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EFFECT OF WATER STRESS ON THE SHOOT MORPHOLOGY AND ROOT ARCHITECTURE OF *Azadirachtaindica* A. Juss. SEEDLING UNDER NURSERY CONDITION

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ABSTRACT

An experiment was conducted from March to October 2015 to find out the responses of shoot morphology and root architecture of *Azadirachtaindica* (neem) seedlings to water stress at the agroforestry and environment research field of Hajee Mohammad Danesh Science and Technology University. There were four treatments; namely- 100% watering, 50% watering, 25% watering and no water (control). Results showed that shoot height of neem seedlings increased after transplanting in the field i.e. from 3 to 6 months of transplantation in different water regimes. After six months of transplantation, the highest shoot height (45.33 cm) was recorded in 100 % watering regime and the lowest (31.0 cm) was found in control. Root collar diameter also varied up to different water levels. Number of branches, sturdiness ratio and central root length showed similar trend of results. In case of biomass allocation, shoot and root dry biomass of neem seedlings increased in all the treatments compared to control. Highest shoot dry biomass was recorded in 50% water level followed by 100 % water level and the lowest was found in 25 % level and control watering regimes. Similar result was found in total dry biomass. In case of root dry biomass, shoot-root ratio and quality index varied insignificant after six months of transplantation. The number of first order lateral roots (FOLRs) recorded insignificant among the watering treatments after 3, 4 and 6 months but it increased after 4 to 6 months in all the water levels except water stress (control) condition. Though mean diameter of FOLRs increased except control but this diameter varied insignificantly over time. The mean length of FOLRs after 3 months varied insignificantly but it varied significantly after 4 and 6 months. Finally, after six months, the full stressed seedlings showed highest length of FOLR(s) and the 100% watering regime recorded lowest length. Increasing the length of FOLRs with the sacrifice of their diameter might be the adaptive mechanism of neem seedlings in water stress condition. Survival rate of neem seedlings was not changed in 100% and 50% watering regimes over time but it decreased sharply in stressed seedlings due to water stress condition after 6 months. Considering the overall results, it can be concluded that neem seedlings can be established to combat desertification with ensuring at least 50% additional water supply at their early stages.

Key words: First order later roots,neem, root architecture, shoot morphology,water stress,

INTRODUCTION

Water stress has a great impact on the physiological process such as plant respiration, plant nutrition, seed germination, dormancy, stomata function, transpiration and so on (Atkinson et al. 2003; Massonnet*et al.* 2007). Plant responses to water stress are usually screened on the level of selected physiological parameters such as water potential, relative water content, stomatal reactions, photosynthesis and osmotic adjustment which have been proven to be good indicators of drought in several studies (Atkinson et al. 2003; Wang 1992; Pretorius 2003). However, the tolerance conditions in which plants grow from the moment of planting might affect their morphology and physiological response.

Bangladesh is an agro-based riverine countrybut many rivers become dry in the winter season (Rana*et al.* 2007). The production of various types of crops is extremely dependent on the amount of the adequate rainfall timely. Adequate rainfall is neither too little nor too much needed for successful agroforestry in Bangladesh. About 80% of the annual rainfall over Bangladesh occurs only during the monsoon. Debnath*et al.* (1995) studied the rainfall characteristics and probabilistic rainfall extremes in Bangladesh during the post-monsoon and early winter season. While Bangladesh is generally unknown for its water scarcity problems, water scarcity is faced in the southwestern and northwestern regions of the country during the dry winter season.

Due to the extreme weather conditions, root systems may not start to grow well initially. As a result, leaf

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flashing may not occur properly. Though plants cannot control environmental temperature, they develop different mechanisms that help to tolerate and survive them in adverse condition (McKersie and Leshem 1994). Some species survive by adopting morphological (Gindaba*et al.* 2004; Aspelmeier and Leuschner 2006) and physiological changes (Lambers*et al.* 1998; Xu and Baldocchi 2003; Aranda et al. 2005; Gindaba et al. 2005) in them. In severe water stressed condition, Gindaba et al. (2004) observed that three deciduous oak species discarded their leaves and reduced leaf area.

For quickly root growth, a seedling mainly depends on its root system activities at the time of out-planting. For bare-root seedlings, minimum damage of rooting system is desirable to plant in the field because their performance can be determined by the root system morphology (Kormanik 1986; Schultz and Thompson 1990). To use bare-root seedlings, sufficient root systems along with large first order lateral roots (FOLR) that grow from the central root should be ensured (Thompson and Schultz 1995). In many excavations, it was observed that these FOLR remained there and new roots grew from them (Thompson and Schultz 1995).

