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Comparative Effects of Boron Toxicity and Deficiency on the Growth, Chlorophyll, Protein and some Cations Accumulation in Zea mays Seedlings

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ABSTRACT: The understanding of the effects of boron (B) toxicity and deficiency on the growth and nutrients accumulation of crop seedlings in the field is required to substantiate the need of adequate dosage for their survival and optimal production. In this study, the susceptibility of *Zea mays* to excess and deficiency of B was obtained by feeding the seedlings with varied concentrations of B in standard nutrient solution. B was applied as Boric acid (H_3BO_3) and at the rate of 0.33ppm (FN/B-optimal dosage) which served as control, 0 ppm (-B) served as B-deficient level, 1.65, 3.30 and 6.60ppm (×5B, ×10B and ×20B respectively) served as toxic levels. Results showed that the number of leaves, leaf area, leaf area ratio, shoot fresh and dry weights, root fresh weight and biomass, chlorophyll a, b and total chlorophyll, calcium (Ca), Magnesium (Mg), nitrogen (N), and protein accumulation were significantly reduced by the application of 3.30 ppm (×10B) and 6.60 ppm (×20B) B dosage. The retardation effects of the ×20B level was not significantly different from ×10B level at $P<.05$ indicating 3.30 ppm (×10B) as the critical level of toxicity for maize seedlings growth and development. The data also showed that toxicity of B was more harmful to juvenile maize than its deficiency since B deficiency diminished just few parameters such as the shoot dry weights, chlorophyll b and total chlorophyll at $P<.05$. Moreover, the study suggested three different optimal concentrations; 0.33, 1.65 and $\geq$ 3.30 ppm respectively for general growth, chlorophyll and carotenoids synthesis in maize seedlings. Lastly, the study emphasized the accumulation of carotenoids and potassium (K) as possible adaptive mechanisms evolved by the maize seedlings to B-stress tolerance and suggested an under-play physiological role for B in the formation and development of the seedlings leaf.

KEYWORDS: Accumulation, Boron, Chlorophyll, Comparative, Deficiency, Protein, Seedlings, Toxicity.

1 INTRODUCTION

The deficiency of a mineral nutrient occurs when an essential element is completely absent or available in insufficient quantity to meet the needs of the growing plant while nutrient toxicity occurs when an element is in excess of plant needs and decreases plant growth, quality and quantity [1]. Boron (B) is one of the important micronutrients of vascular plants whose deficiency or toxicity causes impairment in several metabolic and physiological processes [2,3,4]. According to [5], primary function of B in plants is connected with the cell wall structure and function, that is, cross-linking the cell wall rhamnogalacturonan II (RGII) and pectin assembly. Also, B has been implicated in affecting the growth and yield of crops [6,7]. However, like the other abiotic stressors, B also enhances oxidative damage induced by the formation of reactive oxygen species (ROS), which are strong oxidizers of lipids, proteins, and nucleic acids [8,9]. [10] stated that the maintenance of cell membrane integrity and improvement of cellular defense mechanism is a novelty role of B. Despite the obvious importance of B, the mechanisms of B tolerance to deficiency and toxicity in plants are poorly understood [11,9,12,13]. [14]

noted that the response of crops to B does not only vary with plant species, soil type and environmental conditions, but also its excess/deficiency may affect the availability and uptake of the other plant nutrients.

Crop production, according to [15] is limited worldwide because B in the soil is either insufficient or at toxic levels. Deficiency of B is very common and widespread around the world, causes both quantitative and qualitative losses in crop production [16]. Physiological damages such as; inhibition of root elongation [17,18], loss of integrity and function of membrane, and decrease in cell wall stability resulting in structural damage in plants [19,20,3] had been attributed to the deficiency of B. According to [21], B deficiency first affects the shoot apex and the actively growing leaves with the latter becoming small, dark green, deformed in shape, and brown purple pigmented. Also, the reduction in the cytokinin level as well as the indole acetic acid (IAA) export out of the shoot apex in pea plants grown in B-deficient medium had been reported [22]. In another study, [23] attributed the observed reduction in the photosynthetic efficiency of sunflower leaves to the deficiency of B in the medium. Furthermore, the shoots of plants grown in B deficient medium characteristically had shorter internodes and petioles and stems with bigger diameter [18]. Similarly, toxicity of B constitutes a very serious threat to agriculture in arid and semiarid regions where salt affected soils and saline irrigation water are prevalent [24]. Though, [2] were of the opinion that fertilizers and mining would increase the concentration of B in the soils, irrigation water according to [25] is the most important contributor to high level of B in the soil. In terms of management in crop production system, B toxicity is more difficult than its deficiency which can be corrected through fertilization [26]. However, the fertilization of crop with B to avoid deficiency can result in toxicity since the concentration range between B deficiency and toxicity is narrower than for any other plant essential nutrient [27,28]. Nevertheless, B fertilization is required in boosting the production of many crops [29]. Some of the reported physiological effects of B toxicity include; reduced root cell division [30], decreased shoot and root growth [31,32], reduced vigor, retarded development, chlorotic and necrotic patches in older leaves, inhibition of cell wall expansion, lower leaf chlorophyll contents and photosynthetic rates, deposition of lignin and suberin [33]. Others are; impairment of nitrogen assimilation pathways by affecting key enzymes involved in those processes (Herrera-Rodrı´guez *et al.,* 2010), disruption of RNA splicing [34,12], increased membrane leakiness, peroxidation of lipids and altered activities of antioxidant pathways [35,36].

Among cereal crops, maize (*Zea mays*) in the family poaceae has the highest productivity rate (Nawaz, 2007, unpublished). It is one of the most important food resources of human and second most important crop to soyabean for biodiesel production [37]. As a C_4 crop, it absorbs high amounts of nutrients from the soil and grows under wide range of environmental conditions, majorly for grain and forage consumed by both humans and animals respectively [38]. Because of its great yield potential, a good food source of food for humans and animals and its efficient absorption and maximum use of micro elements in various conditions of soils, this study therefore investigated the effect of the deficiency and supra-optimal concentrations of B on the growth and accumulation of chlorophyll, carotenoids, selected cations, nitrogen and protein in *Zea mays* seedlings.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE AND MATERIALS SOURCES

The experiment was carried out at the Department of Biological Sciences, Wesley University of Science and Technology, Ondo, Nigeria. The seeds of *Zea mays* L. (CV. NS1) were collected from National Horticultural Research Institute (NIHORT) Ibadan.

2.2 SOIL TREATMENT AND GERMINATION OF SEEDLINGS

Seedlings of *Zea mays* L. (‘CV. NS. 1) were utilized in this experiment. Sand was soaked in 1.5N hydrochloric acid for one hour to eliminate microbes and solubilize mineral elements which might be present in it. The acid was drained off, thereafter, the sand was washed with tap water and then double distilled water until the pH of the decantable water was between 6 and 7, which was optimal for the germination and growth of the maize seedlings. *Zea mays* seeds were randomly selected for uniformity on the basis of size and soaked for five minutes in 5% sodium hypochlorite to prevent fungal growth. Thereafter, the seeds were rinsed in running tap water for 5 mins and then thoroughly washed in double distilled water. Ten of these seeds were sown in each of the experimental pots (24 cm diameter × 21cm depth) that were already filled with washed and air-dried sand. Each experimental pot had four holes perforated at the bottom for good drainage. All the pots were initially irrigated with 200 mL of water on a daily basis for nine days. On the ninth day, the seedlings in each pot were thinned down to five uniform seedlings per pot based on uniformity of vigour and transferred to an open environment where the seedlings received approximately eight hours of sunlight daily. Temperature at soil level varied between 27^0C and 32^0C, and 22^0C and 25^0C during the day and night respectively.

