MITIGATION EFFECTS OF WATER-SOLUBLE CHITOSAN ON BORON TOXICITY IN PEPPER PLANTS

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Abstract

Excess boron in soil is often found in dry agricultural areas and is one of the factors limiting crop yields. Polysaccharide-based biostimulants can mitigate the harmful effects of environmental stresses on plants. I performed this study under controlled greenhouse conditions to understand the response of pepper plants under boron stress to treatment with water-soluble chitosan (WSC). I watered plants with 50 or 100 mg/l WSC and 1.5 mM boric acid solution. As a result of WSC application at both concentrations, plants were taller and had more leaves and greater leaf length, width, relative chlorophyll content, and fresh aboveground weight compared with the control. Plants exposed to boron stress had fewer leaves, a lower relative chlorophyll content, and leaf blade damage indicative of boron toxicity. At the same time, boron-exposed plants showed a marked increase in the leaf nitrogen, phosphorus, potassium, and boron contents. Applying WSC at both concentrations modulated boron stress in plants by improving plant growth; reducing boron accumulation in leaves; and increasing the available nitrate nitrogen, phosphorus, and potassium contents in the substrate.

Keywords: oligosaccharides, biostimulants, boron stress, nutrient uptake

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1. Introduction

Biostimulants for plant growth are becoming increasingly popular. The market for these products is growing due to the increasing use of sustainable plant-growing methods and the increased awareness of farmers regarding the benefits of using biostimulants [1]. The organic food industry is the main driving force behind the growth of the biostimulant market, as they are entirely safe for the environment due to their composition. There is a growing emphasis on the importance of a healthy lifestyle and the benefits of eating healthy food [2]. In addition, the expected increase in the world's population to more than 8.5 billion by 2030 emphasises the need to ensure food security, high productivity, and increased crop yields [3].

Chitosan is a plant biostimulant that is safe for the environment and humans. In agriculture and horticulture, chitosan protects plants from diseases and is a biostimulant for growth and development [4, 5]. Chitosan affects many metabolic and physiological processes, and as a result can respond better to environmental stresses [6, 7]. It has been proved that plants treated with chitosan become more resistant to drought, salinity, and heavy metals [8–11]. The problem with the use of chitosan is its poor solubility in water. Modifications of chitosan are carried out to obtain derivatives with increased water solubility and, thus, more potential applications. High-molecular-weight chitosan is used to obtain derivatives that differ from the starting product in terms of the molecular weight and biological, physical, and chemical properties [12]. According to previous studies [13–15], low-molecular-weight chitosan derivatives may exhibit better or similar biostimulatory properties to high-molecular-weight chitosan. Most of the research on the effects of chitosan on plant growth and stress tolerance is on nondegradable biopolymers that have poor aqueous solubility. There are far less data on plant responses to soluble forms of chitosan.

Boron is an essential micronutrient for higher plants and is taken up by the roots, mainly in the form of boric acid. This element is involved in constructing and ensuring the functioning of cell walls and membranes and participates in numerous ion, metabolite, and hormone transport reactions [16]. Boron has an extremely narrow range between deficiency and toxicity. Both boron deficiency and excess have detrimental effects on plant growth and yield [17]. The toxic effects of boron caused by excess boron in soils in arid areas are widely documented. Excess boron in soils has been demonstrated in the Americas (the United States, Mexico, Peru, and Chile), Australia, Asia (Russia, India, Pakistan, Malaysia, Turkey, Israel, Jordan, and Syria), Africa (Iraq, Egypt, Morocco, and Libya), and Europe (Hungary, Serbia, and Italy) [18–20]. Progressive climate change, including water shortages leading to desertification of many areas, contributes to farmland with a boron content that is toxic for plants. Strategies are being sought to effectively protect plants from excess boron in the soil. One of these strategies is to use plant growth regulators and biostimulants that could promote plant growth while reducing the harmful effects of excess boron [21–23].

Pepper (*Capsicum annuum* L.) is a popular vegetable belonging to the Solanaceae family. In temperate climates, peppers are grown from seedlings. A healthy and adequately formed seedling guarantees a high and even pepper yield; hence, methods have been sought to improve its quality parameters and to increase seedling tolerance to unfavourable stress factors [24–26]. This study aimed to determine the effect of applying a water-soluble chitosan (WSC) with a low molecular weight on the growth and mineral status of bell pepper grown under boron stress. I hypothesised that WSC reduces the effects of boric acid application–induced stress in pepper plants.

