

Full Length Research Paper

Influence of watering regime and mycorrhizae inoculations on the physiology and early growth of *Acacia senegal* (L.) Wild.

M. B. Oyun^{1*}, S. A. Adeduntan¹ and S. A. Suberu²

¹Department of Forestry and Wood Technology, Federal University of Technology Akure, P. M. B. 704 Akure, Ondo State, Nigeria.

²Federal Ministry of Environment, Sokoto, Sokoto State, Nigeria.

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Acacia senegal is endowed with features that give it potentials for ecosystem stabilization, anti-desertification and for production of high quality gum arabic. However, the natural population which is becoming low has been traced to poor seed germination and seedling survival. The influence of watering regime and mycorrhiza inoculation on some physiological parameters and early growth of *A. senegal* in the semi arid region of Nigeria was investigated. Inoculated seedlings had higher stem growth, chlorophyll content and leaf number than those without inoculation. Also, seedlings that were watered twice per week had higher stem growth and leaf number than those that were watered daily and once per week. The stomata conductance of *Acacia* seedlings was best for seedling batch that were watered twice weekly without mycorrhiza inoculation and also when inoculated with ectomycorrhiza. However with endomycorrhiza, the seedling batch that received weekly watering had the highest conductance while it was least in the seedling batch that received daily watering. The same trend was observed for the xylem pressure potential. At 16 weeks after planting, seedlings that were watered once weekly wilted irrespective of whether they were inoculated or not. Mycorrhizae inoculation of *A. senegal* seedlings combined with twice weekly watering in the semi arid region of Nigeria will be appropriate for healthy and good seedling performance in the nursery.

Key words: Ecto-and-endo mycorrhiza, xylem pressure potential, chlorophyll, stomata conductance.

INTRODUCTION

Today, *Acacia senegal* is grown primarily for gum, but plays a secondary role in agricultural systems. An estimate of 17,000 tones of gum-Arabic is produced in Nigeria annually which is the second largest production in Africa apart from Sudan (Folorunsho et al., 2002). *A. senegal* is endowed with features that give it potentials for ecosystem stabilization, anti-desertification and for production of high quality gum-Arabic (Awodola and Okoro, 1986). In recent years, high-trade in gum-Arabic and other uses of the species threatened its natural population (Folorunsho et al., 2002). The natural population, which is becoming low, has been traced to poor seed germination and seedling survival (Nautiyal et al., 2002). Poor yield of the species in the semi arid region of Nigeria may be attributed to inadequate physiological

and silvicultural information regarding the species. Hence to adequately integrate *A. senegal* with the other semi-arid trees for the control of desertification and for the production of its very valuable and high-quality gum-Arabic, there is urgent need to delineate its required physiological and silvicultural characteristics. Water is a significant factor in dry land forest nursery and it is critical to tree growth and development in the tropics (Awodola and Nwoboshi, 1993). Water is required by plants for the manufacture of carbohydrates and as a means for transportation of foods and mineral elements. Various vital processes in plants such as cell division, cell elongation, stem as well as leaf enlargement and chlorophyll formation depends on plant water availability (Price et al., 1986). As noted by Levy and Krikum (1983), insufficient water in plants below a critical level is usually demonstrated by changes in all structures leading to the death of the plants. According to Miller (1981), for each ton of vegetative growth, hundreds of tons of water may be

*Corresponding author. E-mail: banjiyun@yahoo.com.

consumed by the growing plant particularly in dry climates. As observed by Awodola (1984), the reduction in relative water contents affects physiological processes and hence plant growth. Similarly, too much water in excess of plant need may retard physiological processes in plants. In particular, stomata conductance which is a numerical measure of the maximum rate of passage of either water vapour or carbon dioxide through the stomata and the xylem pressure potential which is the component of water potential due to hydrostatic pressure that is exerted on water in a cell are influenced by the soil-water balance (Komer, et al., 1979). Huang et al. (1985) reported that root to shoot ratio to be 3.5 times higher in water stressed plants.