2.3 NUTRIENT SOLUTION COMPOSITION AND ALLOCATION OF NUTRIENT REGIMES

The nutrient solution was prepared according to the modified Long Ashton Formula [39]. The nutrient solution composition were given the subscripts; FN (Full Nutrient containing 0.33 ppm B which is the optimal concentration of B) served as the control, -B (that is, FN with 0 ppm B), ×5B, ×10B and ×20B {that is, FN with five (1.65 ppm), ten (3.30 ppm) and twenty (6.60 ppm) times optimal concentration of B respectively}. The pots were then allocated to the control/FN and four different treatments, that is, -B, ×5B, ×10B and ×20B and then arranged in a complete randomized design. Thereafter, each pot was supplied with 200 mL of appropriate feeding solution in the morning and 200 mL of distilled water in the afternoon in order to keep the growth medium moist at all times. On the ninth day when nutrient feeding commenced, seedlings were harvested just before the application of the nutrient solution. Thereafter, harvesting of the plant was on three days intervals for a period of twenty four days.

2.4 MEASUREMENT OF PHYSICAL PARAMETERS

Recording of the following growth parameters (shoot height, number of leaves) data was carried out according to standard methods. The leaf area was determined using the method of [40] and the leaf area ratio calculated. Five shoots in each regime were weighed on Mettler Toledo balance to obtain the fresh weight. The shoots were then packaged separately in envelopes and dried to constant weight at 80^0C in a Gallenkhamp oven (Model IH-150) to obtain the dry weight. Chlorophyll contents of the fresh shoot were extracted with 80% acetone and quantified following the procedure of [41]. For carotenes and xanthophylls extraction, Five gram of maize seedlings leaves was macerated in 20 ml of 80% acetone using a mortar and a pestle. The extract was filtered through a Whatman's No.1 filter paper. 25 ml of petroleum ether was placed in a separating funnel and the acetone extract of the pigment was added. The funnel was gently rotated, releasing the pressure periodically. 35ml of distilled water was poured down the sides of the funnel and the funnel rotated until the upper layer was green; the two layers were allowed to separate before the lower acetone layer was then drawn off. The petroleum ether fraction was later washed with 25 ml of distilled water at three consecutive times and discarded each time. This removed any trace of acetone that remained in the petroleum ether fraction. Twenty five (25) ml of 92% (v/v) methanol was added to the petroleum ether fraction, rotated and then separated into upper and lower fractions (carotenes and xanthophylls). The absorbance of both fractions was determined using Digital Spectrophotometer. Petroleum ether and diethyl ether served as blanks [42].

2.5 EXTRACTION OF MACRONUTRIENTS (K, CA AND MG)

Potassium (K), calcium (Ca) and magnesium (Mg) were measured using a flame photometer (Jenway, PF P7). Percentage nitrogen and protein were determined according to the micro-Kjeldahl nitrogen method as described by [43].

The percentage crude protein accumulation in the shoot of *H. sabdariffa* was estimated using the formulae below.

$$\%\ \text{Total Nitrogen} = \frac{(A-B)\times N \times 14.01 \times 100}{\text{gram of Sample} \times 10}$$

% Crude Protein = % Total Nitrogen × 6.25

Where A = sample reading, B = blank reading; N = Normality of acid used for titration, 100 = conversion to % and 6.25 is the correction factor (F)

2.6 STATISTICAL ANALYSIS

All experiments were conducted in five replicates and the data obtained was subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) software version 20. Treatment means were compared using least significant difference (LSD $P<.05$).

3 RESULTS

3.1 PLANT GROWTH CHARACTERISTICS

There was though no visible symptom of excess or deficiency of B for the first 23 days after planting. However, faint yellow–green coloration at the edge of one of the mature leaf of maize grown in the supra-optimal concentration (×20B) was observed on the last (24th) day of the experiment.

In the control/FN regime, the shoot height and number of leaves on the maize seedlings were 9.84cm and 4.20 respectively (Figures 1&2). These values were reduced to 9.32cm and 3.64 in B-deficient regime. However, application of ×5, ×10 and ×20 optimal concentrations of B respectively increased the shoot height by 15.14, 27.18 and 39.51%. Oppositely, the number of leaves on the seedlings was reduced by 27.62, 42.35 and 47.47% respectively in the ×5B, ×10B and ×20B regime. There was no significant difference between the shoot height of the control seedlings and those of B-deficient and excess B seedlings at *P<.05* while the number of leaves on seedlings treated with ×10B and ×20B were significantly reduced at *P<.05*.

Data illustrated in the Figures 3&4 showed that the leaf area and leaf area ratio of maize seedlings in the control (FN) regime were greater than those of B excess and B-deficient seedlings. Complete absence of boron (-B) as well as application of ×5 optimal concentration of B did not significantly diminish the leaf area and the leaf area ratio of the seedlings. The increase of the applied B up to ×10 optimal concentration however resulted in significant reduction (53.53 and 24.83% respectively) of both parameters at *P<.05* However, the maximum reduction of the leaf area and leaf area ratio was obtained in ×20B-treated regime but the value was not significantly different from that of ×10B-treated seedlings at *P<.05*.

Also, maize seedlings in the control (FN) regime recorded the highest values for shoot fresh and dry weights as well as root fresh weight and biomass (Figures 5-8). While 5.792 and 0.615 g were obtained for shoot fresh and dry weights respectively, the root fresh weight and biomass of the seedlings were 2.841 and 0.272 g respectively. However, seedlings in the ×10B and ×20B regimes recorded a significantly lowered values for these growth parameters at *P<.05* with maximum reduction observed in the ×20B regime. On the contrary, only the shoot dry weight of the seedlings was significantly diminished by the deficiency of B. Statistically, the shoot fresh and dry weights as well as the root fresh weight and biomass of ×10B-treated seedlings were not significantly different from ×20B-treated seedlings at *P*<.05. In the case of application of ×5B dosage, the observed reductions in these parameters were not significant at *P<.05*.

3.2 PHOTOSYNTHETIC PIGMENTS

Figures 9-11 showed that accumulation of chlorophyll a, b and total chlorophyll followed almost the same pattern. The ×20B–treated seedlings recorded the lowest value of chlorophyll a (5.406 µM), b (1.489 µM) and total chlorophyll (7.007 µM) the respective highest accumulations (10.527, 2.845 and 13.372 µM) were obtained in the ×5B-treated regime. In other words, the accumulation of chlorophyll a in the ×5B seedlings was slightly greater than that of the control (FN) seedlings. On the average, the chlorophyll contents in the B-deficient seedlings were slightly reduced compared with the control seedlings. However, formation of chlorophyll in ×10B and ×20B-treated seedlings were significantly reduced at *P<.05.* Comparison of the accumulated chlorophyll in the ×10B-treated seedlings and those of ×20B-treated seedlings showed no statistical significance at *P<.05*.

