GROWTH RESPONSES OF AFRICAN NIGHTSHADES (*Solanum scabrum* MILL) SEEDLINGS TO WATER DEFICIT

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ABSTRACT

Solanum scabrum Mill is widely distributed in Africa especially east Africa. Its leaves and fresh shoots are widely used as a cooked vegetable. Drought is a major abiotic stress factor limiting crop productivity in many arid and semi arid regions of the world. It causes reduction in plant growth, dry matter accumulation, decline in plant water status of the plants or even interfering with chlorophyll synthesis by the leaves. A pot experiment was conducted at Maseno University, botanic garden, to investigate the growth response of *Solanum scabrum* Mill to water deficit. Individual plants were grown in 5 litre plastic pots containing local soil, and watered daily with 300ml of tap water until the start of the treatments. The treatments were three watering regimes [watering daily with 300ml of tap water per pot (X), once per week (Y), and once after two weeks (Z)]. The treatments were laid out in a greenhouse in a completely randomized design with six replicates. Data on leaf number, leaf area, leaf water content, shoot height, shoot and root dry weights, leaf chlorophyll content and Root: Shoot ratio were determined at the end of the experiment. Soil moisture content under different watering regimes was also determined gravimetrically at the end of the experiment. The data collected was subjected to analysis of variance (ANOVA). There were significant differences ($p \leq 0.05$) among treatments in leaf number, leaf area, leaf water content, shoot height, shoot dry weights, root: shoot ratio and chlorophyll a and b concentration among the watering treatments. There was no significant difference in root dry weight. The results of the study demonstrate that *Solanum scabrum* Mill. Seedlings are very sensitive to drought since the stressed seedlings exhibited about 97%reduction in leaf area, 84%reduction in shoot height, 88% reduction in shoot dry weight, and about 29.4% and 63% reduction in chlorophyll a and b contents respectively relative to control plants.

Keywords: *Solanum scabrum*, water deficit, watering regimes, growth.

INTRODUCTION

Among various types of vegetables, leafy vegetables are most commonly consumed in Kenyan daily diet. The importance of leafy vegetables in the developing countries has been recognized only now due to their nutritional and medicinal value (Prasad *et al*., 2008). Green leafy vegetables occupy an important place among food crops as these provide adequate amounts of crude fiber, carotene, a precursor of vitamin A, vitamin C, riboflavin, folic acid and mineral salts like calcium, iron, phosphorous etc. They form cheap and best source of food (Prasad *et al*., 2008).

Water has been described as the single physiological and ecological factor upon which plant growth and development depends more heavily than other factors (Kramer and Boyer, 1995). This can be proved by the fact that not only does water comprise 50-90% fresh weight of all plant tissues but also the distribution of plants on earth's surface is controlled by the availability of water where temperature permits growth (Kramer and Boyer, 1995).

Plants are often subjected to periods of soil and atmospheric water deficit during their life cycle. Any shortage in water supply in relation to the requirement of leaves results in water deficit and plant stress (Maseda and Fernandez, 2006).

A drought is an extended period of months or years when a region notes a deficiency in its water supply. Generally, this occurs when a region receives consistently below average precipitation. It can have a substantial impact on the ecosystem and agriculture of the affected region. Although droughts can persist for several years, even a short, intense drought can cause significant damage and harm the local economy.

Drought is caused by various factors. Generally, rainfall is related to the amount of water vapour in the atmosphere, combined with the upward forcing of the air mass containing that water vapour. If either of these is reduced, the result is a drought. Factors include: Above average prevalence of high pressure systems; Winds carrying continental, rather than oceanic air masses (i.e. reduced water content); Ridges of high pressure areas form with behaviors which prevent or restrict the developing of thunderstorm activity or rainfall over one certain region; El Niño, La Niña (and other oceanic and atmospheric temperature cycles) and global warming; Deforestation and erosion adversely impacting the ability of the land to capture water. Climate change has a substantial impact on agriculture throughout the world, and especially in developing nations (http://en.wikipedia.org/wiki/Drought).

Drought is the major abiotic stress factor limiting crop productivity worldwide, and understanding the physiological and biochemical mechanisms that control drought tolerance is a central question in plant biology. Plant growth in semiarid and tropical regions is often limited by variations in the amount and duration of rainfall. Water stress effects on the shoot and root growth are analyzed to determine relationships with yield and

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plant growth and to evaluate possible drought avoidance mechanisms (Pandey *et al.*, 1984).

