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EFFECTS OF BORON AND SODIUM TOXICITY ON THE GROWTH OF LEAFY AMARANTH (*AMARANTHUS CRUENTUS*)

Adeniran, K.A., *Akpenpuun, T.D., Amodu, M.F. and Samuel, R.T.

[1]Department of Agricultural and Biosystems Engineering, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria.

*akpenpuun.td@unilorin.edu.ng; kadeniran_2003@yahoo.com; mfamodu@yahoo.com; toprich24@gmail.com

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ABSTRACT

The effect of boron and sodium toxicity on the growth of leafy amaranth (Amaranthus cruentus) crop was studied. The study was conducted using a 4 × 2 factorial with completely randomized block design (CRBD) replicated three times. The treatments included: no-boric acid and no-NaCl salts, 5 mM of boric acid and 20mM of NaCl, 15 mM of boric acid and 40 mM of NaCl and 25 mM of Boric acid and 60 mM of NaCl. Interactions between treatments were also considered. Equal amount of water using manual irrigation and the same level of fertilizer were imposed on the treatment using pots. Plant growth parameters such as; plant height, numbers of leaves, leaf area, root length and plant weight were determined and the data was subjected to statistical analysis. The results show that amaranth plant was affected by simultaneous salinity and boron toxicity. Pots treated with 15 mM-boric acid and No NaCl recorded the highest fresh and dry matter accumulation on the seventh week, (52.6 g and 7 g respectively), while treatment 25 mM of Boric acid and 60 mM of NaCl recorded the lowest fresh and dry matter accumulation of 7.8 g and 1.7g respectively for the same period. Plants treated with 25 mM-Boron and 60 mM-NaCl recorded the lowest values of 26.8 cm, 16.1 cm, and 38.5 cm^2 for plant height, root growth and leaf area respectively. The highest plant height and leaf area values of 66.7 cm and 120.2 cm^2 was recorded for plants treated with 15mM of boric acid and No-NaCl during the eight week of growth. The results showed that the low sodium chloride combined with moderate boric acid concentration favoured crop growth and development.

1. INTRODUCTION

Salinity affects plants in different ways such as osmotic effects, specific-ion toxicity and/or nutritional disorders (Lauchi and Epstein, 1990). The extent to which one mechanism affects the plant over the others depends upon many factors including the species, genotype, plant age, ionic strength and composition of the salinizing solution, and the organ in question. Plants undergo characteristic changes from the time salinity stress is imposed until they reach maturity (Munns, 2002).

Boron (B) is a member of the subgroup III of metalloids and has intermediate properties between metals and nonmetals (Marschner, 1995). Boron is essential for plant growth and its availability in soil and irrigation water is an important determinant of agricultural production (Tanaka and Fujiwara, 2007). In soil solution, boron exists primarily as boric acid [$B(OH)_3$], which can be easily leached under high rainfall conditions (Shorrocks, 1997) leading to deficiencies in plant growth. On the contrary, under low rainfall conditions, boron cannot be sufficiently leached and therefore may accumulate to levels that become toxic to plant growth (Reid, 2007).This is common in arid and semiarid regions with high-boron groundwater, where the accumulation of boron in topsoil due to the evaporation of groundwater reaches toxic levels that reduce crop yields (Tanaka and Fujiwara, 2007).

Saline irrigation water contains dissolved sulphates, carbonates, and bicarbonates of calcium and magnesium. While salinity can improve soil structure, it can also negatively affect plant growth and crop yields. Salinity becomes a problem when enough salts accumulate in the root zone and consequently affect plant growth negatively. Excess salts in the root zone hinder plant roots from taking up water from the surrounding soil thereby reducing the amount of water available to the plant. Sodium toxicity has the opposite effect of salinity on soils. The primary physical processes associated with high sodium concentrations are soil dispersion, clay platelet and aggregate swelling. The forces that bind clay particles together are disrupted when too many large sodium ions come between them. When this separation occurs, the clay particles expand, causing swelling and soil dispersion. Soil dispersion causes clay particles to plug soil pores, resulting in reduced soil permeability. When soil is repeatedly wetted and dried and clay dispersion occurs, it then reforms and solidifies into almost cement-like soil with little or no structure (Henderson, 1981). Hence, the aim of this study was to determine the effects of boron and sodium ion toxicity on the growth and yield of leafy amaranth (*Amaranthus cruentus*).