The lowest and highest accumulation of carotenoids was recorded in the control and ×20B-treated seedlings respectively (Figure 12&13). The control seedlings recorded 1.764 and 4.462 µM carotene and xanthophylls contents respectively while 2.110 µM and 4.525 µM were obtained for -B-treated seedlings. However, application of ×5B increased the accumulation of carotene and xanthophylls in the maize seedlings by 100 and 20% respectively while ×10B concentration increased these pigment contents by 205 and 25% respectively. Statistically, the difference between the accumulated carotene in the control seedlings and those of ×10B and ×20B-treated seedlings was significant at *P<.05* However, the increase in the accumulated xanthophylls observed for B-deficient and B-excess seedlings was not significantly different from that of the control seedlings at *P<.05*.

3.3 PROTEIN AND MINERAL NUTRIENTS CONCENTRATIONS

The percentage nitrogen and protein contents in both the control *Zea mays* seedlings was higher than those treated with excess and deficiency of B (Fig 14 &15 respectively). While 0.399 and 2.495% nitrogen and protein contents were recorded for the control seedlings, the application of ×5B slightly boosted these parameters by 5% while absence of B (-B) in the nutrient solution slightly depressed the nitrogen and protein percentages by 5%. On the other hand, significant reductions in these percentages were observed for ×10B and ×20B seedlings at *P<.05*.

Figures 16 showed the positive correlations between the K accumulation and toxicity of B, in other words, the K accumulation in maize seedlings increased with increasing B concentration. The pattern of accumulation of Ca and Mg in these seedlings was similar (Fig. 17&18). Complete deficiency of B in the nutrient solution slightly decreased the accumulation of these mineral nutrients while application of ×5B dosage slightly increased it. However, by increasing B concentrations to ×10 and ×20, the diminutions were significant at *P<.05.*

4 DISCUSSION

The observed discolouration at the edge of one of the mature leaves on the last day of the study was could be attributed to high dosage of B in that regime. This result indicated that juvenile maize seedlings were more susceptible to B toxicity than its deficiency. Earlier workers have reported similar observation in barley [44,45].

The toxic levels of B slightly increased the maize seedlings shoot height but critical observations showed that these seedlings lacked vigour compared with the control seedlings. Similarly, the seedlings in these regimes (×5B, ×10B and ×20B) therefore have probably devoted more of their nutrient for stem extension as apical dominance was more pronounced in them. This was in agreement with the results of [46] and [47] that plants growing in supra-optimal concentration of nutrient respond to nutrient stress by devoting more of their available carbon to shoot growth resulting in elongated stems. Similar non-significant increase in plant height was reported by [29] on fodder beet plant sprayed with 75 and 100 ppm B. The control (FN) seedlings had adequate nutrient supply and so do not require extra carbon for shoot growth; this invariably led to the normal seedlings height, better vigour and short peduncles observed in this regime. Adequate supply of B which played role in synthesis and translocation of sugar could also account for the improved vigour observed in the control seedlings [48]. The reduction in the shoot height of the B-deficient seedlings was similar to the findings of [49] who reported inhibition of the growth of the plant apex which resulted in a relatively weak apical dominance and a subsequent sprouting of lateral buds in B-deficient medium. [26] and [22] stated that B deficiency first affects the shoot apex and the actively growing leaves reducing significantly the cytokinin level and inhibiting the export of Indole Acetic acid (IAA) out of the shoot apex. This phenomenon could probably account for the reduced shoot height observed in this regime. However, it could be due to the reduction in the seedlings meristematic cell division and cell elongation triggered by B-deficiency which according to [50], could result in diminished shoot growth. In addition, the seedlings in B-deficient regime had shorter internodes and petioles and stems with bigger diameter–the characteristics similar to those observed by [18] in some higher plants.

The leaf parameters (number of leaves, leaf area and leaf area ratio) of the control/FN seedlings appear more luxuriant than those in the B-deficient and B-treated regimes. This suggested an under-played physiological role for B in leaf formation and/or development. In other words the dependence of the formation and/or development of leaf on B concentration in juvenile maize were suspected. Earlier workers had reported that normal application of B could increase significantly the leaf area and ultimately the yield of crops compared to treatment with excess B [51,52,53]. B applied at optimal dosage could influence the cell division, transfer of sugars along cell membranes as well as pectin, RNA and DNA synthesis, thus, enhancing optimal growth of the leaves especially the leaf area [50,54]. In B-deficient seedlings, the actively growing leaves could be affected resulting in the reduced leaf area and leaf area ratio. Similarly, the significant diminution in these parameters triggered by the application of ×10B indicated that this concentration (3.30 ppm B) gave adequate concentration for inhibition of *Zea mays* leaf growth. This could result from the impairment of cell division in the leaves or inhibition of shoot and root growth by excess B [33]. These results were similar to the findings of [55] who reported that application of 0.16M concentration of B was more effective in improving the elongation of 4th leaf on rice than 0 ppm whereas ×3B (0.48M) concentration was toxic, decreased the leaf elongation even than 0 ppm. [56] also reported similar findings.

The optimal weights of shoot and root (especially the dry matter yield) rercorded for the control seedlings can be attributed to optimal rate of photosynthesis, adequate nutrient supply and greater leaf surface area. Adequate nutrient supply increased the dry matter production in optimal condition, a situation that corroborated the findings of [57] and [58] where higher dry weight was correlated to optimal leaf expansion rates. It was noted that the magnitude of the decreasing effect of B toxicity on the fresh and dry weight of the seedlings increased up to ×10B (3.3 ppm) concentration and no statistical significance between these values and those obtained for ×20B (6.6 ppm) dosage. This implied that presence of 3.30 ppm B in the soil could seriously hamper the shoot and root yield, hence, the general growth of the seedlings. The diminution in the shoot and root weights of the ×10B and ×20B-treated seedlings might not be unconnected with the lowered leaf area, photosynthetic rates and decreased lignin and suberin contents triggered by excess B in the soil [2,59]. The slight reduction observed for seedlings in the –B regime was not surprising. According to [54], deficiency of only one of the mineral nutrients could potentially depress the growth, thus the biomass production of higher plants. Earlier workers have also reported that deficiency of B inhibits root elongation [17,18,60] which eventually resulted in significant loss of shoot and root yields. This result was consistent with the findings of [61] who reported that shoot and root fresh and dry weights of maize decreased with increasing B applied. [62] and [63] showed respectively that the shoot dry matter yield and root yields of wheat decreased with increasing B application. Similarly, [64] found significant decrease in the shoot dry matter yield of maize plant treated with elevated B concentration. Other workers had also reported similar findings on tomato [65,66], melon [67], tobacco [68] and pea plants [69].

