

## Salinity tolerance of two seedling growth stages of *Annona muricata* L. in Port Harcourt, Nigeria

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### ABSTRACT

This study evaluated seedling growth performances of two growth stages (6 and 12 weeks) of *Annona muricata* at different salinity levels - zero (0.00mgNaCl/l), slight (5000mgNaCl/l), medium (9000 mgNaCl/l) and high (14000mgNaCl/l), 4-weeks and 7-weeks after transplanting (WAT). The experiment was laid out in a Completely Randomized Design (CRD) for each of the two seedling growth stages. One-way analysis of variance was used to test for significant difference in growth attributes of each of the seedling growth stage among salinity levels while t-test was used to test for significant difference in each growth attribute between the two seedling stages. Seedling height and collar diameter in 6-weeks old seedlings 4-WAT reduced significantly ( $p < 0.05$ ) between the high and each of the other salinity levels while leaf number varied significantly ( $p < 0.05$ ) between zero salinity and each of the other salinity levels. At 7-WAT, the reduction in height of the 6-weeks old seedlings was not significant ( $p \geq 0.05$ ) among the salinity levels but the reduction in collar diameter and leaf number was significant ( $p < 0.05$ ). Seedling height of the 12-weeks old seedlings 4-WAT was only significant ( $p < 0.05$ ) among the control, medium and high salinity levels while the reduction in collar diameter varied significantly between the control and each of the other salinity levels. The leaf number varied significantly ( $p < 0.05$ ) between the control and each of the other salinity levels while there was no significant difference among the slight, medium and high salinity levels. At 7-WAT, reduction in height and collar diameter of the 12-weeks old seedlings was not significantly different ( $p \geq 0.05$ ) among the control, slight and medium salinity levels while reduction in leaf number varied significantly among them. At 4-WAT, reduction in seedling height and collar diameter was significant ( $p < 0.05$ ) between the 6 and 12 weeks old seedlings at high salinity while leaf number varied significantly ( $p < 0.05$ ) between them at all the salinity levels. Both seedling stages could not survive at the high salinity 7-WAT; however, the 12-weeks old seedlings performed better at the other salinity levels; and generally had higher survival rates (up to 100% at slight salinity level). It is recommended that 12 weeks old seedlings of *A. muricata* be used for planting in agroecosystems with slight saline soil condition.

**Keywords:** *Annona muricata*, agroecosystems, seedling stage, salinity, seedling growth

## INTRODUCTION

*Annona muricata*, is a very important fruit tree that survives in both tropical and subtropical climates especially in lowlands. Its use for various reasons has been reported by several authors; for instance, it has been reported to be a good source of vitamins B and C (Orwa *et al.*, 2009), as a source of traditional medicine for the treatment of various ailments including cancer (Najmuddin *et al.*, 2016), and as a source of income to farmers (Okigbo and Obire, 2009).

The vulnerability of different areas in many countries of the world, including Nigeria, to climate change impacts, is no longer in doubt as evidences abound. Among the evidences include rising temperatures, rising sea levels, extreme weather events that result to drought, stormy and devastating rainfalls, and inundation. Flooding, as a result of rising sea levels, results in salt water intrusion especially in coastal areas.

Salt water intrusion into hitherto productive agroecosystems especially in coastal areas (Chima *et al.*, 2015) remains a challenge to agricultural productivity, with many parts of Nigeria already affected (Mensah *et al.*, 2006; Adeoti *et al.*, 2010 and Obasi and Akudinobi, 2013). In view of the menace of increasing salinity, the ability of plants to tolerate, adapt and thrive in saline soils remains critical for the productivity of agroecosystems (Mensah *et al.*, 2006; Chima *et al.*, 2015), especially in areas with high vulnerability to salt water intrusion.

One of the factors that affect the adaptability of plants to environmental stress is their stage of development. However, this factor is often ignored by the farmers (Jellani *et al.*, 2016). Hence, apart from the identification of plant species that can adapt to increasing salinity, there is also the need to identify the

ideal growth stages in which they can be planted for optimum survival under saline conditions. This study therefore, evaluated the effect of seedling growth stage on salinity tolerance of *Annona muricata* with a view to providing useful information on the influence of growth stage on the adaptability of its seedlings to various saline conditions.

## MATERIALS AND METHODS

### The Study Area

This study was carried out at the Department of Forestry and Wildlife Management experimental nursery, Choba Park, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria. The University of Port Harcourt is located on latitude 4° 53' 14"N through 4° 54' 42"N and longitude 6° 54' 00"E through 6° 55' 50"E (Chima *et al.*, 2015), and has three campuses - Choba, Abuja, and Delta.

### Seed Collection, Processing and Viability Test

Two mature fruits of *A. muricata* were collected from one healthy mother tree growing within the study area. The fruits were depulped to extract the seeds. The floatation method of testing seed viability was used to detect the viable seeds. Seeds that floated after putting them in a container filled with water were regarded as non-viable and discarded while the seeds that did not float were regarded as viable and used for the experiment.