Azadirachtaindica (A. Juss) is a medium to large, evergreen, deep-rooted tree which belongs to the Meliaceae family. It is native to Indian sub-continent. It is an important multipurpose social-forestry species moderately tolerant to salinity stress (Biswas et al. 2002; Subapriya et al. 2005). The tree has the adaptability to a wide range of climatic, topographic and edaphic factors. It thrives well in dry, stony shallow soils and even on soils having hard clay pan, at a shallow depth. Neem tree requires little water and plenty of sunlight for its survival (Sateesh 1998). Though many research works were conducted on the water stress and growth of plant but very little information is available on the effect of water stress of neem seedlings in container condition. Considering the above facts and phenomena this research was conducted to find out the effect of different water regimes on the shoot morphology, root architecture, biomass allocation and the survival rate of neem seedlings.

MATERIALS AND METHODS

The experiment was conducted in polybag (size 9”×6”) in the research field of Agroforestry and Environment research farm of Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period of July to August. The experimental site was characterized by tropical climate with heavy rainfall from July to August and scanty rainfall in the rest period of the year. About 80% to 90% rainfall is received between June to September. The remaining 10% to 20% rainfall is received during November to April (Zeni *et al.* 2015).

The experiment was conducted in polybags of size 9″x6″ and placed in the field. Neem was selected for its availability and importance. Neem seedlings were collected from government forest nursery of Dinajpur. The experiment was laid out in a Randomized Complete Block Design (RCBD) with four treatments because of field condition. There were three replications for each treatment. The treatments were: Treatment 1 (T_1): (100% water) Well-watered - Regular spot irrigation was applied. Regular watering meaning that the soil was maintained at field capacity (Kaushal and Aussenac 1989). Treatment 2 (T_2): (50% water) 50% water means watering the plants on 1st, 3rd, 5th day and so on upto 6 months.Treatment 3 (T_3): (25% water) 25% water means watering the plants on 1st, 4th, 7th day and so on upto 6 months. Treatment 4 (T_4): control condition (no additional water is applied). Before applying the water stress treatment, initial data of the transplanted seedlings were recorded in April 2015.

Shoot height was measured from the root collar to the base of terminal bud of the living shoot tip (Jacobs *et al*. 2005; Haase 2007). In case of dried/dead shoot, dead part was excluded from the top (Dey and Parker 1997). Root collar diameter was measured by a diameter caliper, 5 mm above the root collar of all the seedlings with an accuracy of 1 and 0.1 mm respectively (Tsakaldimi 2006). For multi-stemmed seedlings, the root collar diameters were calculated Abede (1994) and Stewart and Salazar (1992) according to the formula of :

$$d = \sqrt{d^2{}_1 + d_2{}^2 + ... + d_n{}^2}$$

Where, d = calculated mean diameter and d_1, d_2. . . d_n are the diameters of the 1,2,…n-stems.

The lengths of undercut taproots were then measured from the root collar. First-order lateral roots (diameter ≥ 1 mm) that are equal or greater than 1 mm at junction with taproot were counted and measured (Dey and Parker 1997). Diameters of FOLR were taken at the junction of central roots. The FOLRs whose diameters were less than 1 mm were excluded. Based on the shoot height and collar diameter, measurement of the sturdiness index (shoot height /diameter) was calculated for each seedling (Thompson 1985).

For biomass measurement, the seedlings were divided into two parts: shoot and root system. Both parts were oven-dried at 70°C (Royo *et al*. 2001) for 80 hours until they reached in a constant weight. shoot and root weights were recorded with an electric balance. Then total dry weight (g) was calculated. The root to shoot ratio was calculated by the root and shoot dry weights. The seedling quality index (QI) was calculated using the following equation (Dickson *et al*. 1960):

$$\text{Quality index} = \frac{\text{TotalBiomass(g)}}{\text{Height(cm)}/\text{diameter(mm)} + \text{shootbiomass(g)}/\text{rootbiomass(g)}}$$

Survival rate (%) was taken during data collection. The equality of means of all variables was tested for stress (control) and well-watered treatments. All statistical analyses were carried out using SPSS 19. The significance of relationships was evaluated at $P<0.05$.