Mycorrhiza fungi has been found to increase the drought tolerance of host plants (Pandey, 2000; Osunubi and Mulongoy, 1991), nevertheless, the effect of mycorrhiza on growth and drought tolerance of *A. senegal* is yet to be assessed. Most soils in semiarid zones are marginal and deficient in nitrogen and phosphorus, which are principal elements required for plant growth and development (Shinkafi, 2000). Since poor growth performance of the species on the field is a major constraint to high yield in the semi arid region, identifying efficient nursery management practice through adequate supply of water and other essential nutrient supply mechanism is crucial. The objective of the present study therefore is to investigate the influence of watering regime and mycorrhiza application on the early growth, chlorophyll content, stomata conductance and xylem pressure potential of *A. senegal* in the semi-arid region of Nigeria.

MATERIALS AND METHODS

Experimental site

The nursery experiments were conducted in a screen house at the central nursery of the Federal Ministry of Environment, Sokoto in 2007. Sokoto lies in the North-western Nigeria in the Sudan Savannah vegetation zone. It is located between latitudes 11°30' and 13°50' N and longitude 4° - 6°E (Ogigiri, 1993).

Source and preparation of crude mycorrhiza inoculum

Crude inoculums of *Suillus grevillei* and *Boletus* spp. (ecto- and endo mycorrhiza inoculants) that was used in this study was obtained from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The culture was prepared following the procedure of Osunubi and Mulongoy (1991) and kept properly in a clean sealed container to avoid contamination. The topsoil used was collected from Dundaye village in Sokoto state and sieved to remove the gravels. About 20 kg of the sieved soil was sterilized at 120°C for one hour to kill the micro-organisms. The soil was cooled for 2 days. Four plastic pots (with draining holes at the bottom) measuring 25 cm in length and 15 cm in diameter were then filled with the sterilized soil where millet seeds were sown.

Procedure for mycorrhiza inoculation

150 g *Suillus grevillei* and *Boletus* spp., (starter inoculum) was

applied directly into the seeds of millet sown in the respective pots. One week after emergence, the millet seedlings were thinned to one per pot. The seedlings were grown for three months and were irrigated using distilled water. At the end of the third month, watering was completely withdrawn to induce maximum spore formation. Thereafter the stems of the millet seedlings were removed at the soil level. Then the soil and the roots were harvested and the mixture now referred to as crude inoculums, was used in inoculating *A. senegal* seedlings in this study (Sievarding, 1981).

Experimentation

Standard plastic pot (25 × 15 cm) with drainage holes at the bottom was filled with 4.01 kg of sterilized sandy – loam soil which were irrigated with distilled water just before planting (Awodola, 1984). Thereafter, three viable seeds of *A. senegal* were sown in each pot. The polythene pots were then distributed into nine experimental treatments and labeled as:

NMD: No mycorrhiza with daily watering
 NMB: No mycorrhiza with twice weekly watering
 NMW: No mycorrhiza with weekly watering
 MecD: Ectomycorrhiza with daily watering
 MecB: Ectomycorrhiza with twice weekly watering
 MecW: Ectomycorrhiza with weekly watering
 MEnD: Endomycorrhiza with daily watering
 MenB: Endomycorrhiza with twice weekly watering and
 MenW: Endomycorrhiza with weekly watering.

Ten grammes (10 g) each of the ecto- and endo-mycorrhizae was applied in a ring of 1 cm deep under the seeds of *A. senegal* sown in the respective pots. The experiment was laid out in a completely randomized design with five replicates per treatment. The pots were watered daily for two weeks for the germinated seedlings to establish well. The watering regime (daily, biweekly and weekly watering) of the seedlings actually began after two weeks of growth. The seedlings were later thinned to one seedling per pot. The seedlings were grown for sixteen weeks. The seedlings of the respective treatments were assessed after 4, 8, 12 and 16 weeks for morphological and physiological parameters. The plant height was measured with meter rule, leaf number by physical count and chlorophyll content was determined using spectrophotometer method (Chang and Troughton, 1971).