### Experimental Design

The experiment was laid out in a Completely Randomized Design (CRD) for each of the two seedling growth stages (6 and 12 weeks). Seeds were first germinated in a germination tray containing sterilized sharp sand. Young seedlings were later pricked into 23cm x 22cm x 15cm polybags filled with sterilized forest topsoil and

allowed to grow for a period of 6 weeks before the application of treatments (0.00mgNaCl/l (control), 5000mgNaCl/l, 9000mgNaCl/l, and 14000mgNaCl/l) for the first set of the experiment and 12 weeks for the second set of the experiment. A polybag with seedling was considered as an experimental unit and ten polybags with seedlings (ten replicates) were used for each of the four treatments for each set of the experiment. This gave rise to eighty Polybags (forty for each set of the experiments) used for the study. Fifty (50) millilitres of each of the treatments were administered to the respective seedlings early in the morning and late in the evening every other day until a period of three (3) months for each set of the experiment.

#### Data Collection

Data were collected for a period of three months for each set of the experiment. Data collected include:

1. Seedling survival rate
2. Seedling height
3. Leaf production (number of leaves)
4. Seedling collar diameter

Seedling height was measured from the substrate level to the tip of the youngest leaf; stem collar diameter was measured using a vernier caliper at 1cm above the soil surface while leaf production was determined by directly counting the number of leaves. However, Data analysis was done using data collected for the first seven weeks of the application of treatments because 100% mortality of seedlings recorded under the high saline condition at the seventh week of data collection, could not allow the comparison of growth data for all the treatments beyond seven weeks. In addition, data analysis was done using the differences in the growth of the evaluated attributes from the time of transplanting to 4 and 7 weeks after transplanting, for the two sets of the experiment, respectively.

#### Data Analysis

One-way analysis of variance was used to test for significant difference in growth attributes of the different growth stages of *A. muricata* seedlings among the various treatments four weeks and seven weeks after transplanting. The Duncan Multiple Range Test was used for mean separation where significant differences were observed. The Analysis of Variance was performed using Statistical Package for Social Sciences (SPSS).

T-test was used to test for significant difference in the measured seedling growth attributes of the two seedling growth stages (6 and 12 weeks) at each salinity level (treatment) four weeks and seven weeks after transplanting. Survival rate (SR) was computed for the two growth stages under the different salinity levels (0.00 mgNaCl/l, 5000 mgNaCl/l, 9000 mgNaCl/l and 14000 mgNaCl/l) four weeks and seven weeks after transplanting using the formula below:

$$SR = \frac{n}{N} \times 100$$

Where: SR = Survival rate

n = number of seedling that survived and  
N = number of seedlings transplanted.