2. MATERIALS AND METHODS

2.1. Study Site

The study was carried out using pots filled with sandy-clay loam soil using the experimental plot of the Department of Agricultural and Biosystems Engineering, University of Ilorin, Ilorin, Nigeria. The study was carried out within the period of 30th April, 2012 to 6th July, 2012. The study site is located on latitude 08° 30'N and longitude 04° 35'E with an elevation of about 340 m above mean sea level. Ilorin falls into the Southern Guinea Savannah Ecological Zone of Nigeria with annual precipitation of about 1300 mm. The rainfall pattern is a bimodal distribution. The rainy season starts around March, with a short dry spell in July. The long dry spell begins in November and ends in March.

2.2. Experimental Layout

The total land area of the site was 22.5 m^2. Rain shelter was constructed using bamboo post tied together with ropes and the roof slanting in one direction. It was covered with transparent polythene to shield crops from rainfall and direct sunlight. The pots were perforated at the bottom so as to allow excess water to drain out. The pots were filled with 10 kg of air-dried loamy-sandy soil. The filled pots were then arranged on the field and spaced 10 cm × 10 cm along and within the row between pot of same treatment and 20 cm when separating various treatment and their replicates. Physical and chemical properties of the soil used in the study were determined.

2.3. Experimental Design

The study was conducted using a 4 x 2 factorial experiment using completely randomized block design replicated thrice. The pots were grouped under four categories of treatment (Figure 1). The categories considered include 1, 2, 3 and 4 treated by supplying the same amount of water using manual irrigation. Treatment 1 served as the control, while treatments 2, 3 and 4 were subjected to varied treatment levels. The treatments were as follows:

1. Control; No-Boron and No-Sodium ion treatment.
2. Minimum toxicity; 5mM of Boric acid and 20mM of NaCl.
3. Moderate toxicity; 15mM of Boric acid and 40mM of NaCl.
4. High toxicity; 25mM of Boric acid and 60mM of NaCl.

Interactions between the treatments were also considered. These treatments were introduced four weeks after germination.

Table 1: Treatments imposed

Treatments	Toxicity Hazard
No-Boron and No-NaCl	Control (No-Hazard)
No-Boron and 20mM-NaCl	Minimum toxicity
No-Boron and 40mM-NaCl	Minimum toxicity
No-Boron and 60mM-NaCl	High toxicity
5mM-Boron and No-NaCl	Little toxicity
5mM-Boron and 20mM-NaCl	Minimum toxicity
5mM –Boron and 40mM-NaCl	High toxicity
5mM-Boron, 60mM-NaCl	Moderately High toxicity
15mM –Boron and No-NaCl	High toxicity
15mM-Boron and 20mMNaCl	High toxicity
15mM-Boron and 40mM-NaCl	High toxicity
15mM-Boron and 60mM-NaCl	Very high toxicity
25mM-Boron and No-NaCl	High toxicity
25mM-Boron and 20mM-NaCl	High toxicity
25mM-Boron and 40mM-NaCl	High toxicity
25mM-Boron and 60mM-NaCl	Very high toxicity

Figure 1: Pot arrangement

2.4. Soil Physical Properties

The bulk density of the soil was determined using the oven dry weight while the moisture content was determined using the gravimetric method (USDA/NRCS 2016). Soil samples were weighed on a balance and oven dried at 105°C for 24 hours until a constant weight was attained and allowed to cool at room temperature. The field capacity of the soil was determined by ponding the soil with water and allowing the excess water to drain into a container for three (3) days with surface evaporation prevented by properly covering the bucket. Soil sample were collected at different depths with an auger.

2.5. Crop Management

A predetermined amount (24 g) of amaranth seed was mixed with 200 g of 2 mm sieved air dried soil and planted by broadcasting. The seed germinated within 4-6 days after planting and thinning was done 2 weeks after planting (three plants per bucket). When the plant height was about 6 cm, NPK (15-15-15) fertilizer was added by adopting the optimal rate of 90 kg/ha as recommended (Ojo, 1998). The amount of fertilizer applied was 2.0 g/plant i.e. 6 g/bucket for the three plants stand. Weeding was done manually by hand picking. Random selection of samples on weekly basis was adopted to determine crop growth parameters such as; plant height, leaf area, root length, number of leaves, and plant weight (fresh and dry matter) for all the treatments.