The chlorophyll content is one of the main factors that reflect the photosynthetic rate of crop plants [70]. The optimal synthesis of chlorophyll in control/FN seedlings could be attributed to the protection of the thyllakoid membrane through decreasing the production of oxygen radicals which according to [23] was a novelty role of B in crop plant. Also, the dual effects of B toxicity on accumulation of chlorophyll followed the trend; ×5B > FN > ×10B > ×20B.This indicated that the 0.33 ppm B (in the control/FN regime) recommended as optimal concentration by Long Ashton and modified by [39] was not optimal for the purpose of chlorophyll accumulation since highest chlorophyll contents was recorded in ×5B regime. Though, it was appropriate for the general growth of the seedlings as evidenced from the parameters described above. Compare with the control, the increase in chlorophyll contents of ×5B-treated seedlings could be a response of the seedlings to compensate for the loss of leaf area owing to smaller leaf size. However, there was significant reduction in the chlorophyll contents of the seedlings in the ×10B-treated seedlings, a phenomenon that could be considered a mark of either the commencement of chlorosis or tolerant/critical level of toxicity for maize seedlings. This diminution could be due to oxidative injuries inflicted on the seedlings by B toxicity – injuries which enhanced chlorophyll degradation or inhibited chlorophyll synthesis. [8] and [9,71] had earlier observed an increase in the Malondialdehyde (MDA) and Hydrogen peroxide (H_2O_2) contents of plants exposed to B toxicity and attributed their observations to oxidative stress and membrane peroxidation which could increase the chlorophyll degradation and inhibited chlorophyll synthesis in crops. This result was similar to the findings of [72] who reported a maximum increase in chlorophyll contents of mung bean (*Vigna radiata* (L.) treated with 4μgg^{-1} B concentrations while application of 8, 16 and 32 μgg^{-1} concentrations significantly reduced the chlorophyll contents in the crop. The finding that B-deficient leaves had lower content of chlorophyll pigments is similar to the recent study by [73] who observed a decline in chlorophyll contents of coconut leaflets grown in B-deficient sand and suggested that chlorophyll synthesizing system and/or chlorophyllase activity might have been affected by B deficiency. It was also in congruent with those reported by [74] and [75].

Carotenoids can act as alternative antennas capture light, absorbing the blue region of the spectrum (400-600 nm) and transferring the energy to the chlorophyll. Carotenoids biosynthesis pathway also supplements the chlorophyll contents of plants through the production of the geranyl geranyl pyrophosphate intermediate to make a phytyl group of chlorophyll, thus maintaining the pigments levels and enhanced the growth and yield performance of crops [76]. The above statements probably explains the possible adaptive mechanism evolved by maize seedlings to B toxicity tolerance since the amount of carotene in the B-treated seedlings increased significantly with increasing B levels. However, the main function of carotenoids is to protect the photosynthetic apparatus, dissipating energy to avoid photo-oxidation [77]. Thus, this significant boost in carotene contents of ×10B and ×20B-treated seedlings could be a response of the seedlings to prevent the excessive loss of chlorophyll contents through oxidative injuries mentioned above. The enhancement of the accumulation of the two pigments (carotene and xanthophylls) therefore suggested the direct or indirect involvement of these pigments in the protection against oxidative stress triggered by B toxicity and maintenance of the chlorophyll levels in the juvenile maize growth. The deficiency of B on the other hand could affect photosynthesis directly or indirectly [23] and the first targets were usually the shoot apex and the actively growing leaves [21]. In this study however, it could be deduced that the reduction of the chlorophyll and carotenoids contents constituted the direct primary effects of B-deficiency while the diminished growth and yield in maize seedlings were its secondary or indirect effects on photosynthesis. These results were similar to the findings of [78] and [23] in which both authors respectively attributed the observed reduction in the carotenoids and photosynthetic efficiency of *Taxodium distichum* and sunflower leaves to the deficiency of B in the medium.

The experimental evidence for a direct and specific involvement of B in the nucleic acids metabolism or protein synthesis is scanty [28]. However, the respective optimum value and the slight increase in protein percentage observed for control/FN and ×5B-treated seedlings could be due to increased level of expression of genes related to nitrogen metabolism. Several reports have indicated that B applied at optimal dosage (FN) may influence positively the expression level of genes related to nitrogen metabolism [79,80]. The nitrogen and protein percentage in the B-deficient, ×10B and ×20B-treated seedlings were affected negatively. These reductions according to [26] could result from a decreased nitrate uptake due to lower expression of plasma membrane H^+-ATPase in B-deficient plant. Similarly, [11] affirmed that excess B could potentially cause metabolic disruption by binding ribose RNA, thereby reducing protein synthesis in plant. These results probably explain the basis of the general enhancement observed in the growth and vigour of the control/FN seedlings than the B stressed seedlings. Similar findings were reported by [36]. The authors observed higher amount of soluble protein in the optimal B containing leaves of orange plants irrigated with relatively high and low amounts of B. [81] found an inhibition of DNA within 6 hours of transferring squash plants to the B-deficient media. Several workers had earlier reported significant depression in the leaf nitrogen of crops including maize [82], tomato [83], Sauvignon vine plant [84].

Depending on the concentration of B applied, the interactions of B with other plant nutrients was either antagonistic or synergistic. In this study, while the excess-B-K interaction was synergistic, the interaction of excess-B with Ca, Mg and N was antagonistic. The significant increase in the K concentration observed for ×10B and ×20B seedlings suggested that the survival of B-stressed maize seedlings could be a function of their ability to maintain higher levels of K. In other words, the tendency of the maize seedlings to accumulate K could be considered as an adaptive mechanism to B-stress tolerance. Such positive correlation between K and excess B according to [85] could be due to the increased activation of the ATPase proton pump by B. On the other hand, the lowered concentrations of the Ca and Mg in both regimes treated with excess and deficiency of B suggested that these treatments influenced the membrane permeability by increasing Ca and Mg leakages from the cells of the seedlings. [86] suggested an increase of membrane permeability which resulted in leakages of mineral nutrients as the primary effects of B deficiency. This reduction however could be due to inhibition of the translocation of the two mineral nutrients from the root to the leaves or decrease in number of negative charges and of Ca^{2+}-borate complex in B-deficient maize seedlings. According to [87], the decrease of membrane associated Ca^{2+} in the B-deficient faba bean plants is primarily caused by a reduction in the number of negative charges and of Ca^{2+}-borate complex. [88] also observed inhibition of the translocation of Ca to the upper leaves in B applied tomato. The increased K concentration, according to [89] was the indirect effect of added B, while the decreased concentration of Ca (as well as Mg) at higher levels seems to be the direct effect of B which also resulted in a reduction in growth and yields. Therefore, these results also explained to some extent the deteriorative role of excess and deficiency of B on the growth parameters and chlorophyll contents of the *Zea mays* seedlings. Earlier workers had reported similar findings [82,90,91,84].