RESULTS AND DISCUSSIONS

Morphology after three (3) months: Shoot height of *Azadirachtaindica* seedlings increased from their initial height but they varied insignificantly in different water levels after 3 months of transplantation. Same trends were recorded in case of root collar diameter and sturdiness ratio (Table 1). On the other hand, number of branches varied significantly. Highest number of branches was found in T1 (13.22 cm) followed by T2 (8.88 cm), T3 (7.44 cm) and lowest number of branches was recorded in T4 (6.78 cm). Maximum central root length (29.67 cm) was found in T1 followed by T3 (18.00 cm) and T2 (15.33 cm) where the minimum central root length (12.00 cm) was found in treatment T4. Due to moisture variation, container-grown seedlings may perform differently in the field (Roy et al., 2001).

Morphology after four (4) months*:* Shoot height varied significantly in different water levels after 4 month of transplantation (Table 2). Treatment T_2 (50% water) showed significantly the highest shoot height (33.79 cm) followed by the T_1 (33.25 cm). On the other hand, the treatment T_3 (25% water) and T_4 (control) were recorded comparatively lower shoot height of 26.47 cm and 26.87 cm) respectively. Root collar diameter also varied significantly among the treatments. In case of root collar diameter, T_1 (5.63 cm), T_2 (5.57 cm) and T_3 (5.07 cm) showed significantly the highest root collar diameter than T_4 (3.75). The number of branches also varied significantly in different treatments. Treatment T_1 (10.92) and T_2 (10.57) showed much higher number of branches than treatments T_3 (5.07 cm) but the minimum number of branches was found in treatment T_4 (2.60 cm). Same trend was recorded in case of sturdiness where the maximum sturdiness found in T_4 (9.47 cm). Treatment T_2 (35.33 cm) and T_1 (35.33 cm) showed lengthier central root than the treatment T_3 (28.00 cm) but the minimum length of central root found in treatment T_4 (17.33 cm).

Morphology after six (6) months*:*Shoot height varied insignificantly among the treatments after transplantation in the polybags (Table 3). Higher shoot height was found in treatment T_1 (45.33 cm) and T_2 (44.67 cm) than in treatment T_3 (31.00 cm). Treatment T_4 showed comparatively lower shoot height (36.00 cm) than $T_1$and $T_2$with superior quality. Root collar diameter varied significantly among all the treatments. Root collar diameter of T_1 and T_2 was statistically similar but the quality of T_2 is inferior to T_1. In case of number of branches the treatments T_1, T_2 and T_3 were significantly similar but the number of T_2 (9.00) was greater than T_1 (7.00) and T_3 (7.33). Same trend was recorded in case of sturdiness where all of the four treatments showed almost similar results. In terms of central root length, treatment T_2 showed the maximum length (29.00 cm) followed by T_3 (27.67 cm), T_1 (26.33 cm) and T_4 (24.00 cm).

The present results showed that the shoot height of neem seedlings increased after transplanting in the field i.e. from 3 to 6 months of transplantation in different water stress levels. The highest shoot height (45.33 cm) was found in 100% water treatment and the lowest (31.00 cm) was found in control after six months of transplantation. The shoot height varied over time among different treatments due to the effect of water stress. Similar results were found by Dhillon*et al*. (1995) and Sain*et al*. (2001) in maize. They reported that water stress decreased plant height. In case of root collar diameter, three months to six months of transplantation in polybag the root collar diameter increased over time and varied among different water stress levels. This effect may be due to the vegetative development of the plants that were hampered by non-irrigated condition (Galbiatti 2004). The highest root collar diameter (5.67 mm) was found in 100% water level after six months of transplantation and the lowest (3.90 mm) was recorded in control i.e. full water stress (3.90 mm). On the other hand, number of branches, sturdiness, central root length of neem seedlings from 3 to 6 months of transplantation, increased and varied in different treatments due to different water stress. This result is in accordance with the findings of Palled *et al*. (1985) where they reported that the number of branches per plant increased due to irrigation in black gram plant.

Biomass after three (3) months: After 3 months of seedlings transplantation, shoot and root dry biomass increased from their initial value but they did not vary significantly among the different treatments of water levels. As a result, total biomass, shoot-root ratio and quality index were found statistically similar in different treatments (Table 4).