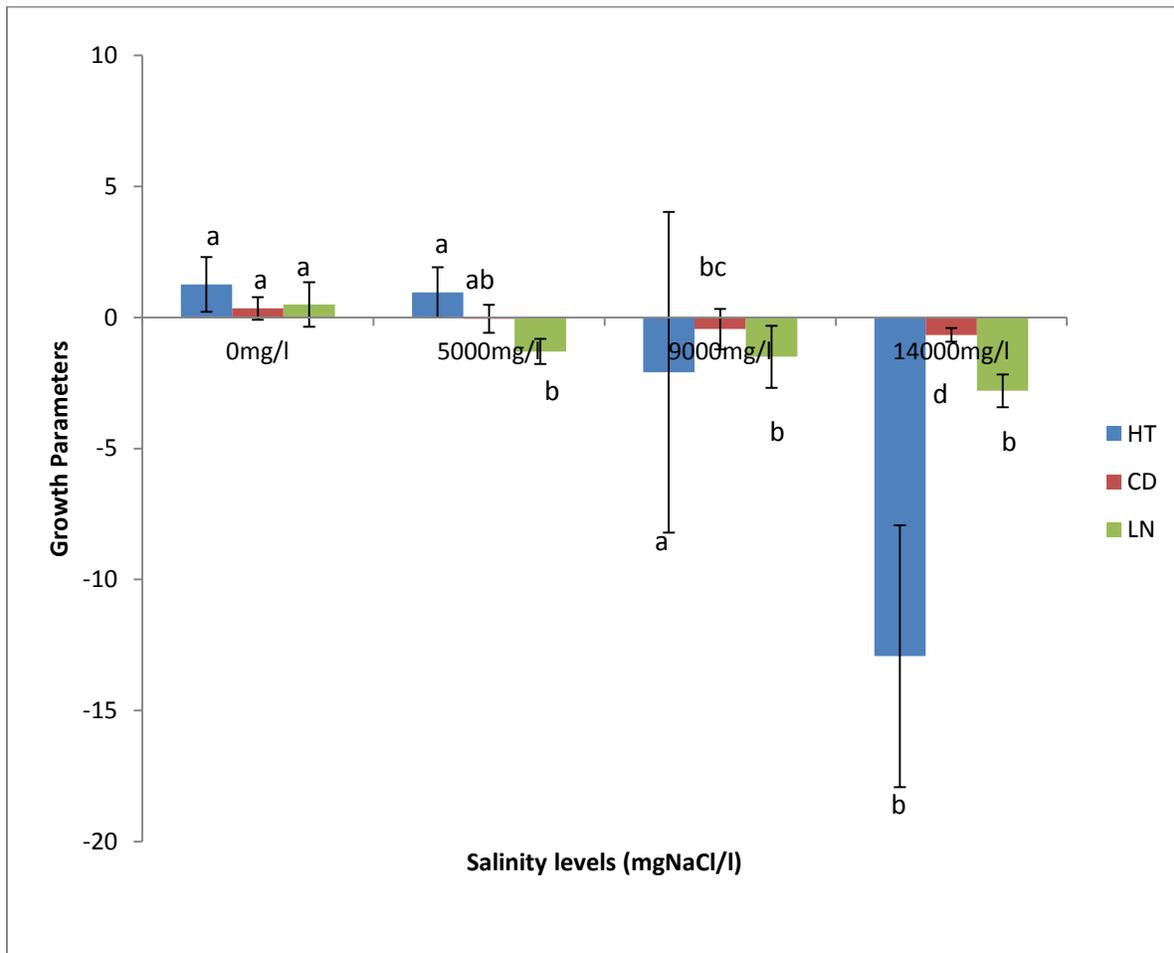
#### RESULTS

##### Effects of different salinity levels on the growth of 6 weeks old seedlings of *A. muricata*

The growth performance of the 6 weeks old seedlings of *A. muricata* at different salinity levels, four weeks after transplanting, is shown in Figure 1. Seedling height, collar diameter and leaf number decreased under the slight, medium and high salinity levels. However, the reduction in both seedling height and collar diameter was only significant between the highly saline condition and each of the other salinity levels (zero/control, slight and medium).

The reduction in leaf number varied significantly between the control and each of

the slight, medium and high salinity levels.



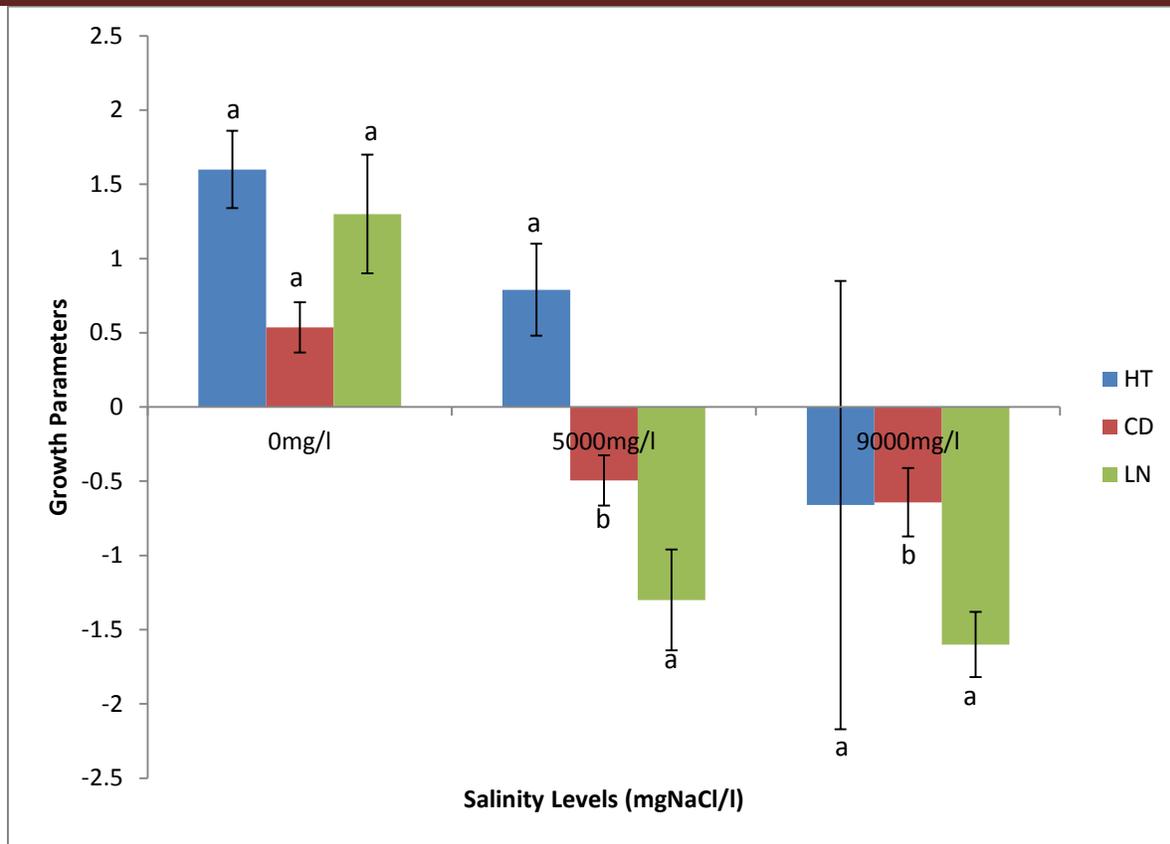
**Fig. 1.** Growth performance of the six weeks old seedlings of *A. muricata* four weeks after transplanting