2. Materials and Methods

2.1. Chitosan

The water-soluble chitosan (WSC) was obtained from the Center of Bioimmobilisation and Innovative Packaging Materials at the West Pomeranian University of Technology in Szczecin, Poland. WSC with a molecular weight (Mw) of \approx 48,000 g/mol, a number average molecular weight (Mn) of \approx 9770 g/mol, and a degree of deacetylation (DDA) of \approx 85% was obtained by a free radical degradation process described by Bartkowiak [27].

2.2. Plant Culture and Treatment

Seeds of the bell pepper cultivar 'Robertina' were sown in mid-March into sowing boxes filled with TS1 medium (0-5 mm light peat, pH 5.7; fertiliser, pH 5.8; PGMix NPK fertiliser 14:16:18 + micro 1 g/m³). The crop was grown in the West Pomeranian University of Technology greenhouse in Szczecin (53°25'N, 14°32'E), where the temperature during the entire experimental period was 20–23°C during the day and 18–20°C at night. Seedlings with developed cotyledons were interplanted individually into 1.7-dm³ pots filled with TS1 medium. The pots were placed on tables and submitted to the natural photoperiod conditions. On days 20, 25, and 30 after sowing, the plants were watered with a WSC solution (50 or 100 mg/l), using approximately 50 cm³ of solution per plant and time. Control plants were watered with water. On days 35, 40, and 45 after sowing, the plants were watered with a solution containing 1.5 mM boric acid, using 50 cm³ of solution per pot. The WSC and boric acid concentrations were determined based on a pilot experiment. Each experimental variant consisted of 16 plants, with 4 plants per replicate. When flowers developed in the apical part of the plants (at day 80 after sowing), the following parameters were determined: height, the number of leaves, and the fresh weight of the aboveground part. The relative chlorophyll content was measured with the SPAD 502 optical apparatus (Minolta, Japan) on three fully expanded leaves of each plant. The collected leaves were dried in the dark for 48 h at 70°C and then ground. After cultivation, substrate samples covering the mixed upper, middle, and lower parts of the root system were taken from the pots.

2.3. Mineral Analysis

In mineralised leaf samples, the total nitrogen content was determined by the titration method (Kjeldahl); the potassium, phosphorus, calcium, and magnesium contents were determined by flame photometry (ASA) using a GENESYS 6 UV-Visible Spectronic spectrophotometer (Thermo Electron Corporation, UK); and the boron content was determined with a colourimetric method. Plant material was mineralised for 1 h in 96% sulphuric acid (H_2SO_4) to determine the total nitrogen, potassium, phosphorus, magnesium, and calcium contents. To assess the boron content of the leaves, samples were mineralised for 8 h in a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) (1:4). The pH in water of the substrate and the content of available nitrate nitrogen ($N-NO_3$) in the substrate were determined by the potentiometric method. In the substrate, the available phosphorus content was determined by a spectrophotometric method, available potassium by flame photometry, and available magnesium and calcium by flame atomic absorption spectrometry (FAAS). Analyses were performed according to previously published methods [28], and each mineral determination was performed in three replicates.

2.4. Statistical Analysis

The results were statistically processed by analysis of variance (ANOVA) for univariate experiments using the TIBCO StatisticaTM Professional 13.3.0 software (TIBCO Statistica,

USA). Multiple comparisons of means were made using Tukey's honest significant difference test to determine differences between means.

3. Results and Discussion

The application of WSC at 50 and 100 mg/l to plants led to a significant increase in their height (by 23.6% and 17.3%, respectively), the number of leaves (by 21.7% and 13.1%, respectively), the relative chlorophyll content (by 18.8% and 16.2%, respectively), and the fresh weight of the aboveground part (by 47.6% and 44.8%, respectively) compared with the control (Table 1). Researchers have previously shown that chitosan stimulates growth and increases plant biomass. Esyanti *et al.* [29] reported that foliar-applied chitosan increased plant height, the number of leaves, and the chlorophyll content of pepper plants. Dung *et al.* [30] evaluated the effects of various chitosan degradation products with an initial Mw of 14.84 kDa on bell pepper growth and yield. These researchers found apparent biostimulatory effects of chitosan derivatives with a Mw of 1–3 and 3–10 kDa on plant growth, biomass gain, and fruit yield. In the case of chili plants [31], of the three tested oligochitosans with a Mw of 7.8, 5.0, and 2.5 kDa, the best was the oligochitosan with a Mw of 2.5 kDa, which significantly increased fresh and dry shoot weight, the total chlorophyll content, and the fresh fruit weight.