Biomass after four (4) months: After 4 months of transplantation, seedling's above ground biomass varied significantly due to water stress in neem seedlings. Shoot dry biomass per plant was recorded highest in T_1 (0.08 g) followed by T_2 (0.07 g), T_3 (0.06 g) and lowest value was found in T_4 (0.04 g) treatments (Table 5). On the other hand, root dry biomass, shoot-root ratio and quality index did not vary significantly among the treatments. Overall, total dry biomass was highest in both T_1 (0.13 g) and T_2 (0.13 g) followed by T_3 (0.12 g) where lowest dry biomass in T_4 (0.07 g) treatment (Table 5).

Biomass after six (6) months: At six months' stage of transplantation only shoot dry biomass varied significantly due to water stress. Highest shoot dry biomass was recorded for treatment T_2 (0.08 g) followed by T_1 (0.07 g) and T_4 (0.05 g) where the lowest amount of shoot dry biomass (0.04 g) was found for treatment T_3. Same trend was observed in case of total biomass. Other parameters like root dry biomass, shoot- root ratio and quality index did not vary significantly at $p<0.05$ (Table 6).

From the results, the shoot dry biomass of neem seedlings increased over time in all the treatments after 3 to 6 months of transplantation. Same result was found for root dry biomass, total dry biomass, shoot-root ratio and quality index as well. Hasan*et al*. (2004) conducted a research in wheat varieties and found that shoot dry biomass increased significantly with increasing the temperature.

Number of FOLRs: Effect of water stress on the number of first order lateral roots ($FLOR_S$) after 3, 4 and 6 months of transplantation has shown in figure 1. After 3 months of transplantation, the number of $FOLR(_S)$ did not vary among the treatments. Though number of FOLR(s) increased from 3 to 4 months and at 6 months, they showed not significant among the different treatments (Figure1).

Diameter of FOLRs: Effect of water stress on the diameter of first order lateral root ($FLOR_S$) after 3, 4 and 6 months of transplantation has been shown in Figure 2. After 3 months of transplantation, diameter of FOLR(s) did not vary significantly among the treatments. After 4 and 6 months of transplantation diameter of FOLRs among the treatments did not vary significantly with each other and they were found statistically same in a same time period (Figure 2).

Length of FOLRs: Effect of water stress on the length of first order lateral roots ($FOLR_S$) after 3, 4 and 6 months of transplantation has shown in figure 3. After 3 months of transplantation, the length of FOLRs did not vary significantly with each other. In 4 months of transplantation the length of FOLR(s) varied significantly among all the treatments (Figure 3). The length of FOLRs was highest (22.9 cm) in T_2, T_3 and T_4 but the growth was lower (15.4 cm and 18.5 cm) in T_3 and T4 respectively than T_2. After 6 months of transplantation, the length of FOLRs did not vary significantly among the treatments. The length of FOLR is lower (15.8 cm) in T_2 than the other treatments. The highest (19.8 cm) length of FOLR(s) found in T_3 followed by T_4 (17.9 cm) and T_1 (16.3 cm).

The number of first order lateral root (FOLRs) did not vary among the watering treatments after 3, 4 and 6 months but increased after 4 to 6 months than their earlier number in all the water levels except water stress (control) condition. Though mean diameter of FOLRs increased except control but this diameter did not vary significantly over time. Thompson and Schultz (1995) recorded more number of FOLR, better growth and survival. Due to moisture stress, some newly grown FOLR might be dead in the hot summer months in our study. The number of diameter of FOLR were class-wise analyzed and showed that in the lower diameter classes of watered seedlings of all species, number of FOLR was more than the higher diameter classes. It is because of in well-watered condition, newly grown roots increased more by getting regular watering but in stressed treatment, newly grown FOLR dried more due to shortage of soil moisture. So, a positive relationship was observed between numbers of FOLRs with the seedling survival i.e. more number of FOLRs with more survival capability in well irrigated seedlings and less number of FOLR with less survival capability was found in the field. Rahman*et al*. (2015) also found similar trend of FOLRs and survival rate in some Mediterranean tree species.

Survival percentage: Survival rate of neem seedlings after 3, 4 and 6 months of transplantation is presented in table 7. After 3 months there was no mortality in T_1 and T_2 but mortality was recorded in T_3 and T_4. After 4 and 6 months' survival rate was same in T_1 and T_2 but it decreased to 20% in T_4 due to water stress condition (Table 7). As there was natural rainfall in the study area, control seedlings did not die completely.