HT = Height (cm); CD = Collar diameter (mm); LN = Leaf number

Means with the same alphabet for each growth attribute are not significantly different ( $p \geq 0.05$ )

The growth performance of the 6 weeks old seedlings of *A. muricata* at different salinity levels seven weeks after transplanting is shown in Figure 2. At seven weeks after planting, all the six weeks old seedlings subjected to high saline condition died. Seedling height, collar diameter and leaf number decreased under the slight and medium salinity levels. However, the

reduction in height under the various salinity levels was not significantly different from each other. The reduction in collar diameter was significantly different between the control (0.00mgNaCl/l) and each of the other salinity levels while there was no significant difference between the slight and the medium salinity levels. The reduction in leaf number was not significantly different.



**Fig. 2.** Growth performance of six weeks old seedlings of *A. muricata* seven weeks after transplanting

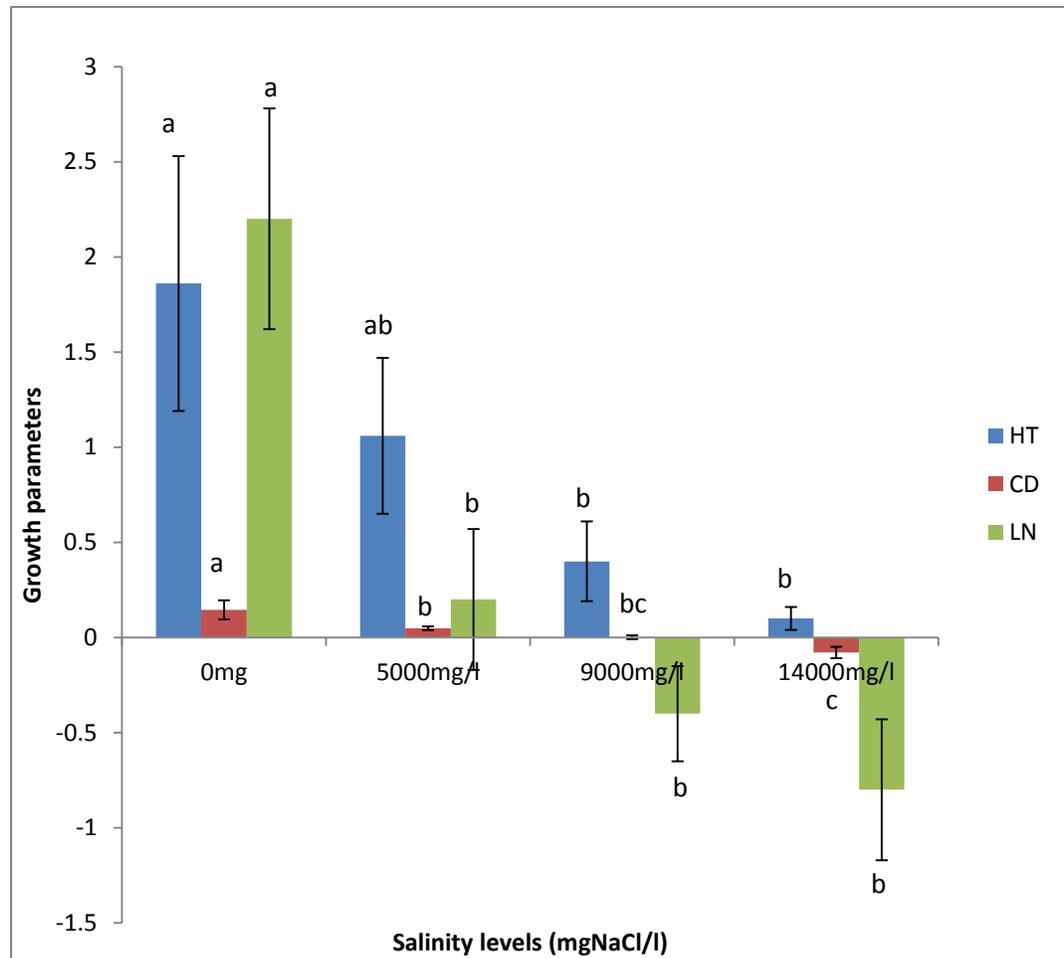
HT = Height (cm); CD = Collar diameter (mm); LN = Leaf number

Means with the same alphabet for each growth attribute are not significantly different ( $p \geq 0.05$ )

**Effects of different salinity levels on the growth of 12 weeks old seedlings of *A. muricata***

The growth performance of the 12 weeks old seedlings of *A. muricata* at different salinity levels four weeks after transplanting is shown in Figure 3. Seedling height, collar diameter and leaf number decreased under the different salinity levels. However, reduction in height was only significant between the control (0.00 mgNaCl/l), and each of the medium and high salinity levels while there was no significant difference between the control and slight salinity level.