BOTANY OF AFRICAN NIGHTSHADES

Origin and geographic distribution

Solanum scabrum occurs as a cultivated vegetable from Liberia to Ethiopia, and south to Mozambique and South Africa. It is very common in lowland as well as highland regions in West and East Africa. The wide range of diversity of *Solanum scabrum* found especially in Nigeria and Cameroon suggests that its origin is likely to be in the warm humid forest belt of West and Central Africa. Outside Africa, *Solanum scabrum* can be found in Europe, Asia, Australia, New Zealand, North America and the Caribbean (http://www.pfaf.org/database/plants).

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Solanales
Family	Solanaceae
Genus	*Solanum* L.
Species	*Solanum scabrum* Mill.

Economic importance

Leaves and fresh shoots of *Solanum scabrum* are widely used as a cooked vegetable. As it has a bitter taste, some people prefer not to use salt. Bitterness is reduced by discarding the cooking water and replacing it with fresh water.

Solanum scabrum is widely used as medicinal plant. Leaf extracts are used to treat diarrhoea in children and certain eye infections and jaundice. In East Africa the raw fruit is chewed and swallowed to treat stomach ulcers or stomach-ache. Infusions of leaves and seeds are rubbed onto the gums of children who have developed crooked teeth. *Solanum scabrum* is used as fodder for cattle and goats. Both the leaves and fruits are a source of dyes. The anthocyanin pigments in the purple to black fruits are used as a dye or as a kind of ink (http://www//database.prota.org/PROTAhtml/).

Properties: The composition of 100 g edible portion of African nightshade leaves is: water 87.8 g, energy 163 kJ (39 kcal), protein 3.2 g, fat 1.0 g, carbohydrate 6.4 g, fibre 2.2 g, Ca 200 mg, P 54 mg, Fe 0.3 mg, β-carotene 3.7 mg, ascorbic acid 24 mg. The dry matter content varies greatly, from 6-18 % depending on plant age, soil moisture and fertilizing. The protein is rich in methionine. The fruit contains about 2.5% protein, 0.6% fat, 5.6% carbohydrate. Green fruits contain comparatively high amounts of the glycoalkaloid solanine and the less poisonous solanidine (http://www.pfaf.org/database/plants).

Effects of water deficit on general plant growth

Water deficit is a problem to plant growth and crop productivity in the vast areas of Kenya. Plants avoid water deficit by developing deep roots or by minimizing water loss (e.g., stomatal closure, small leaves). Water deficit affects growth, development, yield and quality of plants in the greenhouse and field conditions (Luvaha *et al.,* 2008). In plants growing in drying soil, the development of the root system is usually less inhibited than shoot growth, and may even be promoted. Maintenance of root growth during water deficits is an obvious benefit to maintain an adequate plant water supply, and is under genetic control (Sharp *et al*., 2004).

Drought causes reductions of leaf area, dry matter production, decline in plant water status and transpiration. Water deficit significantly reduces dry matter content in plants. Total dry matter reduces by 27-43% under severe water stress. Reduced leaf area is a drought avoidance mechanism, aimed at reducing plant water consumption and hence conserving water during periods of drought. It is achieved through inhibition of leaf expansion and initiation, reduced branching and plant height. Reduced leaf area decreases interception of solar radiation and consequently decreases biomass production for most crops (Masinde *et al*., 2005).

Soil water deficits have been observed to cause reductions in lateral branching, leaf production, shoot height and rates of leaf and shoot expansion in both herbaceous and woody plants (Osorio *et al*., 1998; Ngugi *et al*., 2003; Luvaha *et al.,* 2008). Generally, plants show increased root: shoot ratio under water deficit conditions. This is an adaptation for survival in drought areas since increased root surface area allows more water to be absorbed from the soil (Luvaha *et al.,* 2008).

Effects of water deficit on plant growth and metabolism have been extensively reviewed. At the whole plant level, limited soil water supply may have a strong effect on development, activity and duration of various sources and sink organs (Osorio *et al*., 1998). Under more prolonged water deficit, dehydration of plant tissue can result in an increase in oxidative stress which causes deterioration in chloroplast structure and an associated loss of chlorophyll. This leads to a decrease in the photosynthetic activity (Jafar *et al*., 2004). Total chlorophyll content reduces by 55% compared to well water plants (Kirnak *et a*l., 2001; Cengiz *et al*., 2006).