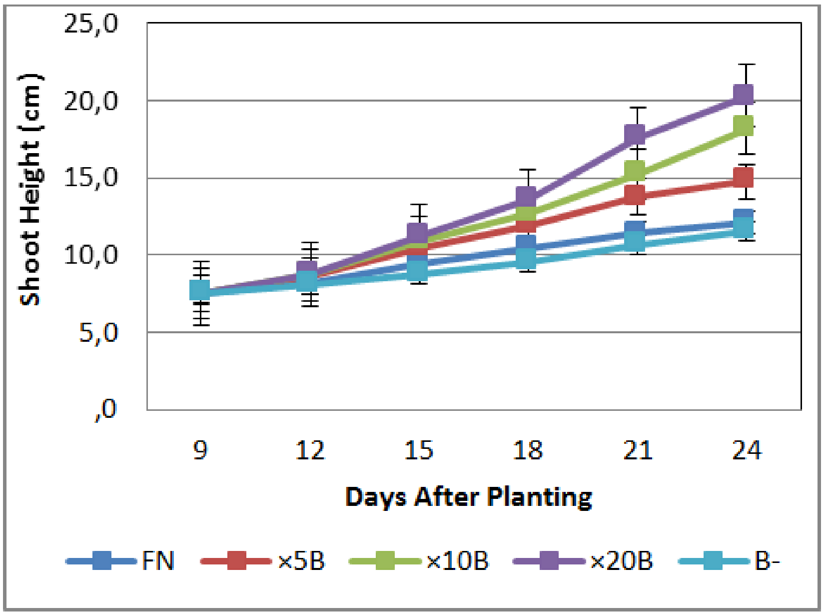

Fig. 1: The Effect of supra-optima concentration and deficiency of boron on the shoot height of Zea mays

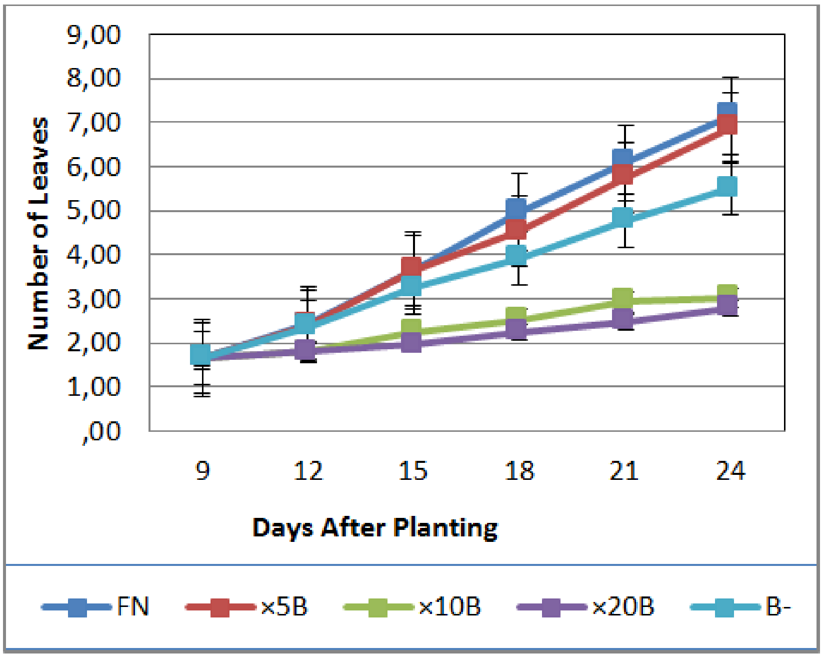

Fig. 2: Variation in the number of leaves on Zea mays seedlings as affected by boron deficiency and toxicity

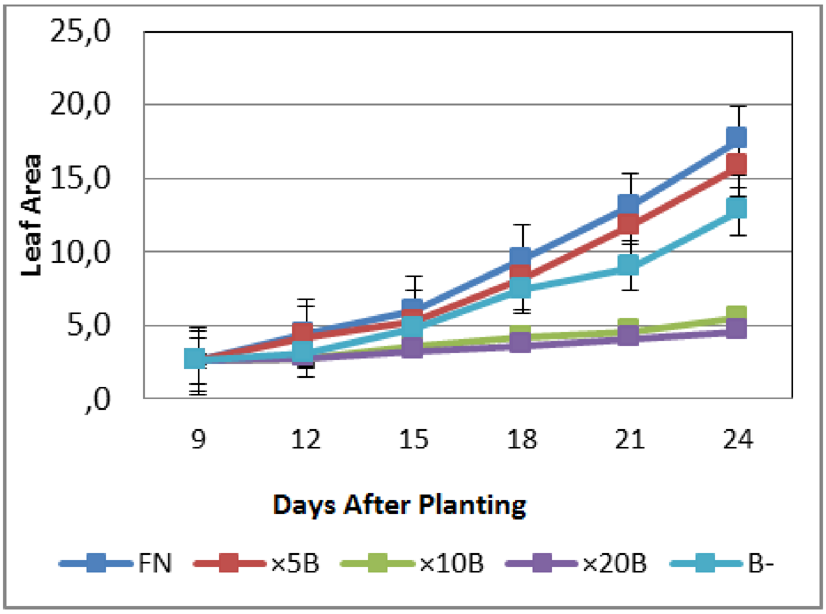

Fig. 3: Leaf area of maize seedlings as affected by the varied doses of boron

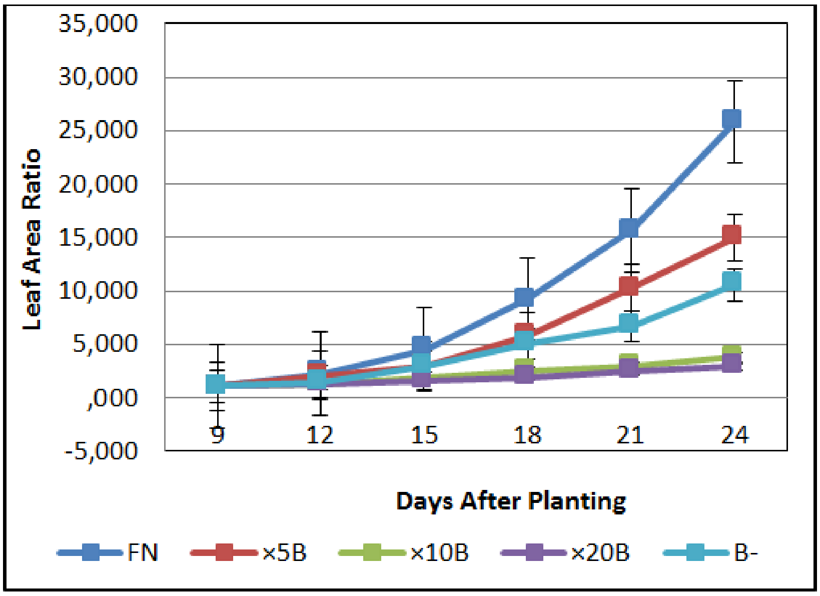

Fig 4: Variation in the leaf area ratio of Zea mays seedlings as affected by boron deficiency and toxicity

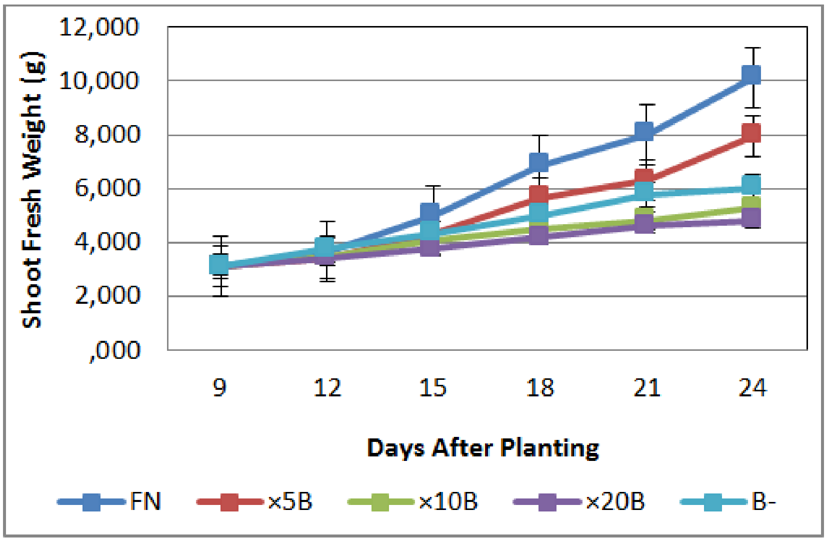

Fig. 5: Time course changes in the shoot fresh weight of Zea mays seedlings subjected to boron nutritional stress