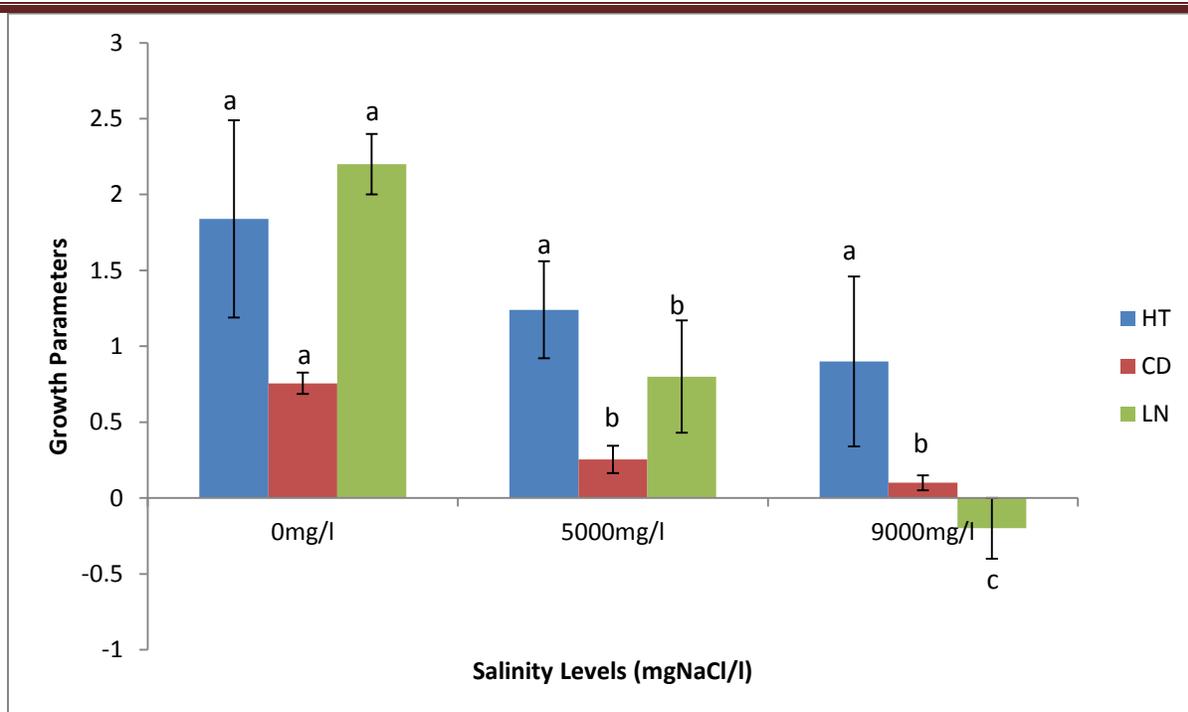
The reduction in collar diameter varied significantly between the control (0.00 mgNaCl/l) and each of the other salinity levels. There was a significant difference in collar diameter reduction between slight and high salinity levels while reduction in collar diameter under medium salinity level was not significantly different from the slight and the high salinity levels. The reduction in leaf number varied significantly between the control (0.00 mgNaCl/l) and each of the other salinity levels while there was no significant difference between each pair of the salinity levels –slight, medium and high.



**Fig. 3.** Growth performance of twelve weeks old seedlings of *A. muricata* four weeks after transplanting  
 HT = Height (cm); CD = Collar diameter (mm); LN = Leaf number  
 Means with the same alphabet for each growth attribute are not significantly different ( $p \geq 0.05$ )

The growth performance of 12 weeks old seedlings of *A. muricata* at different salinity levels seven weeks after transplanting is shown in Figure 4. At seven weeks after transplanting, all the twelve weeks old seedlings subjected to high saline condition also died. Seedling height, collar diameter and leaf number decreased under slight and medium salinity levels. There was no significant difference in seedling height reduction among the control, slight and

medium salinity levels. The reduction in collar diameter was not significantly different between slight and medium salinity levels but it varied significantly between the control and each of the slight/medium salinity levels. Reduction in leaf number varied significantly between the control and each of slight and medium salinity levels, and also significantly between the slight and medium salinity levels.



**Fig. 4.** Growth performance of twelve weeks old seedlings of *A. muricata* seven weeks after transplanting

HT = Height (cm); CD = Collar diameter (mm); LN = Leaf number

Means with the same alphabet for each growth attribute are not significantly different ( $p \geq 0.05$ )

**Effect of growth stage on the salinity tolerance of *A. muricata* seedlings**

The performances of the two growth stages (6 and 12 weeks old) of *A. muricata* seedlings, four weeks after transplanting at different salinity levels is presented in Table 1. Growth in seedling total height and collar diameter was higher in twelve weeks old

seedlings in all the salinity levels although differences in seedling total height and collar diameter were only significant at high salinity level. However, the two growth stages (6 and 12 weeks old) varied significantly in number of leaves for all the salinity levels with the twelve weeks old seedlings performing better.