2.6. Measurement of Crop Growth and Development

The plant height was measured from the surface of the soil on the plant to the terminal bud of the main stem. Leaf area and total numbers of leaves were determined on weekly basis. The root length was measured by gently uprooting the plant from the bucket, rinsing out the soil on the root, and then measuring with the aid of a meter rule. The plant weight was determined by removing the soil particles from its root, gently washing to remove the soil particles completely. The plant was weighed (fresh matter accumulation) and then oven dried at 80^{0}C for 24hr and weighed (dry matter accumulation) on the balance (Dobermann, 2005).

3. RESULTS AND DISCUSSION

3.1. Soil Physical and Weather Conditions

Table 2 shows that the soil contained 74.6% of sand, 23.2% of clay and 2.2% of silt. Using the USDA textural chart for soil classification, the soil is therefore sand-clay loam. The bulk density of the soil was 1.45g/cm^3. The available water needed to bring soil moisture content to field capacity and irrigation frequency were calculated from the moisture content, bulk density and management allowed deficiency (Table 3). Table 4 indicated that high temperatures prevailed during the study with humid conditions in the last three weeks of study.

Table 2: Soil Classification

Soil Property	Percentage (%)
Sand	74.6
Silt	23.2
Clay	2.2
Soil textural class	Sandy-clay loam

Table 3: Available Water

Soil Property	Soil depth		
	0 – 10cm	10 –20cm	20 – 30cm
Average bulk density (g/cm^3)	1.39	1.45	1.51
Available water (%)	27.9	26.9	26.2
Irrigation frequency (days)	7	7	7

The results of the physical properties (Table 2) and the chemical properties (Table 3) of the soil used in the study shows that the low SAR and pH values of the soil conditions before the introduction of the treatments did not affect the toxicity level. Brady and Weil (2002) reported that sandy soils are not affected by the sodium (because of the low clay content).

Table 4: Average meteorological conditions during the period of study

Weeks	Temperatures outside the rain shelter (°C)		Temperatures inside the rain shelter (°C)		Relative Humidity inside the rain shelter (%)	Relative Humidity outside the rain shelter (%)	Rainfall (mm)
	Max	Min	Max	Min			
1	38.3	25.2	32.5	21.6	64.6	69.5	Nil
2	38.4	25.3	32.7	22.0	62.4	67.5	Nil
3	37.8	25.6	31.5	21.7	63.8	68.2	Nil
4	36.7	25.6	31.2	21.8	64.2	68.6	Nil
5	37.4	22.6	31.6	21.6	64.8	69.3	Nil
6	35.5	22.1	30.5	21.2	65.6	69.8	20.5
7	32.7	23.2	30.2	21.1	64.5	71.2	32.6
8	34.8	23.1	31.3	21.4	74.5	76.5	35.4

3.2. Soil Chemical Properties

The results of chemical analysis of the soil sample before imposing the treatments are given in Table 5. Table 5 shows that the soil is acidic, high in organic matter content and had a low sodium adsorption ratio (SAR) of 1.05.

Table 5: Chemical properties of experimental soil

Soil property	Value
pH in water	5.60
pH in KCl	4.75
Organic matter (%)	11.2
Total nitrogen (mL)	1.40
Available phosphorus (mg/kg)	1.83
Sodium absorption ratio (SAR)	1.05
Exchangeable cations (mM/100 g soil)	1.39
Ca^{2+} (mM/100gm of soil)	5.91
Mg^{2+} (mM/100gm of soil)	7.69
Na^{+} (mM/100gm of soil)	2.75
K^{+} (mM/100gm of soil)	2.60

3.3. Plant Growth Parameters

3.3.1. Fresh matter accumulation

Figures 2 and 3 show that initially there was a gradual increase in the weight of both fresh and dry matter accumulation of the experimental crop as the crop grew older, but after week six, a reduction in the weight of the plants was observed. Samples treated with 5 mM-boron and No-NaCl recorded the highest value fresh and dry matter accumulation on the seventh week as 52.6 g and 7 g respectively, while pots treated with 25 mM-boron and 60 mM-NaCl recorded the lowest value of fresh and dry matter accumulation of 7.8 g and 1.7 g respectively for the same period.