The stomatal conductance was assessed at 12 noon using AP4 Porometer while Xylem pressure potential (XPP) was measured using a pressure Bomb with nitrogen gas. Readings were taken at the emergence of water bubbles (Turner, 1982).

Statistical analysis

Data obtained from all the variables in the investigation were subjected to analysis of variance (ANOVA) using AGRES statistical package. Significant difference in the treatments was further subjected to Duncan Multiple Range Test (DMRT) for the separation of treatment means.

RESULTS AND DISCUSSIONS

The combined effects of watering regime and mycorrhiza inoculation on plant height and number of leaf measured at different periods of growth is shown in Figures 1 and 2, respectively. At 2, 8, 12 and 16 weeks after planting (WAP), the plant height and number of leaves varies in

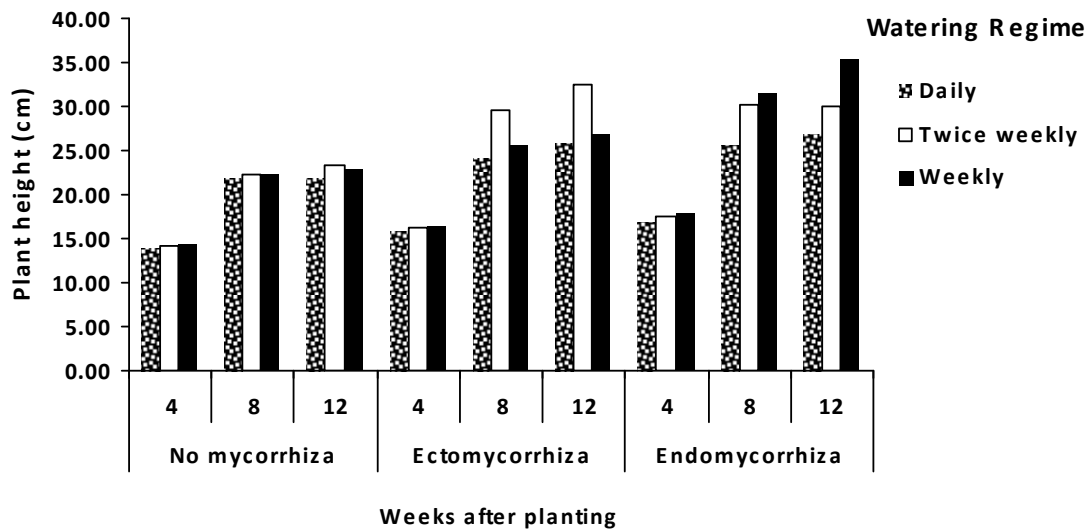


Figure 1. Seedling height of *A. senegal* in response to watering regimes and mycorrhizae inoculation at period after planting.