There was no seedling mortality after 3 months but mortality was recorded after 4 and 6 months of transplantation of neem seedlings. Though after 3 months mean length of FOLR did not vary significantly but it varied after 4 and 6 months. After 6 months, the full stressed seedlings showed highest length of FOLR(s) and the 100% watering regime showed lowest length of FOLRs. Increasing trend of the length of FOLRs with the sacrifice of their diameter might be the adaptive mechanism of neem seedlings in water stress condition. Survival rate of neem seedlings did not change in 100% and 50% watering regimes over time but it was decreased sharply in T_4 followed by T_3 due water stressed condition after 6 months. This finding is in agreement with the findings of Rahman*et al.* (2013).

Table 1. Effect of water regimes on the morphological characteristics (mean±SE) of *Azadirachtaindica*seedlings after three (3) months of transplantation

Treatments	Shoot height (cm)*	Root Collar diameter (mm)	Number of branches	Sturdiness	Central Root Length (cm)
T_1	24.11±2.04	4.77±0.21	13.22a±1.27	5.02±0.31	29.67±5.36
T_2	25.65±1.21	4.88±0.23	8.88b±0.59	5.32±0.23	15.33±7.33
T_3	23.94±1.65	5.04±0.31	7.44b±0.83	4.59±0.34	18.00±6.03
T_4	21.94±1.13	4.65±0.21	6.78b±0.48	4.34±0.14	12.00±1.15
Level of significance	ns	ns	ns	ns	ns

*Here, ns= Not significant at P≤0.05

Table 2. Effect of water regimes on the morphological characteristics (mean±SE) of *Azadirachtaindica* seedlings after four (4) months of transplantation

Treatments	Shoot height (cm)	Root Collar diameter (mm)	Number of branches	Sturdiness	Central Root (cm)
T_1	33.25a±2.17	5.63a±0.15	10.92a±1.40	5.59±0.68	33.33±5.93
T_2	33.79a±2.00	5.57a±.025	10.57a±0.75	5.47±0.52	35.33±2.85
T_3	26.47b±2.22	5.18a±0.32	5.07b±0.88	5.02±0.51	28.00±2.89
T_4	26.87b±2.06	3.75b±0.20	2.60b±0.65	9.47±3.18	17.33±10.40

In a column figures having similar letter(s) do not differ significantly and figure bearing different letters differ significantly by Duncan Multiple Range Test at P≤0.05

Table 3. Effect of water regimes on the morphological characteristics (mean±SE) of *Azadirachtaindica* seedlings after six (6) months of transplantation

Treatments	Shoot height (cm)	Root collar diameter (mm)	Number of branches	Sturdiness	Central root length (cm)
T_1	45.33a±2.91	5.67a±0.34	7.00ab±0.58	8.00a±0.12	26.33a±1.86
T_2	44.67a±0.88	5.27ab±0.13	9.00a±3.06	8.49a±0.30	29.00a±3.51
T_3	31.00b±1.00	4.13bc±0.13	7.33ab±3.33	7.52a±0.41	27.67a±1.45
T_4	36.00ab±5.69	3.90c±0.59	0.00b±0.00	9.32a±0.99	24.00a±3.21

In a column figures having similar letter(s) do not differ significantly and figure bearing different letters differ significantly by Duncan Multiple Range Test at P≤0.05

Table 4. Biomass allocation and quality index of *Azadirachtaindica* seedlings at different treatments afterthree (3)months of transplantation

Treatments	Shoot dry biomass (g)	Root dry biomass (g)	Total dry biomass (g)	Shoot/root ratio	Quality index
T_1	0.06±0.01	0.05±0.01	0.11±0.02	1.21±0.43	0.02±0.01
T_2	0.07±0.00	0.05±0.00	0.12±0.01	1.40±0.07	0.02±0.00
T_3	0.06±0.01	0.04±0.00	0.11±0.01	1.50±0.10	0.02±0.00
T_4	0.05±0.00	0.04±0.00	0.09±0.00	1.25±0.25	0.02±0.00
Level of significance	ns	ns	ns	ns	ns

*Here, ns= Not significant at P≤0.05

Table 5. Biomass allocation and quality index of *Azadirachtaindica* seedlings at different treatments after four (4)months of transplantation