Figures 2 and 3 show that little amount of sodium ion is required by the test crop, *Amaranthuscruentus* for its growth, while the test crop can accommodate high boron ion content. This shows that sodium toxicity seriously affected the crop than boron salinity. This result shows why pots treated with 25 mM-boron and 20 mM-NaCl recorded the highest value fresh and dry matter production, while pots treated with 25 mM-boron and 60 mM-NaCl recorded the lowest fresh and dry matter production values.

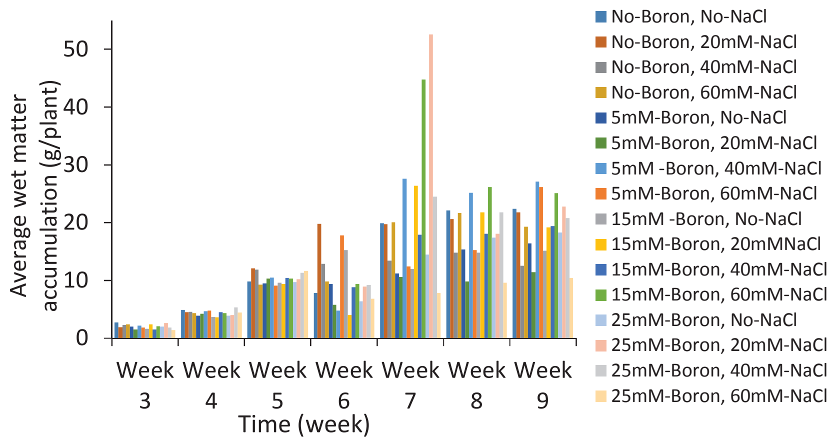

Figure 2: Fresh matter accumulation of plant on weekly basis

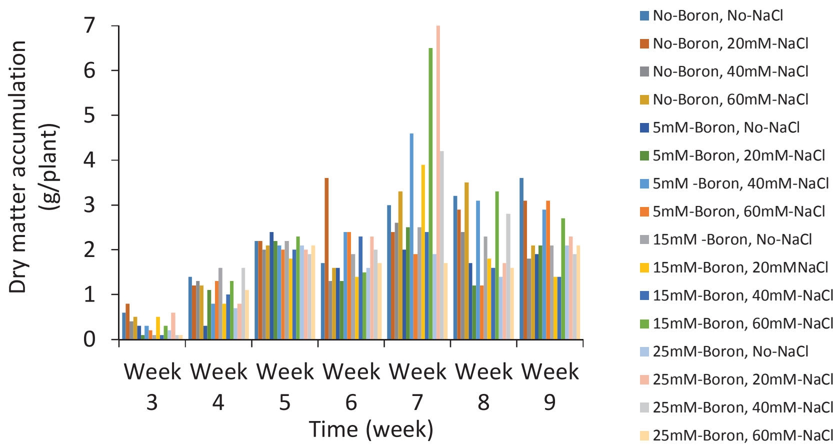

Figure 3: Dry matter accumulation on weekly basis

3.3.2. Effect of toxicity on plant height

From Figure 4, it was observed that during the sixth week, for plant height, pots treated with 25 mM-boron and 60 mM-NaCl recorded the least value of 26.8 cm. The result shows that the plant reacted more to the treatment than others exposed to less lower levels of toxicity. The treatment with 15 mM–boron and No-NaCl produced the highest value 64.2 cm for plant height showing that the low sodium chloride salt neutralised the boric acid level applied. Figure 4 shows that during the ninth week of growth, pots treated with 25 mM-boron and 40 mM-NaCl recorded the lowest value for plant height (39.65 cm).This shows that pots with this treatment were seriously affected by the high NaCl concentration and high boric acid.