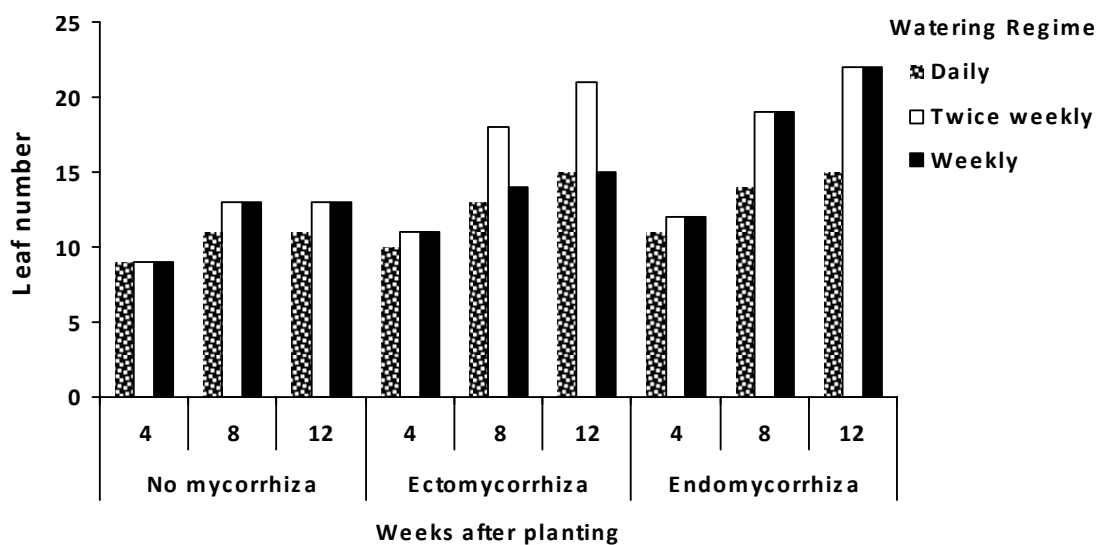


Figure 2. Leaf number of *A. senegal* seedlings in response to watering regimes and mycorrhizae inoculation at different period after planting.

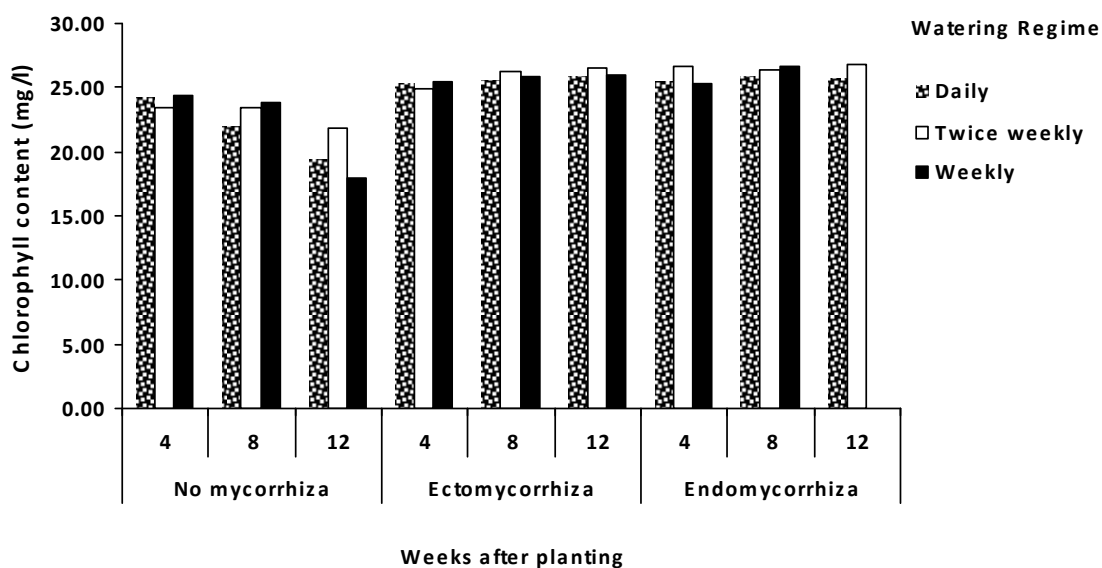
response to watering regime and mycorrhizae inoculation. The plant height and number of leaves of seedlings inoculated with mycorrhizae were higher than the non inoculated seedlings (control). The endomycorrhiza inoculated seedlings have higher plant height and number of leaves than the seedlings inoculated with ectomycorrhiza. Seedlings that were watered twice weekly had higher plant height and number of leaves than those that were watered daily and once weekly. At 16 WAP, the seedlings that were watered once weekly wilted irrespective of whether inoculated or not. At 16 WAP, the root length of the seedlings in response to watering and inoculation

