants are rich in antioxidants like vitamin C, vitamin E, carotenes, polyphenols and many other such compounds which cause a decrease in disease risk. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxides, hydroperoxides of lipid hydroxyls and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Sarmistha Saha, and Ramtej J. Verma2014).

The present study was undertaken to examine the possible alleviating roles of the extracts from buds of *Syzygium aromaticum* and from the bark of *T. arjuna* due to their antioxidant potential (Divya G and Mukesh K 2017), in combating B toxicity in growing rice seedlings. The parameters such as root shoot length, fresh and dry weights of roots and shoots, chlorophyll content, ultrastructural changes in stomata, production of reactive oxygen species (ROS), induction of oxidative stress and status of the activities of antioxidative enzymes were examined in rice seedlings growing in hydroponics in presence of toxic levels of boron.

II. Materials and Methods

Preparation of Syzygium Aromaticum Bud Extract

Buds of *S. aromaticum* were procured from local market. After drying, buds were ground to the fine powder. 1 g of powder was homogenized in 100 ml of double distilled water and placed on the shaker at room temperature for 24 h. The mixture was centrifuged at 12,000 × g for 15 min to remove debris, and the dark brownish supernatant was collected and stored at 4 °C. Stock solution with concentration 10 mg ml^{-1} was prepared. For alleviation experiments, extracts with concentration 0.5 mg ml^{-1} were used.

Preparation of Terminalia Arjuna Bark Extract

The bark of *Terminalia arjuna* (Ta) was collected from Banaras Hindu University, Varanasi (India) campus. After drying, Ta bark was ground to fine powder. Ta bark powder weighing 5 g was mixed with 100 ml of double distilled water and placed on the shaker for 72 h. The mixture was centrifuged at 12,000 × g for 15 min to remove debris, and the supernatant was collected and stored at 4°C. For use, the extract was evaporated; the residue was weighed (3.2 mg) and dissolved in 1 ml water, giving a stock solution with a concentration (w/v) of 3.2 mg ml^{-1}.

Plant Materials and Boron Treatment

Seeds of Indica rice (*Oryza sativa L.*) *cv.* Malviya-36 procured from Banaras Hindu University farm were used. Rice seeds were germinated on moistened filter paper at 28ºC for 5 days in a BOD cum Humidity Incubator (Maheshwari *et al*. 2009). Thereafter, seedlings were placed in hydroponics in plastic pots containing 200 ml Yoshida nutrient solution (Yoshida et al. 1971), which served as control. Nutrient solutions containing either 1.5 mM boron or 1.5 mM B and 5 ml *Sa* working solution (*Sa* + B), as well as 1.5 mM B and 1 ml *Ta* extract (Ta + B) served as treatment solutions. Pots were kept in green house for 8 days for the growth of the seedlings at 28 ± 1ºC under 80 % relative humidity and 12 h light/dark cycle with 190-200 µmol $m^{-2}s^{-1}$ irradiance. Seedlings were taken out at the 8th day of growth, treatment and all analyses and determinations were done in triplicate.

Rice Seedling Vigor, Boron Uptake and Relative Water Content

Lengths and fresh weights of roots and shoots of the seedlings were determined at the 8th day of treatment using 10 random samplings in triplicate. To determine dry weights, plant samples were oven dried at 70°C for 3 days and subsequently weighed. The concentration of absorbed boron in roots and shoots was determined using inductive coupled plasma-optical emission spectrometer (ICP-OES, Optima 7000 DV, Perkin Elmer) following the protocol of Moore et al. (1986). To determine B, plant samples were washed thoroughly with ion-free water (Milli-Q) and oven dried at 70°C for 3 days. Dried samples weighing 150 mg were used for B quantification. Samples were ground to fine powder and digested in a di-acid mixture (nitric acid-perchloric acid). Digested samples were diluted to 25 ml with ion-free water and filtered with 0.45 µm Whatman filter paper. The filtrate was then subjected to boron analysis using ICP-OES.

Relative water content (RWC) was determined in control and boron treated seedlings according to Weatherley (1950). RWC was calculated using the formula: RWC = (FW−DW) / (TW−DW) × 100, where FW = fresh weight, DW = dry weight and TW = turgid weight.

Ultrastructure Studies of Stomata using Scanning Electron Microscopy and Energy Dispersive X-Ray Analysis

To examine the effect of excess boron on ultra structural changes in the stomata, leaves from 8-day control and treated seedlings were used. A thin slice of each leaf was fixed in 2.5% glutaraldehyde in 100 mM sodium-phosphate buffer (pH 7.2). Fixing and serial dehydration were done following Pathan *et al*. (2008). Critical point drying, mounting, coating with gold and viewing were done using standard operating procedures of Sophisticated Analytical Instrument facility of IIT Madras, India. The material was observed at 20 KV using SEM (FEI Quanta FEG 200-High Resolution Scanning Electron Microscope). Same samples were used to check the ultrastructural changes of the elemental composition by using energy dispersive X-ray analysis (EDXA).