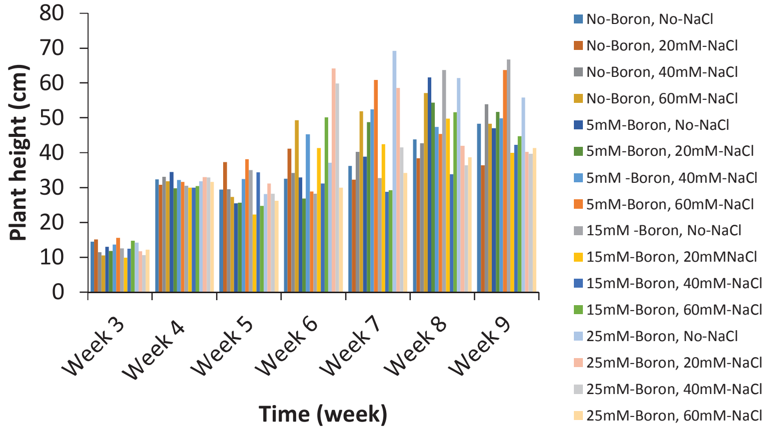

Figure 4: Average plant height

3.3.3. Effect of toxicity on root growth and leaf production

Figure 5 shows that during the eighth week of growth, pots treated with 25 mM-boron and 60 mM-NaCl recorded the lowest value 16.1 cm for root length. This result shows that the treatment seriously affected the growth of the test crop. This is reflected in low value (15 cm) of the number of leaves (Figure 6) and the leaf area, 38.5 cm^2 (Figure 7). The highest value of 23.3 cm was recorded for plants treated with 25 mM-boron and 20 mM-NaCl during the eight week of growth, showing that low sodium chloride application favours root growth. The same treatment, 25 mM-Boron and 20mM-NaCl recorded again very high values (82 cm) in Figure 5cm and 102cm^2 in Figure 7 for number of leaves and leaf area respectively. From Figures 4 to 6, it was observed that during the ninth week, the plant had grown to its third stage of growth and development, hence there was no increase in the plant growth parameters, indicating that; at the final growth stage of the plant life span, the plant height, root length, leaf area remain constant and flowering begins.

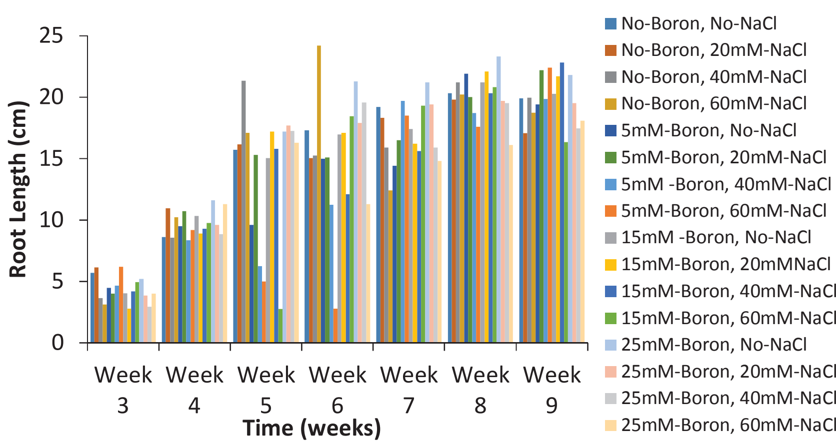

Figure 5: Effects of toxicity on root length

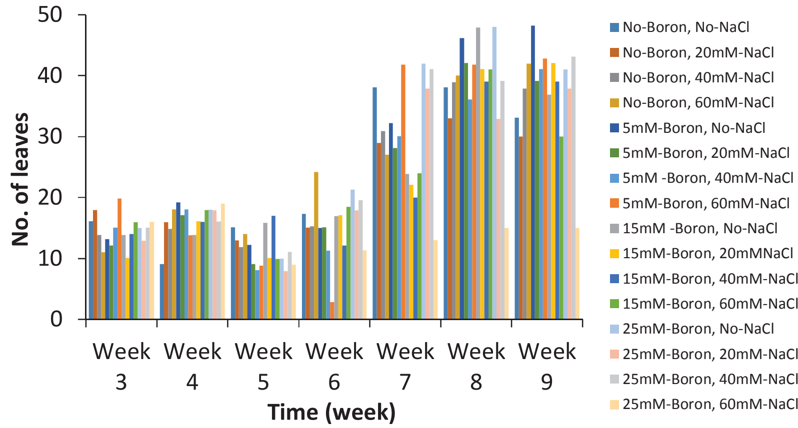

Figure 6: Effects of toxicity on leaf production

Figures 4 to 7 confirmed the results that plant growth indices used such as plant height, number of leaves, leaf area and root length were very high on pots tread with 25mM-boron and No-NaCl and least for those treated with 25 mM-boron and 60 mM-NaCl. Therefore, high NaCl content in the treated pots affected the crop more than high boron ion concentration. Quddus *et al*., (2014) reported that high seed yields were obtained with high concentrations of zinc and boron and low yield for no-zinc and no-boron tests. The high values of the regression coefficient, R^2=0.725 and R^2=0.646 for fresh and dry matter accumulation respectively reveals that the predicted values were close the real values.