**Table 1:** T-test showing performances of different growth stages of *Annona muricata* seedlings at various salinity levels four weeks after transplanting

|                     | 0 mg/l NaCl            |                        | 5000 mg NaCl/l          |                        | 9000 mg/l NaCl          |                         | 14000 mg/l NaCl         |                         |
|---------------------|------------------------|------------------------|-------------------------|------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
|                     | 6 weeks                | 12 weeks               | 6 weeks                 | 12 weeks               | 6 weeks                 | 12 weeks                | 6 weeks                 | 12 weeks                |
| Total height(cm)    | 1.26±0.33 <sup>a</sup> | 1.86±0.46 <sup>a</sup> | 0.96±0.30 <sup>a</sup>  | 1.06±0.27 <sup>a</sup> | -2.09±1.94 <sup>a</sup> | 0.40±0.14 <sup>a</sup>  | -12.9±1.58 <sup>a</sup> | 0.10±0.04 <sup>b</sup>  |
| Collar diameter(mm) | 0.35±0.14 <sup>a</sup> | 0.15±0.03 <sup>a</sup> | -0.05±0.17 <sup>a</sup> | 0.05±0.01 <sup>a</sup> | -0.44±0.24 <sup>a</sup> | 0.00±0.01 <sup>a</sup>  | -0.67±0.08 <sup>a</sup> | -0.08±0.02 <sup>b</sup> |
| No. of leaves       | 0.50±0.27 <sup>a</sup> | 2.20±0.39 <sup>b</sup> | -1.30±0.15 <sup>a</sup> | 0.20±0.25 <sup>b</sup> | -1.50±0.37 <sup>a</sup> | -0.40±0.16 <sup>b</sup> | -2.78±0.22 <sup>a</sup> | -0.89±0.26 <sup>b</sup> |

Means on the same row with same alphabet for each salinity level are not significantly different ( $p \geq 0.05$ )

The growth performance of the two seedling stages (6 and 12 weeks old) of *A. muricata* seedlings seven weeks after transplanting at different salinity levels is presented in Table

2. There was no significant difference in seedling total height for both growth stages under the different salinity levels, although, the twelve weeks old seedlings generally

had higher mean values. However, the two growth stages varied significantly in collar diameter at slight and medium salinity

levels. Leaf number for the two seedling growth stages varied significantly at the control, slight and medium salinity levels.

**Table 2:** T-test showing performances of different growth stages of *Annona muricata* seedlings at various salinity levels seven weeks after transplanting

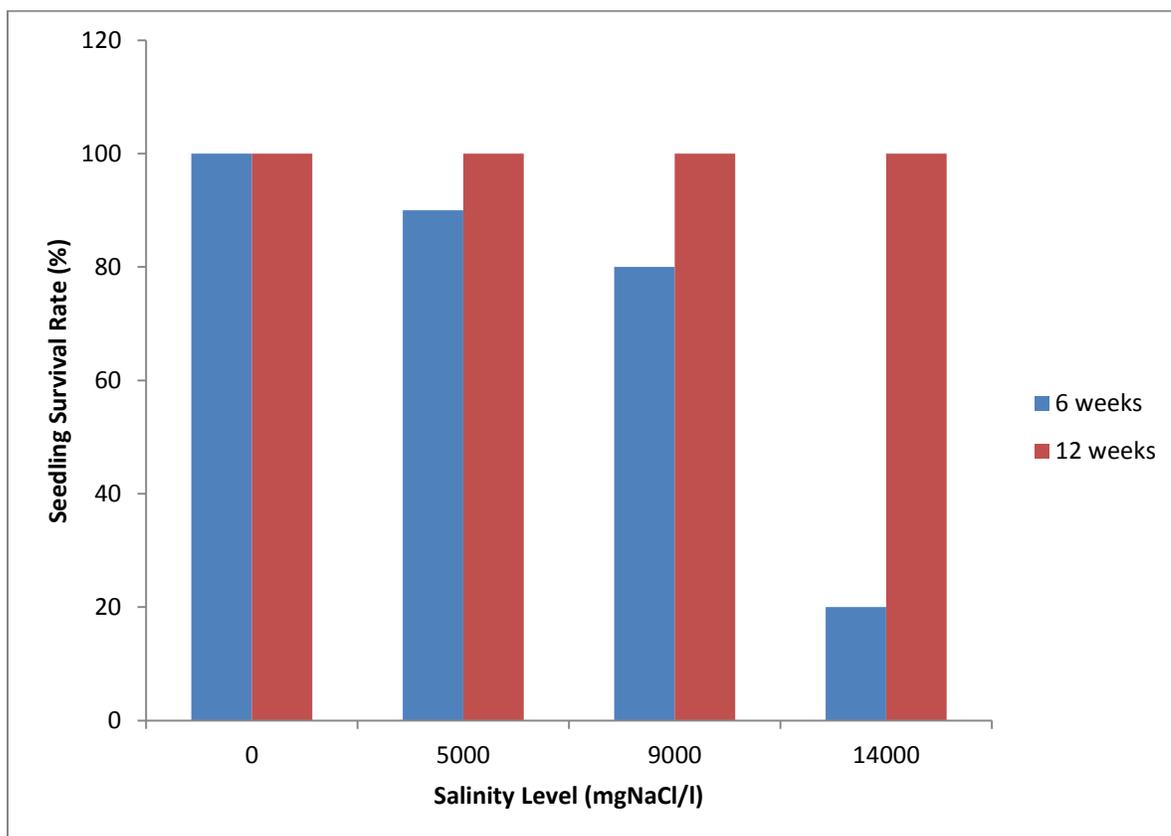
|                     | 0 mg/l NaCl            |                        | 5000 mg/l NaCl          |                        | 9000 mg/l NaCl          |                         |
|---------------------|------------------------|------------------------|-------------------------|------------------------|-------------------------|-------------------------|
|                     | 6 weeks                | 12 weeks               | 6 weeks                 | 12 weeks               | 6 weeks                 | 12 weeks                |
| Total height(cm)    | 1.60±0.26 <sup>a</sup> | 1.84±0.44 <sup>a</sup> | 0.79±0.31 <sup>a</sup>  | 1.24±0.21 <sup>a</sup> | -0.66±1.51 <sup>a</sup> | 0.90±0.37 <sup>a</sup>  |
| Collar diameter(mm) | 0.54±0.17 <sup>a</sup> | 0.76±0.05 <sup>a</sup> | -0.50±0.17 <sup>a</sup> | 0.25±0.06 <sup>b</sup> | -0.64±0.23 <sup>a</sup> | 0.06±0.03 <sup>b</sup>  |
| No. of leaves       | 1.30±0.40 <sup>a</sup> | 2.20±0.13 <sup>b</sup> | -1.30±0.34 <sup>a</sup> | 0.80±0.25 <sup>b</sup> | -1.60±0.22 <sup>a</sup> | -0.20±0.13 <sup>b</sup> |