Determination of Levels of Superoxide Anion, H_2O_2 and Lipid Peroxidation in the Seedlings

The rate of superoxide anion radical production in both roots and shoots of control and treated seedlings was

measured following the method of Mishra and Fridovich (1972). This was done by observing the auto-oxidation of epinephrine in terms of the rate of adrenochrome formation and was expressed as the change in absorbance at 480 nm min^{-1} g^{-1} per tissue fresh weight. An increase in absorbance was recorded at 30 s intervals up to 5 min at 480 nm using a spectrophotometer (Model SL 177, ELICO Ltd, India).

H_2O_2 production in the seedlings was determined spectrophotometrically using titanium sulfate, as described by Jana and Choudhuri (1981). Fresh root and shoot samples weighing 150 mg were homogenized in 3 ml of 50 mM sodium phosphate buffer (pH 6.5). After centrifugation at 22,000×*g* for 10 min, the supernatant was used to calculate H_2O_2 content by reaction with titanium sulfate. The intensity of yellow color developed was recorded at 410 nm and the amount of H_2O_2 produced was calculated using extinction coefficient 0.28 μM^{-1} cm^{-1} and expressed as nmol g^{-1} of tissue fresh weight.

The level of lipid peroxidation products was measured in terms of thiobarbituric acid reactive substances (TBARS), following the procedure of Heath and Packer (1968). Fresh root and shoot samples weighing 200 mg were ground in 3 ml of 0.25 % (w/v) 2-thiobarbituric acid (TBA) and 10 % trichloroacetic acid (TCA) using a mortar and pestle. The homogenate was heated for 30 min at 95°C and then quickly cooled in an ice bath and centrifuged at 22,000 × *g* for 10 min. The absorbance of the supernatant was recorded at 532 nm, and nonspecific turbidity was corrected by subtracting the absorbance of the same at 600 nm. The concentration of the lipid peroxides was calculated and expressed as TBARS in terms of nmol g^{-1} fresh weight using extinction coefficient 155 $mM^{-1}cm^{-1}$.

Determination of Antioxidant Enzyme Activity

The activity of the enzyme superoxide dismutase (SOD) was assayed in the root and shoot of the seedlings following the method of Beauchamp and Fridovich (1971). Fresh tissues weighing 200 mg were homogenized using chilled mortar and pestle in 2 ml of 100 mM potassium-phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 0.1 % (v/v) Triton-X-100 and 2 % (w/v) polyvinyl pyrrolidone (PVP). The homogenate was centrifuged at 22,000 × *g* for 10 min at 4°C and the supernatant was dialyzed in cellophane membrane tubing in cold room for 4 h against the extraction buffer, with 3 to 4 changes of the buffer. SOD activity was assayed using 3 ml assay mixture containing 5 mM sodium carbonate-bicarbonate buffer (pH 9.8), 0.1 mM EDTA, 0.6 mM epinephrine and dialyzed enzyme. Epinephrine was added as the last component. The adrenochrome formation for 30 min was recorded at 475 nm in a UV-Vis spectrophotometer (Varian, Cary 50 Bio, Australia). One unit of SOD activity is expressed as the amount of enzyme required to cause 50 % inhibition of epinephrine oxidation under experimental conditions.

Catalase activity was determined following the method of (Beers and Sizer 1952). Fresh samples weighing 200 mg were homogenized using a chilled mortar and pestle in 2 ml of 50 mM Tris-HCl buffer (pH 8.0) containing 0.5 mM EDTA and 2 % PVP. The homogenate was centrifuged at 22,000 × *g* for 10 min at 4°C and dialyzed. The assay mixture in a total volume of 3 ml contained 2 ml of 100 mM potassium-phosphate buffer (pH 7.0), 900 µl of 200 mM H_2O_2 and 100 µl enzyme. The decomposition of H_2O_2 was followed at 240 nm (extinction coefficient of 0.036 mM) by observing the decrease in absorbance using a UV–Vis spectrophotometer (Varian, Cary 50 Bio, Australia). Enzyme specific activity is expressed as µmol H_2O_2 oxidized min^{-1} mg^{-1} protein.

Statistical Analysis

All experiments were performed in triplicate. The results are expressed as mean ± S.D. of three independent determinations. The mean differences among control and treatments were compared by one factorial ANOVA followed by Tukey's multiple range test. Asterisks (*) were used to denote the level of significance of the difference between control and B treatments as *$p \leq 0.05$ and ** $p \leq 0.01$.

III. Results

Effect of Syzygium Aromaticum and Terminalia Arjuna Extracts on Vigor, RWC, and Boron Uptake in Boron-Treated Seedlings

Effects of excess boron on seedling vigor were examined in terms of plant height, fresh weight and relative water content. When rice seedlings were raised in hydroponics for 8 days in presence of 1.5mM boron, a consistent decline in plant height, fresh weight and RWC was observed in the seedlings due to boron treatment (Fig1). In seedlings grown for 8 days in presence of 1.5mM B, 41% ($p \leq 0.01$) reduction in length of roots and 29% ($p \leq 0.05$) reduction in length of shoots was observed compared to controls.