treatments varies significantly (Table 1). However the seedlings that were watered twice weekly has higher ($P < 0.05$) seedling root length for both ectomycorrhiza (12.65 cm) and endomycorrhiza (13.06 cm) inoculation treatments than those that were watered daily which were 9.96 cm and 10.77 cm for ectomycorrhiza and endomycorrhiza treatments respectively. The chlorophyll content, stomata conductance and xylem pressure potential of *A. senegal* seedlings in response to watering regime and mycorrhizae inoculation at different periods of growth are shown in Figure 3, Tables 2 and 3 respectively.

Table 1. Root length (cm) of *A. senegal* seedlings in response to watering regime and mycorrhizal inoculation at harvest (16 WAP).

Watering regime	Mycorrhiza inoculations	Root length (cm)
Daily	No mycorrhiza	8.57f
Biweekly	No mycorrhiza	9.17e
Daily	Ectomycorrhiza	9.96d
Biweekly	Ectomycorrhiza	12.65b
Daily	Endomycorrhiza	10.77c
Biweekly	Endomycorrhiza	13.06a
S.E.		3.27

Means in a column followed by the same letter(s) are not significantly different using DMRT at 5% level, WAP = Weeks after planting.

**Figure 3.** Chlorophyll content in the leaves of *A. senegal* seedlings in response to watering regimes and mycorrhizae inoculation at different period after planting.**Table 2.** Stomatal conductance ($\text{mmol m}^{-2}\text{s}^{-1}$) of *A. senegal* seedlings at 12 noon in response to watering regime and mycorrhizal inoculations at different periods of growth.

Watering regime	Mycorrhizal inoculation	Stomatal conductance ($\text{mmol m}^{-2}\text{s}^{-1}$)			
		4 WAP	8 WA P	12 WAP	16 WAP
Daily	No mycorrhiza	33.51k	35.22k	37.39k	39.18k
Biweekly	No mycorrhiza	48.63k	51.75j	58.20j	62.83i
Weekly	No mycorrhiza	64.32i	68.53i	72.46h	Died
Daily	Ectomycorrhiza	74.32h	78.14h	82.76g	84.37g
Biweekly	Ectomycorrhiza	116.22d	122.15d	130.50c	132.54c
Weekly	Ectomycorrhiza	93.69f	101.42e	103.33e	sd
Daily	Endomycorrhiza	92.89f	98.72f	103.21e	105.49e
Biweekly	Endomycorrhiza	135.84c	143.20b	145.72b	147.55b
Weekly	Endomycorrhiza	148.11b	155.10a	158.51a	sd
S.E.		4.33	3.53	2.46	1.84

Means in a column followed by the same letter(s) are not significantly different using DMRT at 5% level. WAP = weeks after planting, Sd = seedling death.

Table 3. Xylem pressure potential of *A. senegal* seedlings in response to watering regime and mycorrhiza inoculation at different periods of growth.

Watering regime	Mycorrhiza inoculation	Xylem pressure potential (Mpa)			
		4 WAP	8 WAP	12 WAP	16 WAP
Daily	No mycorrhiza	-2.07a	-2.27a	-2.31a	-2.38a
Biweekly	No mycorrhiza	-1.17c	-1.28b	-1.37b	-1.42b
Weekly	No mycorrhiza	-1.35b	-2.21a	-2.97a	sd
Daily	Ectomycorrhiza	-0.98d	-1.23b	-1.29b	-1.38b
Biweekly	Ectomycorrhiza	-0.98d	-1.22b	-1.27b	-1.29b
Weekly	Ectomycorrhiza	-0.98d	-0.99e	-0.96d	Sd
Daily	Endomycorrhiza	-1.15c	-1.38b	-1.61b	-1.84b
Biweekly	Endomycorrhiza	-0.88d	-1.19c	-1.23b	-1.53b
Weekly	Endomycorrhiza	-0.36e	-0.57d	-1.12c	sd
S.E.		3.38	1.79	2.54	1.95

Means in a column followed by the same letter(s) are not significantly different using DMRT at 5% level, WAP = weeks after planting, Sd = seedling death.