In the present study, I found that WSC at 50 and 100 mg/l significantly increased the leaf nitrogen (by 56.2% and 26.1%, respectively) and boron (28.3% and 32.7%, respectively) contents. In addition, WSC at 50 mg/l increased the leaf potassium content (by 28.3%), and WSC at 100 mg/L increased the phosphorus (by 55.5%) and magnesium (36.9%) contents (Table 2). These findings confirm previous studies [32–34] that have shown chitosan can improve the efficiency of root uptake of minerals, contributing to better plant nutrition and increased yield. The increased relative chlorophyll content and biomass after chitosan application in the present study may be due to the high nitrogen content in the leaves. Nitrogen is one of the most critical factors modifying plant growth and development processes [35].

Treatment	Relative chlorophyll content [SPAD]	Plant height [cm]	Number of leaves	Fresh weight [g]	
Control	44.4 ^b	37.1 ^{bc}	23.9 ^b	52.5°	
WSC 50 mg/l	52.7ª	45.9ª	29.1ª	77.5ª	
WSC 100 mg/l	51.6ª	43.5 ^{ab}	27.0 ^{ab}	76.0ª	
B stress	37.3°	35.0°	19.8°	55.9 ^{bc}	
WSC 50 mg/l + B stress	39.8°	45.0ª	22.9 ^{bc}	71.4ª	
WSC 100 mg/l + B stress	44.5 ^b	43.0 ^{ab}	23.1 ^{bc}	69.3ab	

Table 1. The effects of water-soluble chitosan (WSC) and boron (B) stress on the relative
chlorophyll content, the plant height, the number of leaves, and the fresh weight
of the aboveground part of pepper plants.

Note. Means followed by the same letter are not significantly different (p < 0.05) according to Tukey's honest significant difference test.

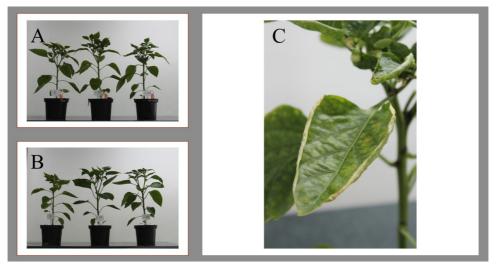
Treatment	N [%DM]	P [%DM]	K [%DM]	Ca [%DM]	Mg [%DM]	B [mg/kg DM]
Control	2.03°	0.27°	4.12 ^d	6.63ª	0.34°	19.1 ^d
WSC 50 mg/l	3.17ª	0.33 ^{bc}	5.30 ^{bc}	4.39°	0.39 ^{bc}	24.5 ^{bc}
WSC 100 mg/l	2.56 ^b	0.43 ^{ab}	4.75 ^{cd}	4.42 ^{bc}	0.46 ^{ab}	25.4 ^b
B stress	2.60 ^b	0.44 ^{ab}	5.65 ^{ab}	5.83 ^{abc}	0.37 ^{bc}	50.5ª
WSC 50 mg/l + B stress	2.69 ^b	0.48ª	6.03 ^{ab}	5.89 ^{ab}	0.45 ^{ab}	20.4 ^{cd}
WSC 100 mg/l + B stress	2.60 ^b	0.45 ^{ab}	6.35ª	5.39 ^{abc}	0.49ª	19.4 ^d

 Table 2.
 The effects of water-soluble chitosan (WSC) and boron (B) stress on the mineral content in the leaves of pepper plants.

Note. Ca, calcium; DM, dry mass; K, potassium; Mg, magnesium; N, nitrogen; P, phosphorus. Means followed by the same letter are not significantly different (p < 0.05) according to Tukey's honest significant difference test.

Boron application led to a significant reduction in the number of leaves (by 17.2%) and the relative chlorophyll content (by 16.1%) compared with plants not submitted to boron stress. Excess boron did not significantly alter the plant height and fresh weight of the aboveground part (Table 1). Boron-exposed plants showed a marked increase in the leaf nitrogen (by 28.3%), phosphorus (59.3%), potassium (36.9%), and, as expected, boron (164%) contents compared with the control (Table 2). The results confirm reports of the harmful effects of boron stress on a plant's growth and physiological status [19, 20]. Excess boron disrupts the ionic homeostasis of plant cells, which can consequently lead to changes in nutrient content [36]. In the case of the pepper cultivar 'Balga', treatment of plants with 100 µM boron resulted in visible symptoms of toxicity, reduced photosynthesis, and reduced dry matter [37]. Specific symptoms of boron toxicity occur mainly on mature leaves in the form of chlorosis, spotting, or necrosis of leaves at the edges and tops [38], as confirmed by the present study. Indeed, the toxic effects of boron were noticeable on pepper leaves in the form of discolouration and deformation, as well as drying of the leaf blade edges (Figure 1). This permanent damage to cells, tissues, and organs due to boron application was most likely the cause of the stunted plant growth.