Root and shoot fresh biomass also declined significantly in the seedlings after boron treatment (Fig. 1). Seedlings grown for 8 days under 1.5 mM B showed 52% ($p \leq 0.01$) reduction in fresh biomass of roots and 39% ($p \leq 0.05$) reduction in fresh biomass of shoots compared to controls. Similarly, RWC also declined in B-treated seedlings compared to controls. Due to the presence of *Sa*, and *Ta* extracts in B containing medium, considerable alleviation in the B-induced decrease in growth parameters was observed. Seedlings grown in presence of *Sa* and *Ta* extracts in B containing medium showed greater root and shoot length compared to boron treated seedlings, indicating that *Sa/Ta* extract alleviated the growth inhibition of rice due to B. Treatment with *Sa* and *Ta* extract caused 30 and 23 % restoration in length of root and 35 % and 27 % restoration in the length of shoot respectively compared to length of root and shoot in B treated seedlings at 8 days of growth.

Similarly, 31 % and 28 % restoration in the fresh biomass of roots and 24 % and 18.5 % restoration in the fresh biomass of shoots was observed in 8 days grown seedlings treated with B + *Sa* and B + *Ta* respectively compared to boron treated seedlings. Due to the presence of *Sa* and *Ta* in the medium along with boron, a considerable recovery in the RWC of seedlings was observed. However, treatment with *Sa* and *Ta* alone had

no evident effect on the growth status of rice seedlings compared to the control seedlings grown in Yoshida nutrient solution (Data not shown here).

Effect of Sa and Ta Extract on Boron Uptake in Rice Seedlings

Uptake of B by growing rice seedlings as examined by ICP-OES analysis revealed that B was readily taken up by the seedlings from the growth medium (Fig. 2). After its uptake, B was more localized in roots than in shoots. Seedlings grown for 8 days in presence of 1.5 mM B showed 595 µmol g^{-1} dry wt B in roots and 413 µmol g^{-1} dry wt B in shoots. However, with the addition of Sa and Ta extracts in the growth medium in presence of 1.5 mM B, there was a marked decline in B uptake, compared to B uptake in 1.5 mM B treated seedlings.