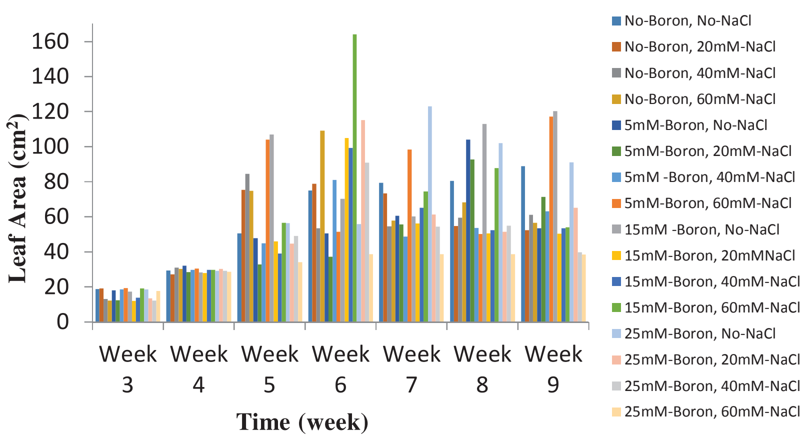

Figure 7: Effects of toxicity on leaf area

3.3.4. Statistical analysis

The statistical result obtained for plant growth parameters at the end of each week within the period of observation from the third week to the ninth week are presented in Tables 6 to 8. Table 6 shows that fresh matter accumulation increased rapidly during the weeks at 1% significant level. The plant grew gradually with respect to the age. Table 7 shows that dry matter accumulation also increased at 1% significant level while the effect of treatment on the

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results obtained was significant at 10% level. These values show that the plant responded to the fertilizer applied than the treatments.

Table 6: Statistical analysis of treatment means on fresh matter accumulation

Source of Variation	SS	Df	MS	F	P-value	F crit
Treatment	647.7357	47	43.1824	1.3905	0.174 ^{ns}	1.8018
Weeks	4469.5755	5	893.9151	28.7854	0.001*	2.3366
Error	2329.0861	235	31.0545			
Total	7446.3974	287				

*significant at 1% level, ns not significant

Table 7: Effect of treatment means-dry matter accumulation of plant

Source of Variation	SS	Df	MS	F	P-value	F crit
Treatment	13.8929	47	0.9262	1.6450	0.0819***	1.8018
Weeks	82.1683	5	16.4337	29.1872	0.001*	2.3366
Error	42.2283	235	0.5630			
Total	138.2896	287				

*significant at 1% level, ***significant 10% level

3.3.5. Predicting fresh and dry matter accumulation

Data presented in Figure 2 was used to predict fresh matter accumulation. Using the t-test as shown in Table 8, the rate of fresh matter production was determined. A predictive equation of the form shown in Equation (1) was developed.

$$Fresh\ matter = -2.579375 + 0.0542*treatment + 3.7351*Time \quad (R^2=0.725) \qquad (1)$$

Table 8: Statistical analysis on growth parameters

Groups	Count	Sum	Average	Variance
Plant height	48	769.80	48.11	75.14
Root Length	48	317.40	19.84	3.91
Number of Leaves	48	604.20	37.76	45.54
Leaf Area	48	1108.75	69.30	552.83
Fresh matter	48	310.40	19.40	25.74
Dry matter	48	38.52	2.41	0.36