Treatments	Shoot dry biomass (g)	Root dry biomass (g)	Total dry biomass (g)	Shoot/root ratio	Quality index
T_1	0.08a±0.01	0.05a±0.00	0.13a±0.01	1.60a±0.06	0.02a±0.00
T_2	0.07ab±0.01	0.06a±0.01	0.13a±0.02	1.17a±0.17	0.02a±0.00
T_3	0.06ab±0.01	0.06a±0.01	0.12ab±0.02	1.05a±0.52	0.02a±0.00
T_4	0.04c±0.01	0.04a±0.01	0.07b±0.02	1.02a±0.13	0.01b±0.00

In a column figures having similar letter(s) do not differ significantly and figure bearing different letters differ significantly by Duncan Multiple Range Test at P≤0.05

Table 6. Biomass allocation and quality index of *Azadirachtaindica* seedlings at different treatments after six (6)months of transplantation

Treatments	Shoot dry biomass (g)	Root dry biomass (g)	Total dry biomass (g)	Shoot/root ratio	Quality index
T_1	0.07ab±0.01	0.07a±0.01	0.13ab±0.01	1.04a±0.21	0.02a±0.00
T_2	0.08a±0.00	0.07a±0.01	0.14a±0.01	1.17a±0.10	0.01a±0.00
T_3	0.04bc±0.01	0.04a±0.01	0.08b±0.01	1.00a±0.19	0.01a±0.00
T_4	0.05bc±0.01	0.04a±0.03	0.09ab±0.03	1.25a±0.97	0.01a±0.01

In a column figures having similar letter(s) do not differ significantly and figure bearing different letters differ significantly by Duncan Multiple Range Test at P≤0.05

Table 7. Survival rate (%) (mean±SE) of the seedlings in different time period after transplanting

Treatments	3-months *	4- months	6- months
	Mean survival %	Mean survival %	Mean survival %
T_1	100.0a±0.0	100.0a±0.0	100.0a±0.0
T_2	100.0a±0.0	100.0a±0.0	100.0a±0.0
T_3	95.0ab±2.9	95.0ab±2.9	95.0ab±2.9
T_4	90.0b±2.9	60.0c±2.9	20.0c±0.0

*In a column figures having similar letter(s) do not differ significantly and figure bearing different letters differ significantly by Duncan Multiple Range Test at P≤0.05

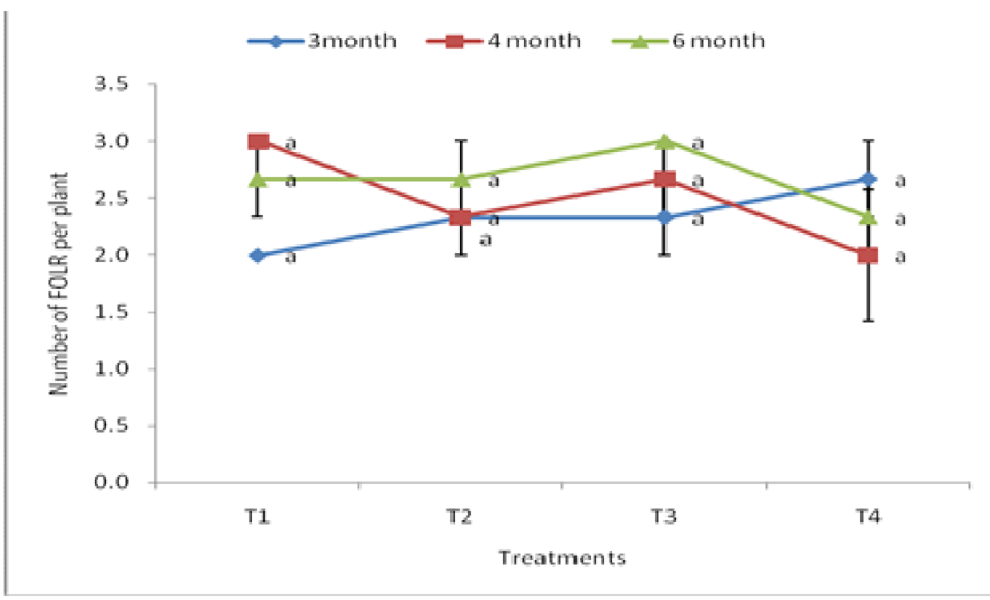

Figure 1.Effect of water stress on the number of first order lateral roots (FOLR≤1mm). Note: T1=100% water, T2=50% water , T3=25% water , T4=control (No water)