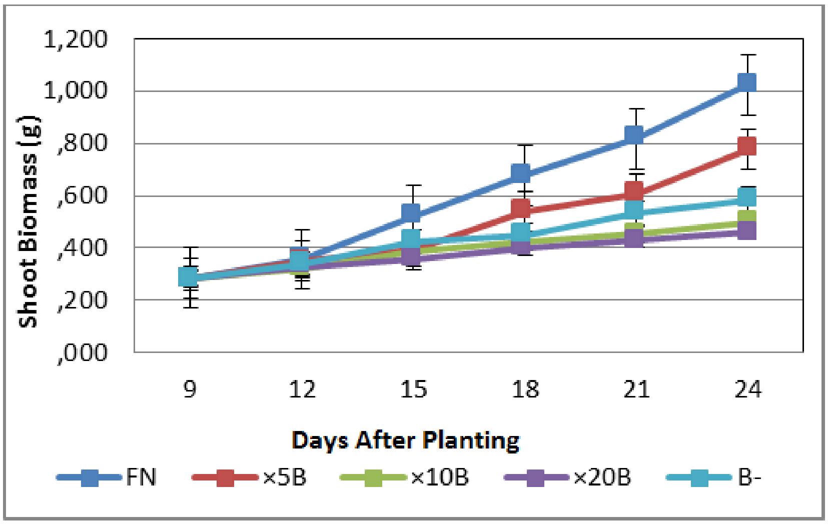

Fig. 6: Time course changes in the shoot biomass of Zea mays seedlings subjected to boron nutritional stress

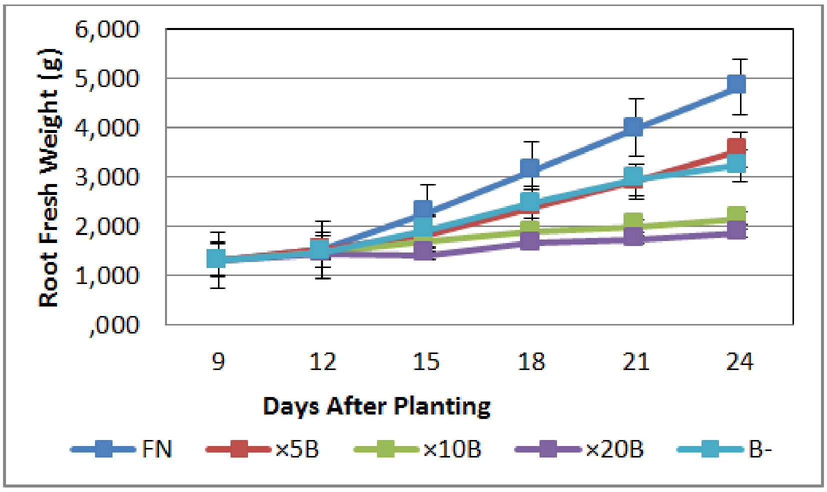

Fig.7: Effect of boron nutritional stress on the root fresh weight of Zea mays seedlings

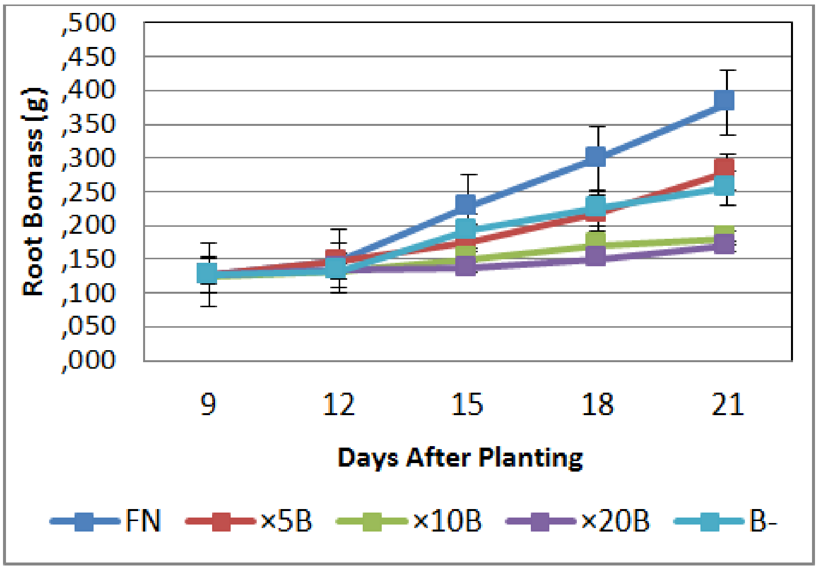

Fig. 8: Effect of boron deficiency and toxicity on the root biomass of Zea mays seedlings

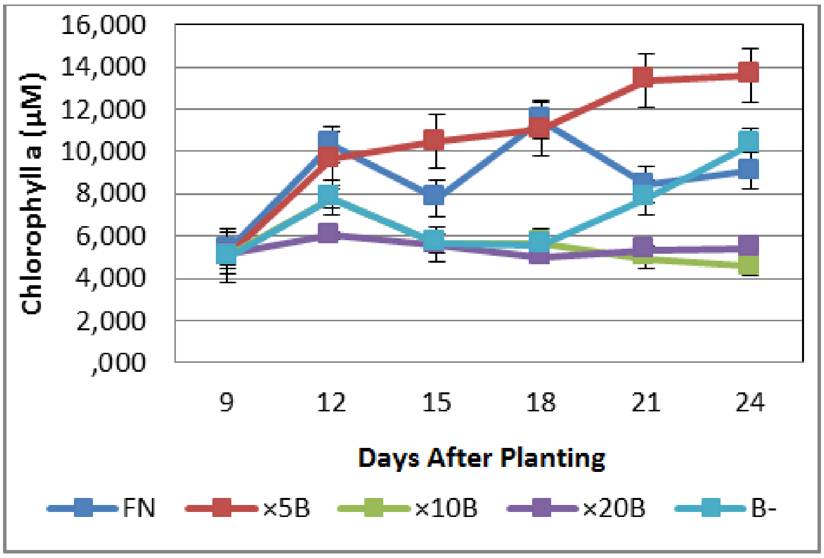

Fig. 9: Time-course accumulation of chlorophyll a in boron nutritional stressed Zea mays