Plants watered with the WSC solution at both concentrations (50 and 100 mg/l) and subjected to boron stress had significantly increased height (by 28.6% and 22.9%, respectively) and fresh weight of the aboveground part (by 27.6% and 23.9%, respectively) compared with plants submitted to boron stress alone. In addition, plants under boron stress and treated with 100 mg/l WSC had an increased number of leaves (17.0%) and relative chlorophyll content (by 19.5%) compared with plants not treated with the biopolymer. WSC applied at 50 and 100 mg/l to plants under boron stress reduced boron accumulation in leaves. Plants treated with boric acid alone had a leaf boron content of 50.5 mg/kg dry mass, while leaves of plants treated with boron and WSC at 50 and 100 mg/l contained 25.5 and 25.4 mg/kg dry mass of boron, respectively (Table 2). WSC may have inhibited the uptake of excessive amounts of boron by the leaves, thus protecting the plants from stress. The available literature lacks information on the effect of low-molecular-weight chitosan on pepper plant growth under excess boron conditions. In the case of cucumbers, researchers showed that foliar application of chitosan mitigated the harmful effects of boron stress [39]. Specifically, chitosan improved growth and increased the chlorophyll content and photosynthetic activity.



- Figure 1. The effects of water-soluble chitosan (WSC) and boron stress on the growth of pepper plants. (A) Left to right: nontreated control, WSC 50 mg/l, and WSC 100 mg/l. (B) Left to right: boron stress, WSC 50 mg/l + boron stress, and WSC 100 mg/l + boron stress. (C) Visible symptoms of boron toxicity.
- **Table 3.** The effects of water-soluble chitosan (WSC) and boron (B) stress on the pH andavailable nutrients (mg/kg) of the substrate after harvest.

Treatment	рН	N [mg/kg DM]	P [mg/kg DM]	K [mg/kg DM]	Ca [mg/kg DM]	Mg [mg/kg DM]
Control	5.54 ^b	35.0°	72.0 ^d	72.3°	1568 ^b	134 ^b
WSC 50 mg/l	5.53 ^b	55.5 ^b	92.5°	129 ^b	1659 ^{ab}	215ª
WSC 100 mg/l	5.43 ^b	66.5ª	95.5°	131 ^b	1557 ^b	179 ^{ab}
B stress	5.71ª	32.0°	109 ^{bc}	75.5°	1887ª	187ª
WSC 50 mg/l + B stress	5.45 ^b	53.0 ^b	139ª	189ª	1645 ^{ab}	201ª
WSC 100 mg/l+ B stress	5.45 ^b	61.0 ^{ab}	127 ^{ab}	139 ^b	1814 ^{ab}	232ª

Note. Ca, calcium; DM, dry mass; K, potassium; Mg, magnesium; N, nitrogen; P, phosphorus. Means followed by the same letter are not significantly different (p < 0.05) according to Tukey's honest significant difference test.

Analysis of the composition of the substrate after cultivation showed that as a result of watering the plants with boric acid, the pH of the substrate increased to pH 5.71 compared with the control (pH 5.54). In the variants where boron and additional WSC were applied at both concentrations (50 and 100 mg/l), the pH of the substrate did not differ significantly from the control (Table 3). The increased substrate pH after boron application may result from the increased calcium content in the substrate, as confirmed by the results. The substrate in which plants treated with boron alone grew had the highest calcium content (1887 mg/l). When WSC was applied at 50 and 100 mg/l in addition to boron, the calcium content of the substrate decreased slightly to 1645 and 1814 mg/l, respectively. In all variants where WSC was applied, the substrate's nitrate nitrogen, phosphorus, and potassium contents increased. These findings support published reports that chitosan

increases soil nutrient abundance [5–7]. This may be related to the fact that chitosan is a nitrogen- and carbon-rich polymer that stimulates microbial activity in the soil, thus improving the abundance of available macronutrients in soil [40, 41].

4. Conclusion

In conclusion, the WSC tested in the study at both concentrations (50 and 100 mg/l) promoted the growth of pepper plants and increased the macronutrients in the substrate and leaves. Under boron stress conditions, WSC inhibited excessive boron accumulation in the leaves, consequently mitigating the harmful effects of boron stress in pepper plants. Moreover, under boron stress conditions, applying WSC contributed to increase the abundance of the available nitrate nitrogen, phosphorus, and potassium while maintaining the pH of the substrate. The mechanisms by which WSC could mitigate boron stress are complex and require further research.

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