At 2, 8, 12 and 16 WAP, the chlorophyll of the seedlings vary in response to watering regime and mycorrhiza inoculation (Figure 3). Accordingly, the chlorophyll content of seedlings inoculated with mycorrhizae was greater than the non inoculated seedlings (control). The endomycorrhiza inoculated seedlings gave a higher chlorophyll content and number of leaves than the ectomycorrhiza inoculated seedlings. Seedlings that were watered twice weekly had higher chlorophyll content than the daily and weekly watered seedlings.

The stomata conductance of the seedlings inoculated with mycorrhizae were higher ($P < 0.05$) than the non inoculated seedlings at the different period of growth assessment (2, 8, 12 and 16 WAP). At 12 WAP, the stomata conductance of the seedlings that were watered weekly ($72.4 \text{ mmol}^{-2} \text{ s}^{-1}$) was higher ($P < 0.05$) than those that were watered twice weekly ($58.2 \text{ mmol}^{-2} \text{ s}^{-1}$) which also was higher ($P < 0.05$) than those watered daily ($37.39 \text{ mmol}^{-2} \text{ s}^{-1}$) for the non inoculated seedlings. For the seedlings inoculated with ectomycorrhiza, the stomata conductance of the seedlings that were watered twice weekly ($130.50 \text{ mmol}^{-2} \text{ s}^{-1}$) was higher than those that were watered weekly ($103.33 \text{ mmol}^{-2} \text{ s}^{-1}$). For the endomycorrhiza inoculated seedlings, those watered weekly had higher stomata conductance ($158 \text{ mmol}^{-2} \text{ s}^{-1}$) than the once watered twice weekly. For both ecto- and endo – mycorrhizae inoculated seedlings the stomata conductance of the daily watered ones was least throughout the period of plant growth. At 16 WAP, the seedlings that were watered weekly wilted irrespective of whether inoculated or not.

The xylem pressure potential of the seedling also varies significantly ($P < 0.05$) in response to watering regime and mycorrhizae inoculation. As shown in Table 3, the seedling batch that was watered twice weekly had higher xylem pressure potential than either the daily or weekly watering without mycorrhizae inoculation while

those watered once per week had the lowest. Throughout the period of the growth trial, the seedlings without mycorrhiza inoculation had lower xylem pressure potential than the mycorrhizae inoculated seedlings irrespective of the watering regime. The seedlings inoculated with endo mycorrhiza have significantly lower xylem pressure potential than the ectomycorrhiza inoculated seedlings. The seedlings inoculated with mycorrhiza have improved growth more than the non-inoculated seedlings. It was also observed that seedlings that were watered twice weekly (slightly stressed) had enhanced height growth and produced more leaves than those watered daily and weekly. Also, slightly water-stressed mycorrhizae treated seedlings twice weekly watering had higher mean growth parameters than non-mycorrhiza (control) and highly water-stressed (weekly watering) mycorrhizae treated seedlings. The results showed significant difference in plant height between water-stressed (weekly watering) and slightly water-stressed (twice weekly watering) plants inoculated with either of the mycorrhizae types (ectomycorrhiza or endomycorrhiza). The growth response of *A. senegal* was better with endomycorrhiza than with ectomycorrhiza. These findings support the earlier observations by Levy and Kirkum (1983); Read and Boyd (1986) and Shinkafi (2000) that mycorrhiza inoculation increased soil water extraction and root hydraulic conductivity. Shinkafi (2000) also noted higher growth attributes from endomycorrhiza treated seedlings of *Acacia albida* than the ectomycorrhiza treated ones. Under serious water stress, the seedlings stopped growing, leaves turned yellow, wilted and eventually died. This observation supported the findings of Mulongoy et al. (1988) who reported that growth parameters were reduced under drought stressed condition in *Acacia auriculiformis*. Abubakar (2002) also observed that *A. senegal* seedling showed high sensitivity to water stress at seedling stage and indicated

that most of the growth parameters studied performed better in unstressed conditions.