Means on the same row with same alphabet for each salinity level are not significantly different ( $p \geq 0.05$ )

**Effect of seedling growth stage on the survival rate of *A. muricata* at different salinity levels**

The survival rate of the two growth stages (6 and 12 weeks) of *A. muricata* seedlings under the different salinity levels four weeks after transplanting revealed that the 12

weeks old seedlings had 100% survival rate in all the salinity levels. However, the six weeks old seedlings had 100% survival rate in the control while survival rate varied from 90% at slight to 80% at the medium and as low as 20% at high salinity levels (Figure 5).



**Fig. 5.** Seedling survival rate at four weeks after transplanting

The survival rate of the two growth stages of *A. muricata* under the different salinity levels seven weeks after transplanting is presented in Figure 6. The twelve weeks old seedlings seven weeks after transplanting had 100% survival rate in the control and slight salinity levels, 60% survival rate in

the medium salinity level and 0% survival rate in the high salinity level. The six weeks old seedlings had 90% survival rate in the control, 70% and 60% survival rates in the slight and medium salinity levels, respectively and 0% survival rate in the high salinity level.

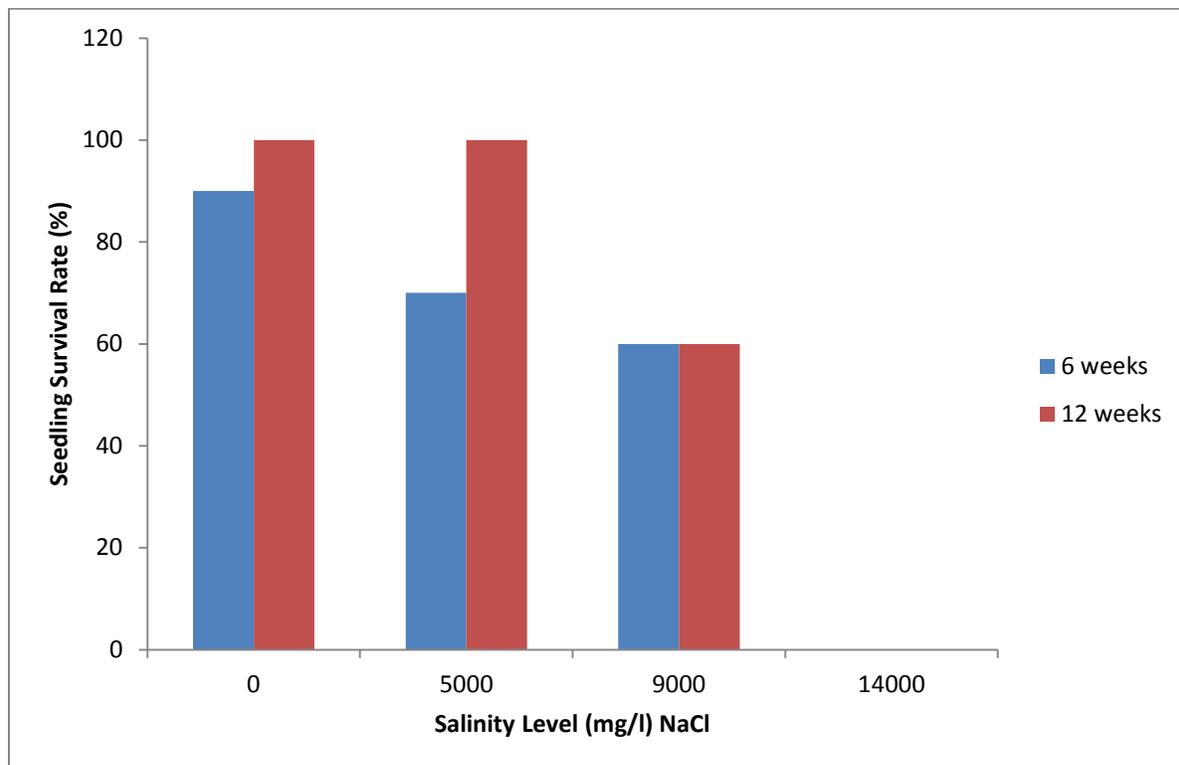


Fig. 6. Seedling survival rate at seven weeks after transplanting

## DISCUSSION

The reduction in the various growth attributes of *A. muricata* seedlings with increasing salinity could be attributed to physiological stress. Ashrafa and Harrisb (2004) observed that stress due to salinity in soil or water could be detrimental to crop productivity while Nawaz *et al.* (2010) attributed reduction in plants growth following increased salinity to the inability of plants to absorb water due to salinity stress.

The height of seedlings is considered a useful criterion for understanding the effect of salinity at seedling establishment stage (Unal *et al.*, 2001; Chima *et al.*, 2015). The reduction in seedling heights at different salinity levels when compared with the control (0 mg/l NaCl) underscores the detrimental effect of salt stress. Several other studies such as Dantas *et al.* (2005), Mathur *et al.* (2006), Jamil *et al.* (2007), and Taffouo *et al.* (2010) have also reported reduction in height in other plant species due to increase in salinity levels.

The inability of plants to absorb water and loss of moisture in response to high salt concentration of the soil, may also lead to a reduction in the collar diameter. In this study, collar diameter of seedlings under different salinity levels equally decreased as compared to the control for the two seedling growth stages that were considered. The significant reduction in collar diameter between the two growth stages at high salinity (four weeks after transplanting) and also at different salinity levels (except control) seven weeks after transplanting, clearly indicates that both period of exposure to salinity stress and the level of salinity negatively affects collar diameter increment in *A. muricata* seedlings. Isarat et al. (2018) had reported that increasing salinity levels reduced collar diameter in *Azadirachta indica* (Neem) seedlings with the highest reduction caused by the highest salinity level, as was also observed in this study. Other authors like Jaleel et al. (2008), and Abdel-Azim and Ahmed (2009) have also reported reduction in collar diameter due to salinity stress, in other species.