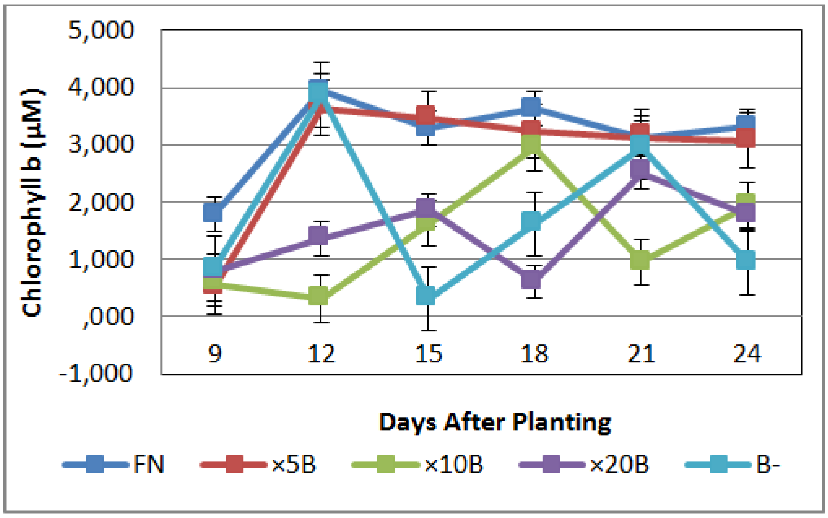

Fig. 10: Time-course accumulation of chlorophyll b in the boron nutritional stressed Zea mays seedlings

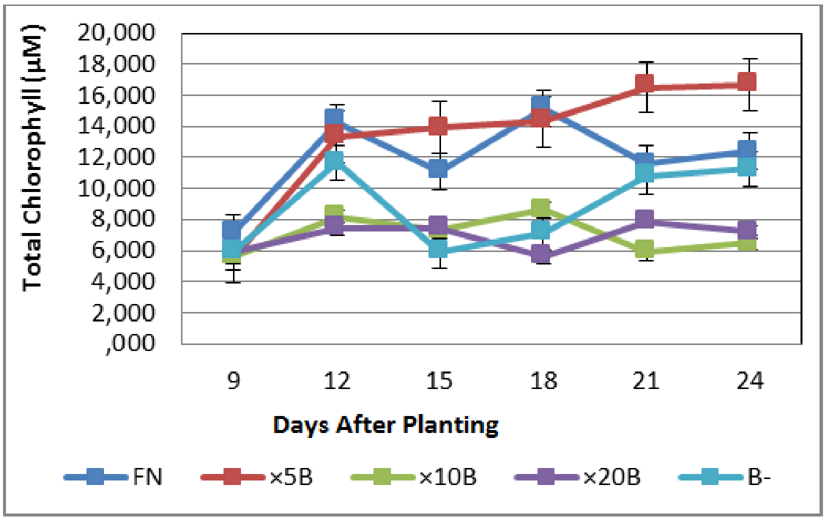

Fig. 11: Time-course accumulation of total chlorophyll in the boron nutritional stressed zea mays seedlings

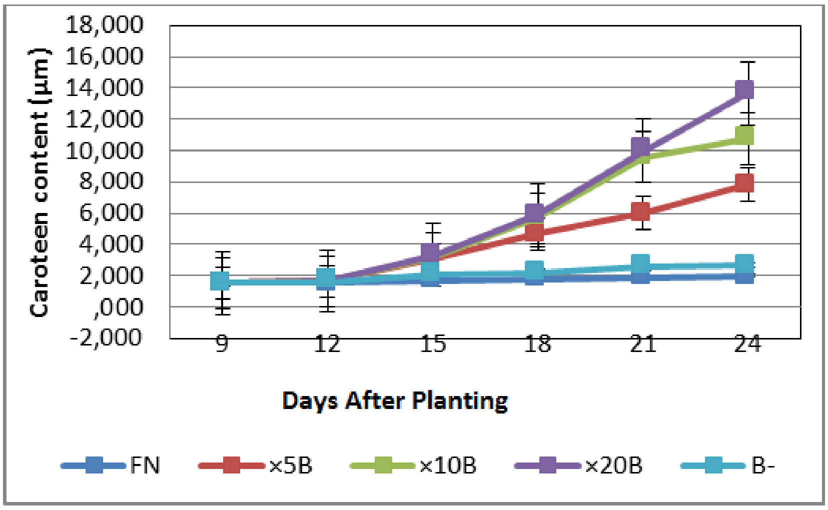

Fig. 12: Variation in the carotene content of Zea mays seedlings as affected by deficiency and toxicity of boron

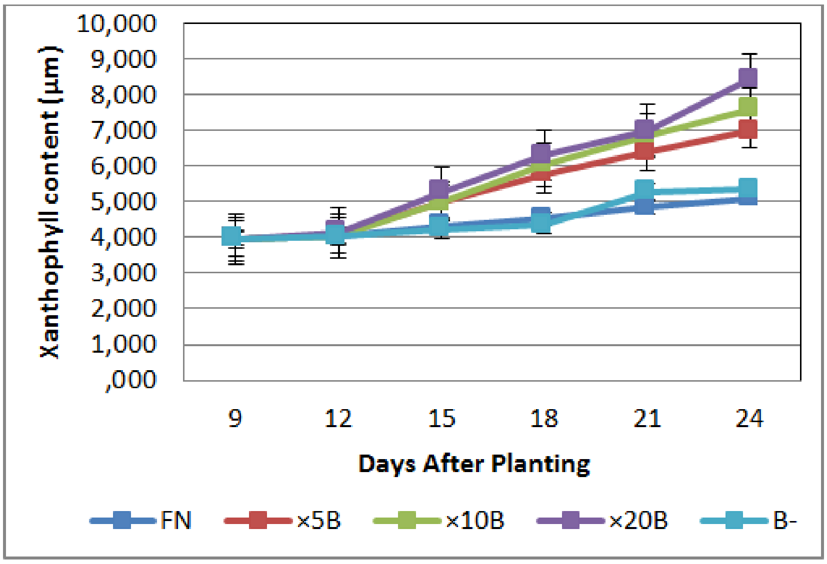

Fig. 13: Variation in the Xanthophylls content of Zea mays seedlings as affected by boron deficiency and toxicity

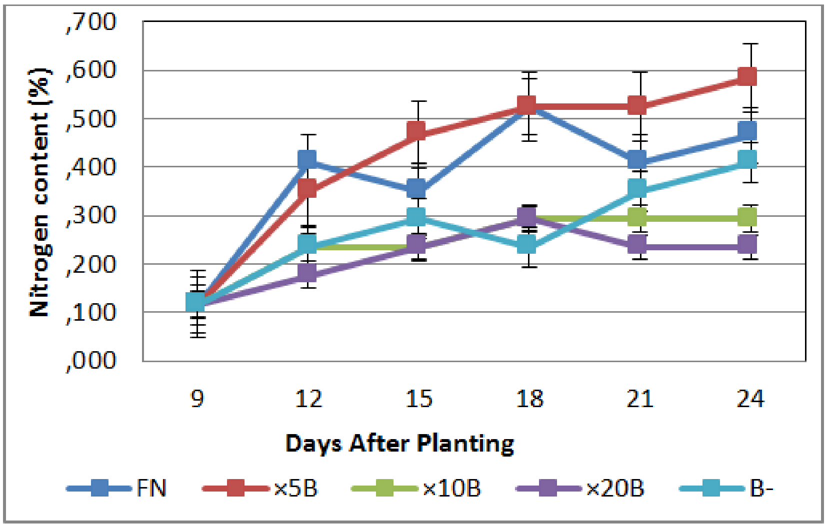

Fig. 14: Effect of deficiency and toxic concentrations of boron on the nitrogen content of Zea mays seedlings