Mycorrhizae inoculations whether ecto- or endomycorrhiza combined with different watering regime were found to have significant influence on the growth of *A. senegal*. This result supported the observation by Awotoye et al. (1992) who noted that slightly stressed mycorrhizae inoculated seedlings had higher mean growth values than the non-mycorrhiza (control) and water stressed *Leuceana leucocephala*.

In conformity with the findings of the present study, Awotoye et al. (1992) and Osundina (1995) who worked on *Parkia biglobosa* observed that *A. senegal* had enhanced leaf number, increased collar diameter, higher shoot height and other growth parameters largely due to the presence of mycorrhiza. Mycorrhizae inoculations as observed in the present study increased stomata conductance and xylem pressure potential. This finding corroborates with the earlier report by Nelson and Safir (1982) and Levy and Krikum (1983). This therefore suggests that the ecological range of *A. senegal* could be extended to marginal lands characterized by low soil moisture with endomycorrhiza and ectomycorrhiza improving growth. This is because the association between the seedlings and the mycorrhizae will enhance growth and good seedling vigor and improve the plant physiological activities like stomata conductance and xylem pressure potential which are directly related to soil-water balance and growth determinant in water-stressed environment. This is evident from the increase in plant height, collar diameter and leaf number of inoculated seedlings more than the plants not inoculated which were both subjected to weekly watering (highly stressed). Levy and Krikum (1983) and Read and Boyd (1986) observed higher soil water extraction and root hydraulic conductivity in mycorrhiza inoculated plants. Partially stressed *A. senegal* seedlings that were watered twice a week were significantly higher in terms of growth than non-inoculated plants receiving the same watering frequency. In general, the interaction between mycorrhiza and drought was found to be significant as a result of positive mycorrhiza effect on young seedlings of *A. senegal*. No growth depression was observed, rather the growth of the inoculated seedlings was significantly enhanced. The interaction between mycorrhiza and water-stress resulted in a positive growth performance of all the plant parameters measured, indicating that both endo- and ecto-mycorrhizae were not parasitic on *A. senegal*.

Total chlorophyll content of the leaves of *A. senegal* seedlings inoculated with mycorrhiza, particularly those inoculated with endomycorrhiza and watered twice weekly was significantly higher than what was observed in other treatments. It was also observed that the total chlorophyll content of all the seedlings gradually increased with the age of the seedlings within the same treatment. These findings agreed with the work of Bello (2005) who in a similar study discovered that mycorrhiza inoculation enhanced total chlorophyll content of *A. senegal*

and *Acacia nilotica*.

As observed in this study, the root length of seedlings increase with increased water stress. Thus those seedlings that were watered twice weekly had higher root length than those watered daily. Similar findings were reported by Fisches (1980). The enhanced root growth in response to stress condition may be due to high osmotic adjustment in the root cells which may likely resulted to rapid elongation of the root meristem.

Conclusion

It is evident from the results of this study that mycorrhiza inoculation (particularly endomycorrhiza) is required for healthy and adequate growth of the seedlings of *A. senegal* in the nursery. From the study, it can be concluded that twice weekly watering is most adequate for tending the seedling of *A. senegal* in the nursery. This is evident because daily watering produced fragile seedlings that may not be able to withstand the harsh drought condition in the field while the weekly watering regime resulted to stress condition that wilted the seedlings even in the nursery before transplanting into the field. It is also noticeable from this study that mycorrhizae inoculation (particularly endomycorrhiza) enhanced growth performance and seedling survival of *A. senegal* in the nursery.

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