As observed by Munns (2002), the detrimental effects of salinity may progress and become obvious with time especially in more sensitive species and may culminate in the yellowing or death of older leaves. That was also observed in this study. In addition, the older leaves of *A. muricata* dropped first before the younger ones as observed by Munns (2002). Similar observations of leaf abscission due to salinity stress had been reported by Mehari et al. (2015) on *Acacia nilotica* and *A. tortilis*. The reduction in number of leaves of *A. muricata* seedlings increased with increased salinity for the two growth stages, corroborating the findings of Nazari (2007), Ghavami and Ramin (2008), Nouman et al. (2012), and Isarat et al. (2018). Higher accumulation of NaCl in the cell walls and cytoplasm of the leaves could

be the reason for the decrease in number of leaves at increasing salinity (Tabatabaie and Nazari, 2007; Isarat et al., 2018). The rates of leaf abscission in *A. muricata* seedlings at different salinity levels decreased with increase in seedling age, with the 12 weeks old seedlings showing greater resilience to leaf abscission when compared to the 6 weeks old seedlings.

Increase in salinity adversely affected the survival rates of the two growth stages of *A. muricata* seedlings but the 12 weeks old seedlings had higher survival rates than the six weeks old seedlings. Liu et al. (2010), Ahmad et al. (2014) and Louhaichi (2015) had equally observed a decrease in seedling survival rates with increased salinity levels on *Helianthus annuus*, cotton and *Artemisia herba-alba*, respectively.

The ability of plants to develop adaptability and acclimatise with age (Saiema et al., 2013) has been identified as the reason for higher tolerance to higher salinity with increase plants' ages. This explains why the 12 weeks old seedlings survived better than the 6 weeks old seedling both at four and seven weeks after transplanting. Although all the seedlings at both growth stages died at the high salinity level, the 12 weeks old seedlings maintained 100% survival rate under the slight salinity level at 7 weeks and as well throughout the three-month period of the study.

## CONCLUSION

Saline conditions significantly reduced growth in *A. muricata* seedlings and the rate of reduction increased with an increase in the salinity level. Increase in age from 6 to 12 weeks increased the survival rate of *A. muricata* seedlings (with 100% survival recorded at slight salinity level), and also significantly increased seedling growth under different salinity levels. It is therefore,

recommended that twelve (12) weeks old seedlings of *A. muricata* be integrated in agroecosystems with slight saline soil condition. Further studies should be conducted with older seedlings to ascertain if they will perform better at higher salinity levels.

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