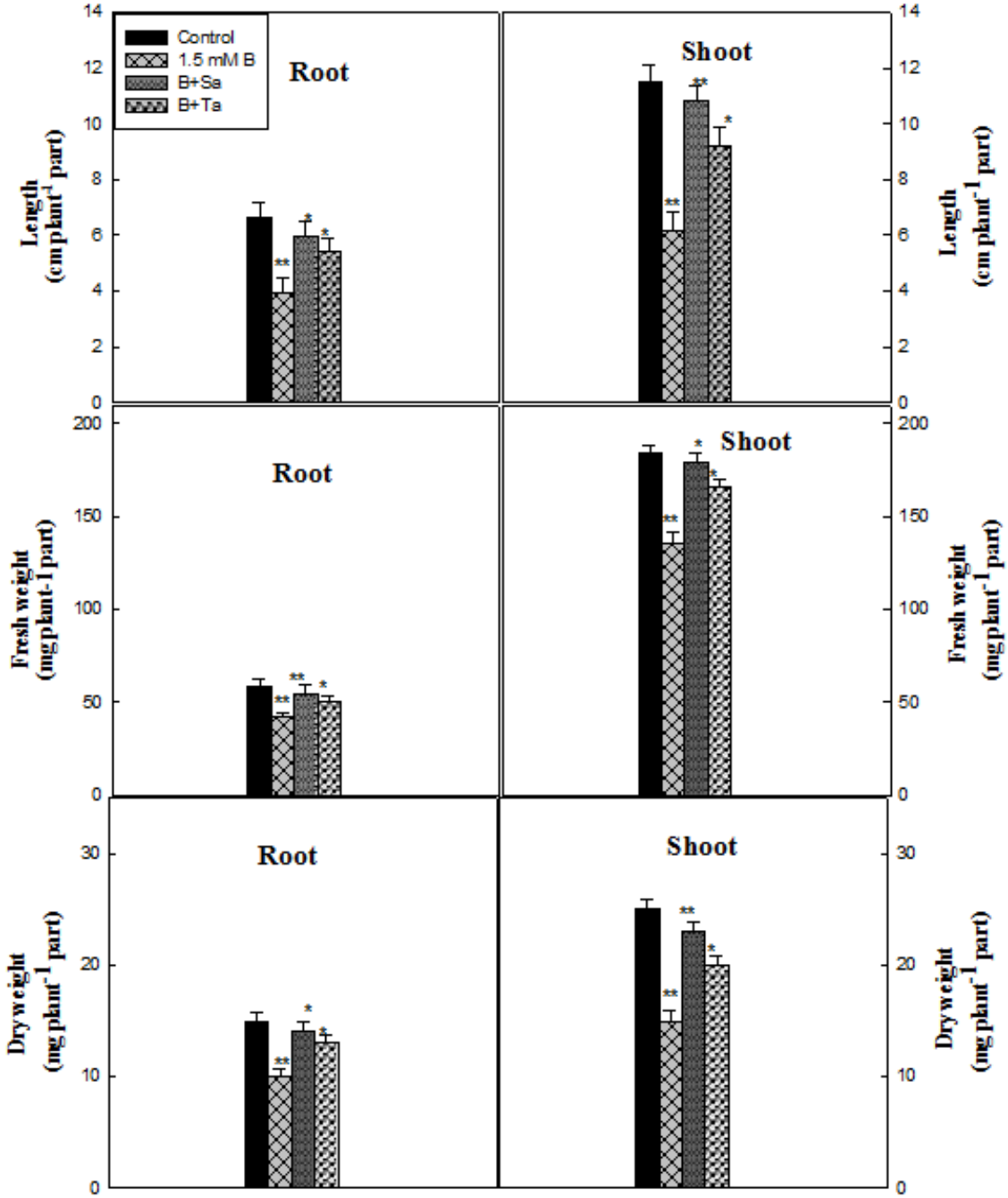

Fig. 1. Effect of Boron (1.5mM), B + Sa and B + *Ta* in the growth medium on length, fresh weight and dry weight of roots and shoots of rice seedlings. Control represents seedlings grown in the nutrient solution, whereas B + *Sa* represents nutrient solution containing *Syzygium aromaticum* bud extract, whereas B + *Ta* represents nutrient solution containing *Terminalia arjuna* bark extract. Values are mean ± S.D. based on three independent determinations and bars indicate standard deviations. Asterisks (*), (**) indicate values that differ significantly from controls at $p \leq 0.05$, $p \leq 0.01$ respectively according to Tukey's test.

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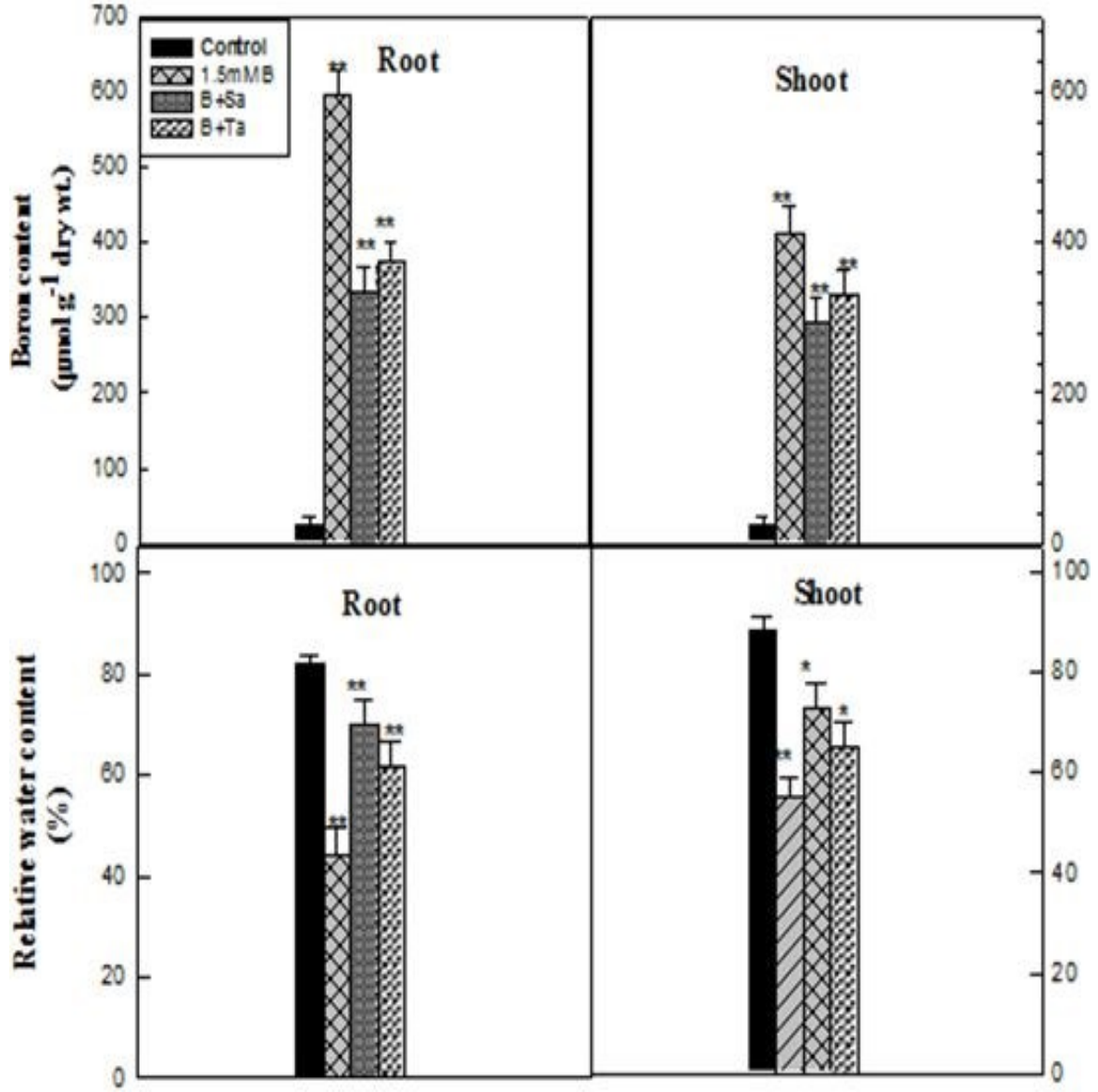