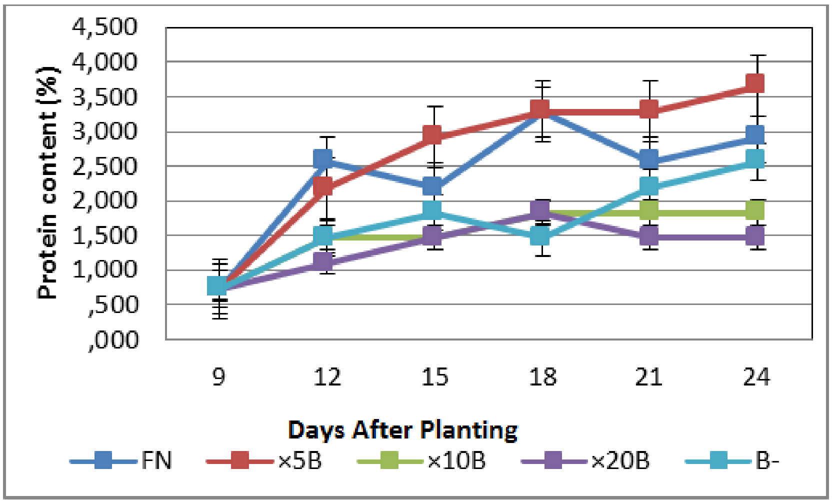

Fig. 15: Time-course accumulation of protein contents in boron nutritional stressed Zea mays

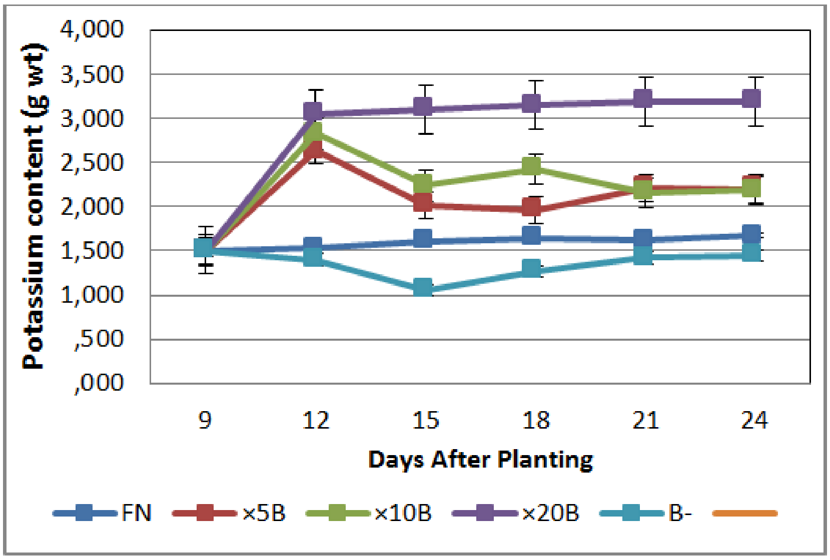

Fig. 16: Variation in the potassium contents of Zea mays seedlings as induced by boron deficiency and toxicity

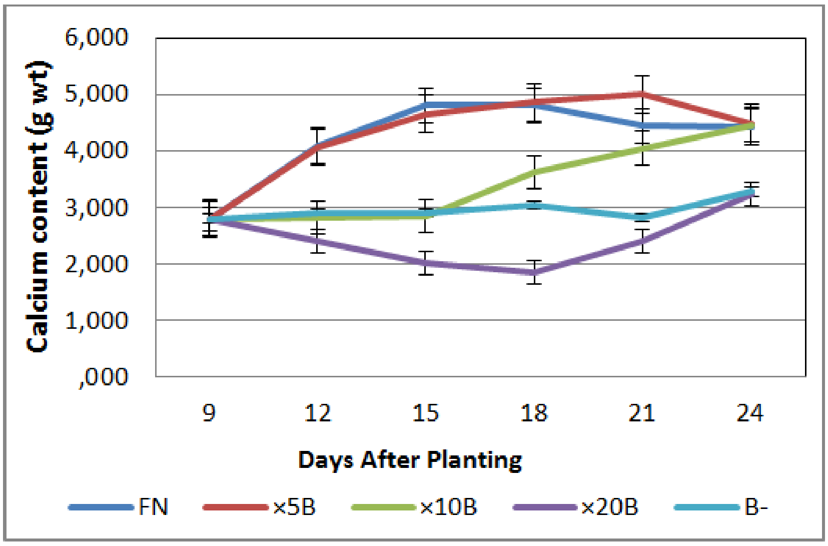

Fig. 17: Effect of boron nutritional stress on the calcium contents of Zea mays seedlings

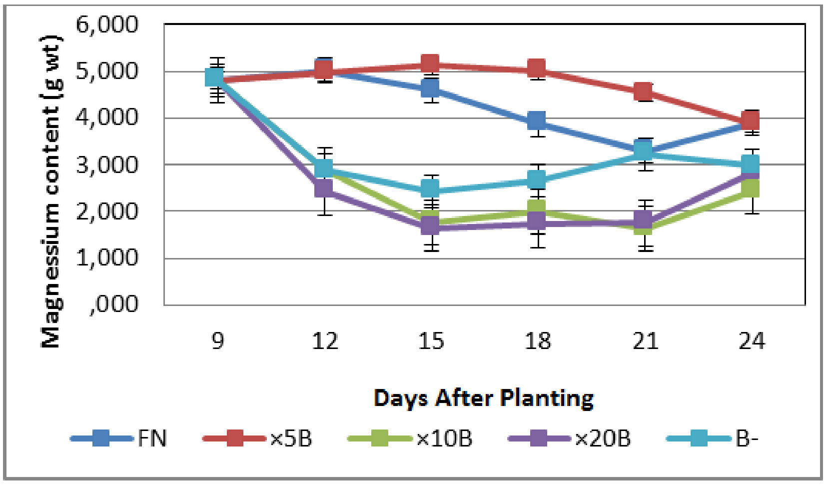

Fig. 18: Effects of varied doses of B on Magnessium concentrations in Zea mays seedlings

5 CONCLUSION

This study had shown that that toxicity of B was more harmful to juvenile maize than its deficiency and presented 3.30 ppm (×10B) concentration of B in the soil as the critical level of toxicity for maize seedlings. The result also showed that B toxicity was more harmful to juvenile maize growth than its deficiency and therefore recommended three different optimal concentrations; 0.33, 1.65 and $\geq$ 3.30 ppm respectively for general growth, chlorophyll and carotenoids synthesis in maize seedlings. The data presented here suggested an under-play physiological role for B in the seedlings leaf formation and development. It also emphasized accumulation of carotenoids and potassium (K) as adaptive mechanisms evolved by the maize seedlings to B-stress tolerance. Further study to validate these hypotheses is therefore recommended.

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