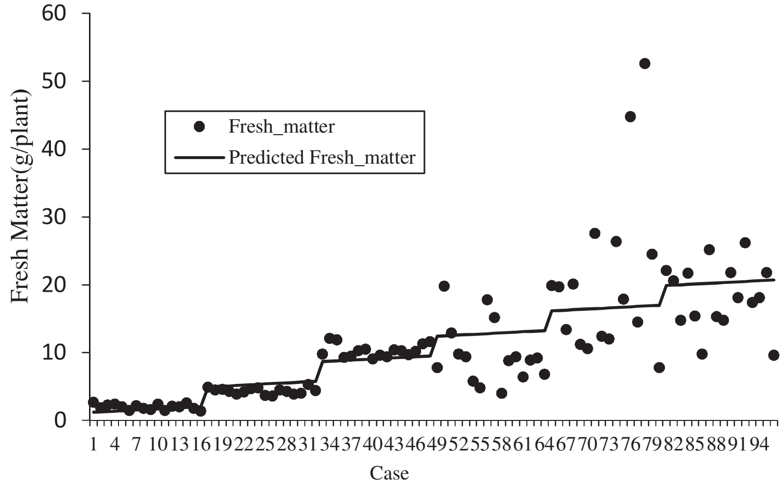

Figure 8: Observed and predicted fresh matter

The values of the predicted fresh matter production using Equation (1) were compared with the ones presented in Figure 2 and the results are shown in Figure 8.
Data presented in Figure 3 were used to predict dry matter production. Using the t-test as shown in Table 8, rate of dry matter production was done. A predictive equation (Equation 2) was developed.

$$Dry\ Matter = 0.2513-0.0020*Treatment + 0.4539*Time \qquad R^2=0.646 \qquad (2)$$

The values of the predicted dry matter production using Equation (2) were compared with the ones presented in Figure 3. The relationship between both set of values is shown in Figure 9.

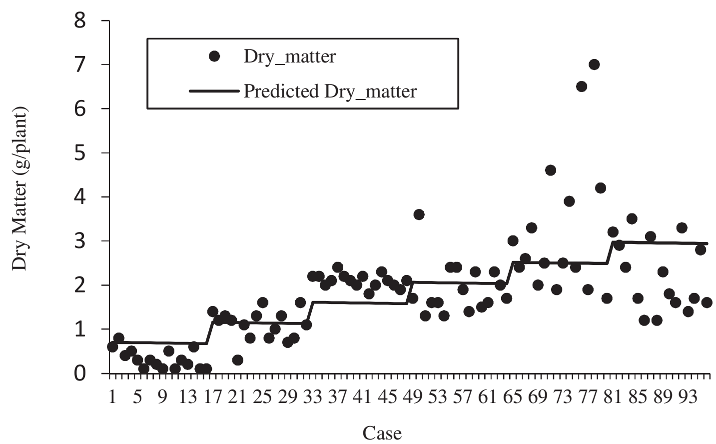

Figure 9: Observed and predicted dry matter

Table 9: Effects of treatment and time on fresh matter accumulation

Measured parameters	P_{left} value	P_{right} value	Std Error	-95%	95%	t Stat
Fresh matter	-2.5794	0.1654	1.8450	-6.2432	1.0845	-1.3980
Treatments	0.0543	0.6917	0.1365	-0.2167	0.3253	0.3978
Period	3.7352	0.0000	0.3684	3.0036	4.4667	10.1392

Table 10: Effect of time on dry matter accumulation of plant

Measured parameters	P_{left} value	P_{right} value	Std Error	-95%	95%	t Stat
Dry matter	0.2513	0.3695	0.2786	-0.3020	0.8045	0.9018
Treatment	-0.0020	0.9225	0.0206	-0.0429	0.0389	-0.0975
Period	0.4539	0.0001	0.0556	0.3435	0.5644	8.1597

The effects of treatment and time on fresh and dry matter accumulation are as shown in Tables 9 and 10. Both Tables show that differences in results of treatments imposed are significant at 95% confidence while changes in the period have no effect.

4. CONCLUSION

From the results of this study, deduction can be made, that amaranth plant growth is very rapid at early stage of life, more sensitive to sodium chloride toxicity than that of boric acid. At week nine, the highest plant height was 66.65 cm for pots treated with 15mM–Boron and No-NaCl and the least plant height of 9.90 cm was obtained for pots treated with 15mM –Boron and No-NaCl at week three. Combined boron toxicity and NaCl salinity caused severe toxic effects on growth. The study shows that availability of boron and sodium chloride in soil and irrigation water is an important determinant of agricultural production.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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