Fig. 2. Effect of Boron (1.5mM), B+Sa and B+*Ta* in the growth medium on boron content and relative water content of roots and shoots of rice seedlings at 8 days of growth. Control represents seedlings grown in Yoshida nutrient solution, whereas B + *Sa* represents nutrient solution containing *Syzygium aromaticum* bud extract, and B + *Ta* represents nutrient solution containing *Terminalia arjuna* bark extract. Values are mean ± S. D. based on three independent determinations and bars indicate standard deviations. Asterisks (*), (**) indicate values that differ significantly from controls at $p \leq 0.05$, $p \leq 0.01$ respectively according to Tukey's test.

Effect of Sa and Ta on Guard Cell Ultrastructure of B-Treated Rice Seedlings

The ultrastructure of guard cells from leaves of rice seedlings subjected to B stress was examined by SEM. The guard cells of control plants showed an organized structure with intact cell wall (Fig. 3). The Guard cells from B-treated seedlings showed irregular cell wall structure or diminished borders compared to control seedlings. The guard cells appeared comparatively healthier and considerably regained their shape in seedlings grown in the presence of *Sa* + B and *Ta* + B compared to B-treated seedlings

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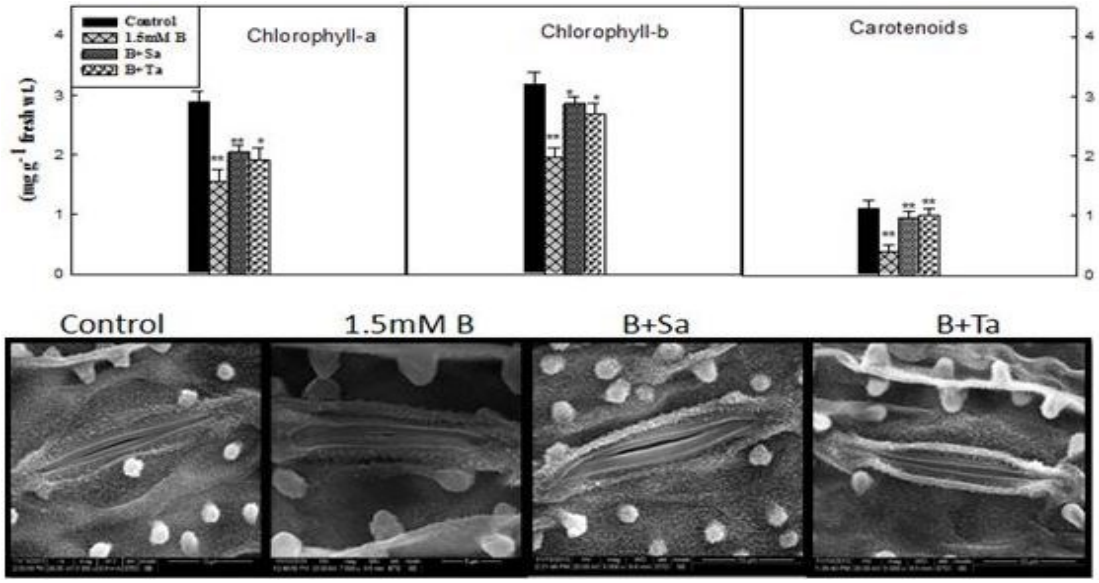

Fig. 3. Effect of Boron (1.5mM), B+Sa and B+*Ta* in the growth medium on contents of Chlorophyll a, Chlorophyll b and carotenoids and ultrastructural changes in stomatal guard cells in shoots of 8 day grown rice seedlings. Control represents seedlings grown in the nutrient solution, whereas B + *Sa* represents nutrient solution containing 1.5 mM B and*Syzygium aromaticum* bud extract, whereas B + *Ta* represents nutrient solution containing 1.5 mM B and *Terminalia arjuna* bark extract. Control represents seedlings grown in nutrient solution. Values are mean ± S. D. based on three independent determinations and bars indicate standard deviations. Asterisks (*), (**) indicate values that differ significantly from controls at $p \leq 0.05$, $p \leq 0.01$ respectively according to Tukey's test and *Ta*+B compared to B-treated seedlings (Fig. 3).