Soil moisture content decreases with increasing water deficit. It is important to maintain proper soil moisture since there is a very close relationship between the soil moisture and crop yield. There is low increase in plant height under extreme deficit possibly due to reduced cell turgor which affects cell division and expansion (Luvaha *et al*., 2008). Drought is a major problem in arid and semiarid areas of Kenya and has contributed to increased food insecurity and poor human health. Although African nightshades are very important indigenous vegetables, literature on growth responses to

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water deficit and its adaptations to drought is lacking. There is need to grow crops that can withstand drought conditions to ensure constant food supply. African nightshade is an important indigenous vegetable and can highly contribute to food security in Africa. The vegetable is highly nutritious as well as medicinal. There have been relatively few studies on the growth responses of indigenous vegetables including African nightshades (*S.scabrum*) to water deficit (Masinde *et al*., 2005; 2006); and information is needed in order to understand how well these plants can grow and survive in arid and semiarid areas which experience unreliable rainfall. It was hypothesized that water deficit affects growth and yield of African nightshades (*Solanum scabrum* Mill). The main objective of the study was to investigate the growth response of African nightshades seedlings (*S.scabrum*) to water deficit.

The Specific objectives were:

- To determine the effect of water deficit on growth parameters of African nightshades (*S.scabrum* Mill).
- To determine the effect of water deficit on leaf chlorophyll content of African nightshades (*S.scabrum* Mill).
- To determine the effect of water deficit on leaf water content of African nightshades (*S.scabrum* Mill).

MATERIALS AND METHODS

Experimental materials

The experiment was conducted under greenhouse conditions at Maseno University, botanic garden. Seeds were obtained from the botanic garden. About 2.5 kg of garden soil was put in each of 5L plastic pots. 10 viable seeds were planted per pot. The soil is classified as Acrisol (Mwai, 2001; Musyimi, 2005), well drained and acidic with a pH range of 4.6-5.4. The plants were thinned to two seedlings per pot 10 days after germination.

Experimental design and treatments

Plants were subjected to three watering regimes [High soil water (X)-watered every day, moderate soil deficit (Y)-watered once per week, and severe water deficit (Z)-watered after two weeks]. The water content of the soil varied according to table1 below. 300ml of tap water were added to every pot during the experimental period. The treatments were replicated six times and pots arranged in a completely randomized design inside a greenhouse. Before the onset of water treatments, soil moisture was kept high by watering daily all the pots. Weeds were controlled by hand pulling. Data collection commenced after two weeks since the initiation of the experiment.

Table-1. Water content of the soil according to the various watering regimes.

Watering regime	**Soil moisture** (%)
Watered daily	127.167a
Watered once per week	85.833b
Watered after two weeks	69.000b
LSD	20.54

Means followed by the same letters down the column for watering regimes are not significantly different at $p \leq 0.05$), Means of six replicates.

Growth measurements

Data on growth was collected every week up to the end of the experiment, and included the following parameters:

Leaf number

The number of fully expanded mature leaves on the main stem and branches were counted and recorded.

Shoot height

Shoot height was measured using a meter rule from the stem base up to the shoot apex.

Leaf area

Leaf area was calculated using the following formula according to Jose *et al*. (2000).

$A_L = 0.73\ (L_L X\ W_L)$, where L_L is the leaf length and W_L is the maximum width measured for each leaf on each plant.

Leaf water content

Leaf water content was measured using gravimetric method according to Farnsworth and Meyerson (2003), by weighing fresh leaves immediately following removal from harvested stems and re-weighing them following oven drying.

$$L.W.C = \frac{(\text{Fresh weight} - \text{Dry weight})}{\text{Dry weight}}$$

Where L.W.C is the leaf water content

Root and Shoot dry weights

The whole plant was uprooted, rinsed, separated into shoot and root and oven dried for 48 hours at 72^0c. The root and shoot dry weights were measured with an electronic weighing balance.

Root: Shoot ratio

The root: shoot ratio was determined at the end of the experiment using the following formula:

$$\text{Root: Shoot} = \frac{\text{Root dry weight}}{\text{Shoot dry weight}}$$

Soil moisture content

Soil moisture content was determined according to Brock and Galen (2005). 40g of soil sample from each treatment was taken from each pot, sealed in a plastic bag, weighed (fresh weight), oven dried at 100^0c for 48 hours and reweighed (dry mass). Soil water content was estimated as:

$$S.M.C = \frac{(\text{Wet mass-Dry mass})}{\text{Dry mass}}$$

Chlorophyll concentration

Chlorophyll concentration was determined using the method described by Adelusi *et al.* (2006). The sixth fully expanded leaf from the shoot apex was sampled from all the treatments. 0.5g of *Solanum scabrum* leaves were ground in 20ml 80 %(v/v) acetone using mortar and pestle. The resulting supernatant was read at 664 and 647 nm using UV-visible spectrophotometer. Chlorophylls a and b contents were determined using the following equations:

Chlorophyll a =*13.19 A664-2.57 A647* (mg g^{-1}fresh weight)
Chlorophyll b =*22.1 A647-5.26 A664* (mg g^{-1} fresh weight)
A664 is the absorbance at 664 nm
A647 is the absorbance at 647 nm

Data analysis

Analysis of variance (ANOVA) was carried out on the data collected using a SAS statistical computer package, the treatment means were separated using the least significant difference (L.S.D.) test.