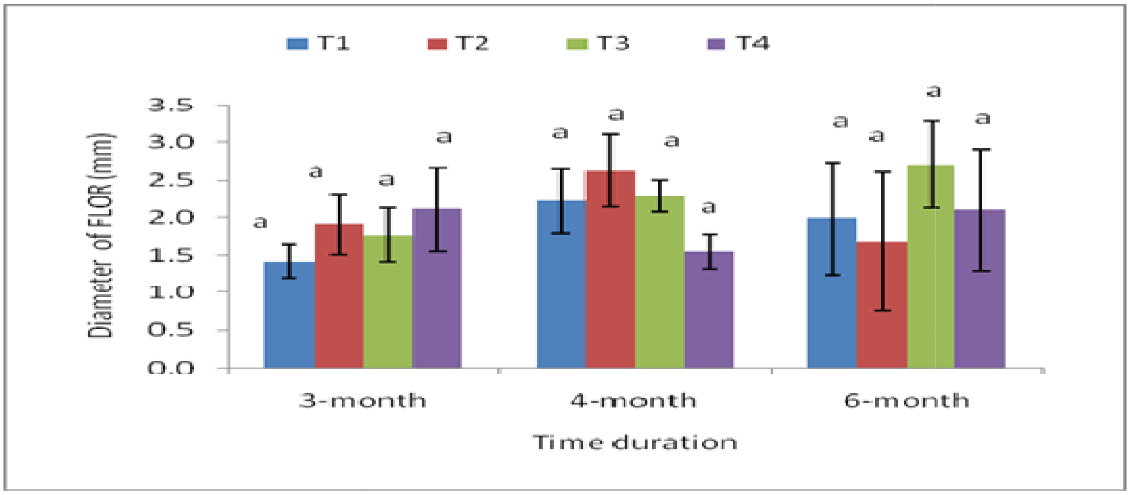

Figure 2. Effect of water stress on the diameter of first order lateral roots (FOLR≤1mm). Note: T_1=100% water, T_2=50% water , T_3=25% water , T_4=control

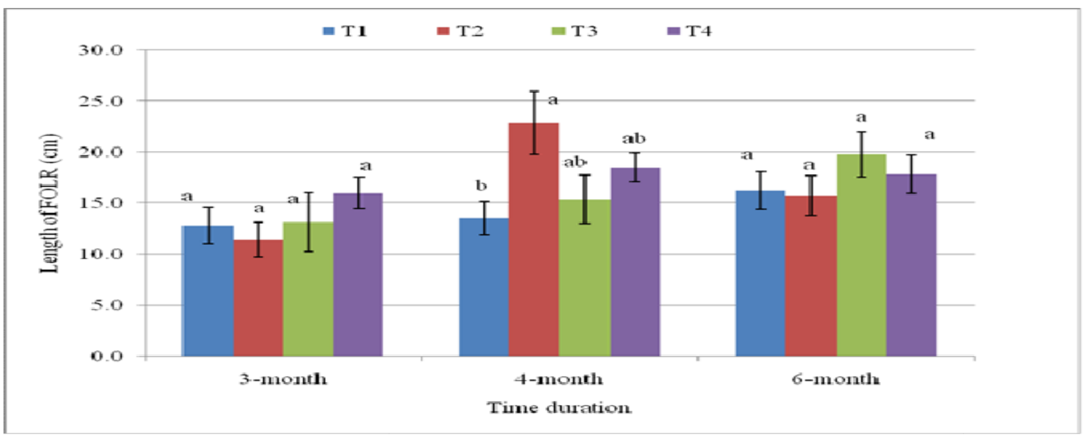

Figure 3. Effect of water stress on the length of first order lateral roots (FOLR≤1mm). Note: T_1=100% water, T_2=50% water , T_3=25% water , T_4=control (no water).

CONCLUSION

From the results and foregoing discussion, it is clear that water stress has a great effect on root architecture and shoots morphology of neem (*Azadirachtaindica* A. Juss) seedling. Among the four treatments, the highest shoot height was in T_1 treatments (100% water) after six months of transplantation in polybags and the highest survival percentage was in the treatments of 100% and 50% watering regimes in 3,4 and 6 months of transplantation. The overall results concluded that neem seedlings can be established in water stressed condition with ensuring at least 50% additional water supply in the polybags at early stages.

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