Effect of *Sa* and *Ta* treatment on B-induced generation of ROS and lipid peroxidation Boron treatment of 1.5 mM for 8 days caused enhanced generation of $O_2^{\cdot -}$ in both roots and shoots of the rice seedlings compared to controls (Fig. 4). Rice seedlings exposed to B for 8 days showed 142 % ($p \leq 0.01$) increased $O^{2\cdot}$−level in roots and 136 % ($p \leq 0.01$) increased level in shoots compared to controls. Due to the presence of Sa and Ta in B containing medium, 40 and 35 % reduced level of $O_2^{\cdot}$- production was observed in roots and 29 % and 33% ($p \leq 0.05$) reduced production in shoots as compared to the $O_2^{\cdot}-$ level in B alone-treated seedlings. Similarly, increased H_2O_2 production was observed in both roots and shoots of B treated seedlings compared to controls (Fig.4). With 1.5 mM B treatment for 8 days, the level of H_2O_2 production increased by 39 % ($p \leq 0.01$) in roots and 64 % ($p \leq 0.01$) in shoots. There appeared suppression of B-induced H_2O_2 production due to the presence of *Sa* and *Ta* in the medium. Treatment with Sa and Ta reduced H_2O_2 accumulation by 31 % and 18 % respectively in roots and 26 % and 22 % respectively in shoots in 8-day grown seedlings compared to the H_2O_2 level in B alone-treated seedlings. The level of H_2O_2 was higher in shoots than in roots in both control as well as B-treated seedlings (Fig. 4). The production of TBARS determines the level of lipid peroxidation. Our results showed that TBARS content increased in the seedlings due to B treatment indicating that B excess induces lipid peroxidation in rice seedlings (Fig. 4). In 1.5 mM B grown seedlings, TBARS content increased by 38 % ($p \leq 0.01$) in roots and 42 % ($p \leq 0.01$) in shoots at the 8 days of growth as compared to control seedlings. In the presence of Sa and Ta in the B containing medium, there appeared significant reduction of TBARS production as compared to B-treated seedlings. Seedlings grown in the presence of *Sa*+B and *Ta*+B showed 29 % ($p \leq 0.05$) and 26 % ($p \leq 0.05$) respectively decreased level of TBARS in roots and 31 % ($p \leq 0.01$) and 24 % ($p \leq 0.05$) decreased level of TBARS respectively in shoots as compared to B-alone treated seedlings.

Effect of Sa and Ta on B-induced alteration in activity of antioxidative enzymes

B treatment of 1.5 mM caused an increase in SOD activity in both roots and shoots of the rice seedlings (Fig. 5). The activity of the enzyme under both controls and B treatments was always higher in roots than in shoots. The presence of *Sa* and *Ta* along with B in the growth medium led to considerable decline in B-induced increased SOD activity. In 8-day grown seedlings, B treatment caused 58 % ($p \leq 0.01$) increased SOD activity in roots and 134 % ($p \leq 0.01$) increased activity in shoots compared to controls The presence of Sa and Ta in B containing medium led to 34%($p \leq 0.01$) and 24 % ($p \leq 0.05$) lesser increase in SOD activity in roots and 51 %($p \leq 0.01$) and 38%($p \leq 0.01$) lesser increase in shoots compared to B-treated seedlings at 8 days of growth. B treatment caused the increase in CAT activity in 8-day grown rice seedlings. (Fig.5). The presence of *Sa* and *Ta* in B containing nutrient medium caused a considerable restoration in CAT activity with the values near to controls.

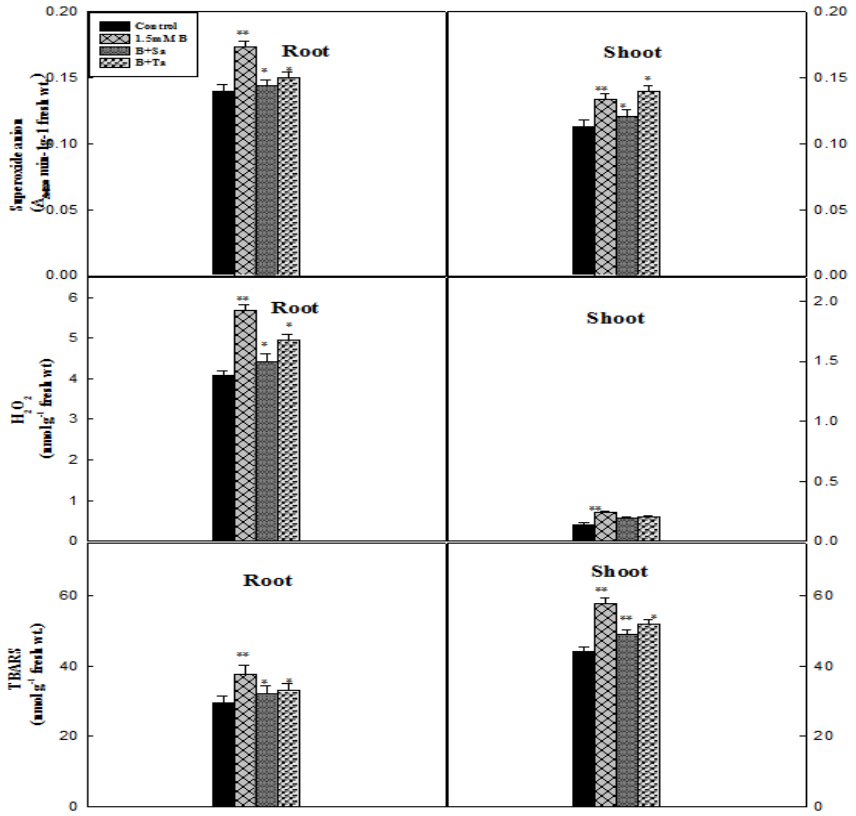

Fig. 4. Effect of Boron (1.5 mM), B + *Sa* and B + *Ta* in the growth medium on the production of superoxide anion, H_2O_2 generation and lipid peroxidation in rice seedlings. Control represents seedlings grown in the nutrient solution, whereas B + *Sa* represents nutrient solution containing 1.5 mM B and *Syzygium aromaticum* bud extract and B + *Ta* represents nutrient solution containing 1.5 mM B and *Terminalia arjuna* bark extract. Values are mean ± S. D. based on three independent determinations and bars indicate standard deviations. Asterisks (*), (**) indicate values that differ significantly from controls at $p \leq 0.05$, $p \leq 0.01$ respectively according to Tukey'stest.

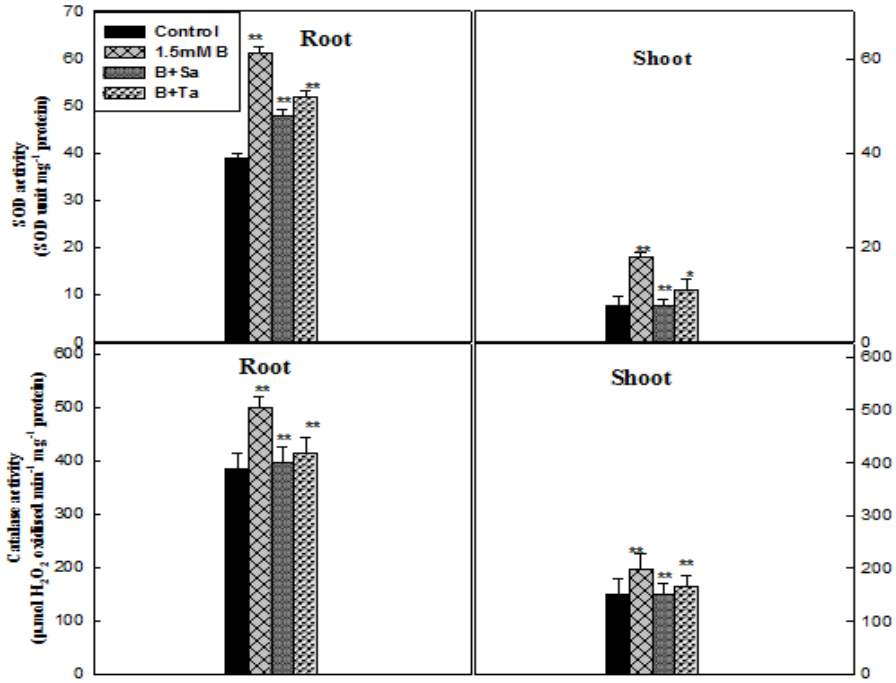

Fig. 5. Effect of Boron (1.5 mM), B + Sa and B + Tain the growth medium on the activity of antioxidative enzymes SOD and catalase in 8 day grown rice seedlings. Control represents seedlings grown in the nutrient solution, whereas B +*Sa* represents nutrient solution containing 1.5 mM B and*Syzygium aromaticum*bud extract and B + *Ta* represents nutrient solution containing 1.5 mM B and *Terminalia arjuna* bark extract. Values are mean ± S. D. based on three independent determinations and bars indicate standard deviations. Asterisks (*), (**) indicate values that differ significantly from controls at $p \leq 0.05$, $p \leq 0.01$ respectively according to Tukey's test.

DISCUSSION

The effects of B toxicity in plants depend on intracellular B concentration (Hayes and Reid 2004). The extent of toxicity also varies according to the type of plants (Ferreya*etal.* 1997) and among the plants of same species (Nable 1988). We experimented with a B-sensitive rice cv. Malviya-36. The visible effects of B toxicity observed in our studies with rice seedlings were chlorosis, suppressed growth and reduced fresh biomass. Results indicated that vigor of rice seedlings decreased when raised in hydroponics containing 1.5mM H_3BO_3 in the growth medium. Decrease in root and shoot length of rice seedlings due to B toxicity in our studies could possibly be due to limiting cell elongation rather than cell division due to excess B (Noble *et al.* 1997, Brown *et al.* 2002). The decline in fresh and dry weight in wheat and rice plants was observed with an increase in B concentration in the growth medium (Muhammad *et al.* 2013). Boron treatment alone resulted in a decrease in RWC in our studies. The decrease in RWC was more pronounced in roots than in shoots. The possible reason of decreased RWC in boron treated seedlings could possibly due to decreased leaf potential caused by B toxicity. It has been shown that several metals and metalloids, when taken in excess by plants, cause decreased RWC in the tissues (Yadav *et al.* 2010).The extent of damage in rice seedlings due to B toxicity was reduced when the treatment medium contained Sa or Ta. A considerable restoration in root-shoot length, fresh weight and RWC was observed compared to control values when the growth medium contained Sa, along with 1.5 mM B. *S. aromaticum* extract is known to contain phenolic compounds such as flavonoids, hydroxybenzoic acids, hydroxycinnamic acids and hydroxy phenylpropanes. The presence of Eugenol which is the key bioactive compound of *S. Aromaticum* (Anna et al 2017) may contribute as the major component for protecting the plant against boron toxicity. Among phenolic acids, gallic acid is found in higher concentration. However, other gallic acid derivatives such as hydrolyzable tannins are also present (Walter *et al.* 2011). Other phenolic acids found in clove are the caffeic, ferulic, elagic and salicylic acids. These phenolic compounds are known to have antioxidant activity and also they act as metal chelators (Zahra *et al.*2015).Similar to *Syzgiumaromaticum*, *Terminalia arjuna* bark aqueous extract also contains a large number of polyphenols, for instance, gallic acid, ellagic acid, etc. which function as metal chelators (Saha *et al.* 2012). Therefore, it is possible that these molecules form complexes with boron leading to decreased absorption of B by plant roots. This could be one of the reasons for the reduced toxicity symptoms of B in the seedlings in the presence of Sa and Ta extracts in the growth medium. However, the alleviating effect was more evident with *Syzygium. aromaticum* bud extract as compared to *Terminalia arjuna* bark extract.

Chlorosis is the first visible symptom of boron toxicity (Wang *et al.* 2011). Our study showed a significant decline in chlorophyll content in rice seedlings grown under B toxicity. Reduction in the level of chlorophyll pigments could possibly be due to their decreased synthesis or due to structural damage to thylakoids (Landi *et al.* 2013). Application of Sa and Ta extract caused significant restoration of photosynthetic pigments. Boron toxicity is also known to interfere with the stomatal conductance (Lovatt and Bates 1984).This can be well correlated with our findings of SEM analysis of the leaves of rice seedlings where we observed marked distortion of guard cells under B toxicity. Guard cells play a vital role in the stomatal conductance as well as opening and closing of stomatal aperture. Distortion of guard cells under B toxicity could contribute to reduced transpiration rate. We observed decreased photosynthetic rate in rice seedlings exposed to B. A significant recovery in the photosynthetic rate and related parameters such as transpiration rate and stomatal conductance with the application of Sa or Ta extracts in B-stressed seedlings suggests that Sa and Ta extracts have the potential to alleviate B toxicity effects in rice seedlings by restoring photosynthesis.

We observed increased production of reactive oxygen species (ROS) upon exposure of rice seedling to excess B for 8 days. Similar to other abiotic stresses (drought, salinity, cold, heat and heavy metals), excess B also results in the production of ROS such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide, and free singlet oxygen (Siddiqui *et al.* 2013, Archana and Pandey 2015). Overproduction of ROS triggers harmful effects on cellular processes, including oxidative damage to nucleic acids, proteins, lipids, and resulting in the alteration in activities of antioxidative enzymes (Molassiotis *et al.* 2006, Tombuloglu *et al.*2012). Superoxide radicals ($O_2^{\bullet -}$) are noxious by products of oxidative metabolism and can interact with H_2O_2 to form highly reactive oxygen species, which are thought to be primarily responsible for oxygen toxicity in the cell (Mittler 2002). To overcome any abiotic stress-induced oxidative damage, plants use their own antioxidative defense system. The primary enzymes which contribute to antioxidative defense are SOD and catalase. The activities of both SOD and catalase increased in our studies when rice seedlings were exposed to excess B, though enhancement was more pronounced in roots than in shoots. The conversion of superoxide radical into less reactive form H_2O_2 is catalyzed by the enzyme SOD. In our studies, we found an increase in the expression level of SOD. Moreover, Ardic *et al.* (2009) reported that the increase in SOD activity under stress was due to the induced synthesis of new isoforms of the enzyme rather than an increase in the level of constitutive iso enzymes. It has been shown that SOD activity is strongly regulated in response to B excess (Garcia *et al.* 2001, Karabal *et al.* 2003). At the same time when the level of H_2O_2 exceeds the optimum concentration, catalase becomes active and catalyzes the conversion of H_2O_2 into the water and molecular oxygen. The increase in catalase activity was evident in our study on B treatment of rice seedlings. Wang *et al.* (2011) reported an increase in the activity of catalase in peer plants upon H_2O_2 accumulation. We noticed increased $O_2\bullet$-, H_2O_2 and increased lipid peroxidation marked by increased level of TBARS in B treated seedlings. Application of both Sa and Ta in the

growth medium caused the lesser production of H_2O_2 and TBARS compared to the levels in B treated seedlings. On application of both the natural plant extracts in the growth medium the activity of enzymes SOD and catalase declined compared to the activities in B treated seedlings, indicating that both of these extracts help in lowering the production of ROS in the seedlings and also help in maintaining the requisite antioxidative potential in B stressed rice seedlings. Thus our results suggest that both of these plants extracts *Syzygium aromaticum* and *Terminalia arjuna* have the potential to alleviate B toxicity in rice seedlings by preventing oxidative damage and protecting photosynthetic machinery. However, among these two plant extracts *S.aromaticum* shows greater potential in alleviating the agony of B toxicity in rice seedlings.

CONCLUSION

It appears that the antioxidative and chelating properties of the phenolic compounds of *Sa and Ta* extracts could be responsible for its protective action against B toxicity in rice seedlings. The results suggest that *Syzygium aromaticum* bud aqueous extract has better alleviating capacity than *Terminalia arjuna* bark aqueous extract and can be used to alleviate boron toxicity in rice plants.

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