RESULTS

Leaf number

Leaf number was significantly ($p \leq 0.05$) affected by watering regime. There was significant difference ($p \leq 0.05$) in leaf number among the treatments (Table-2). Production of leaves decreased with increasing water stress, about 65% decrease in highly stressed plants compared to the control treatment.

Leaf area

Leaf area was significantly ($p \leq 0.05$) affected by water stress. Leaf expansion was highest in the plants watered daily with no significant difference between the plant watered weekly and after two weeks (Table-2). Leaf area was reduced by about 97% in highly stressed plants compared to the control treatment.

Leaf water content

Watering regime significantly ($p \leq 0.05$) affected leaf water content. Leaf water was reduced by about 93% in highly stressed plants compared to the control (Table-2).

Shoot height

There was significant difference ($p \leq 0.05$) in shoot growth among the treatments. Shoot height was lowest in the most stressed plants (Tables, 3 and 5), with a reduction of about 84% compared to the control treatment.

Shoot dry weight

Shoot dry weight was significantly affected ($p \leq 0.05$) by watering treatments. Shoot dry weight was highest in plants watered daily (Table-3) and reduced by about 88% in plants watered after two weeks.

Root dry weight

There was no significant difference ($p \leq 0.05$) in root dry weight among the treatments. However plants watered daily had relatively higher root dry weight (0.30 cm) compared to the most stressed plants (0.11cm) (Table-3).

Root: shoot ratio

Root: shoot ratio increased with increase in soil water deficit (Figure-1). The most stressed plants had root: shoot ratio of 2.2 while the control treatment had a ratio of 0.7

Chlorophyll concentration

Chlorophyll concentration was significantly ($p \leq 0.05$) affected by watering regime. Chlorophyll synthesis was significantly reduced in highly stressed plants (Tables, 4 and 5). Chlorophyll a concentration reduced by about 29.4% of control treatment while chlorophyll b concentration reduced by 63% of the control plants.

Table-2. Effect of different watering regimes on leaf number, leaf area and leaf water content of African nightshades after 35 days since the initiation of the treatments.

Watering regime	Leaf number	Leaf area (cm^2)	Leaf water content
Watered daily	21.833a	77.442a	7.6000a
Watered once per week	12.833b	68.75b	1.2667b
Watered after two weeks	7.667c	18.82c	0.5167c
LSD	3.636	14.059	1.5046

Means followed by the same letters down the column for watering regime are not significantly different at ($p \leq 0.05$). Means of six replicates.

Table-3. Effect of watering regimes on shoot height, shoot dry weight, and root dry weight of African night shades after 35 days since the initiation of the treatments.

Watering regime	**Shoot height** (cm)	**Shoot dry weight** (g)	**Root dry weight** (g)
Watered daily	40.500a	0.45000a	0.3000a
Watered once per week	9.500b	0.16667b	0.2500a
Watered after two weeks	6.417b	0.05167b	0.1133a
LSD	5.6778	0.2154	0.3569

Means followed by the same letters down the column for watering regime are not significantly different at (p≤0.05). Means of six replicates.

Table-4. Effect of watering regimes on chlorophyll content of African nightshades after 35 days since the initiation of the treatments.

Watering regime	**Chlorophyll *a*** (mgg^{-1}fresh weight)	**Chlorophyll *b*** (mgg^{-1}fresh weight)
Watered daily	0.042400a	0.44762a
Watered once per week	0.021933b	0.33725b
Watered after two weeks	0.012450b	0.28200c
LSD	0.0098	0.0422

Means followed by the same letters down the column for watering regime are not significantly different at (p≤0.05). Means of six replicates.

Table-5. Analysis of variance of leaf number, leaf area, leaf water content, shoot height, shoot dry weight, root dry weight, soil moisture content, chlorophyll a, and chlorophyll b concentration.

Parameter	**Source**	**df**	**MS**	**F**	**Pr > F**
Leaf number	Model	7	99.1269841	12.41	0.0003
	Error	10	7.9888889		
	Replicate	5	15.4222222	1.93	0.1759
	Treatment	2	308.3888889	38.60	0.0001
Coeff var = 20.03005					
Leaf area	Model	7	3159.34017	26.45	0.0001
	Error	10	119.44260		
	Replicate	5	137.51567	1.15	0.3957
	Treatment	2	10713.90142	89.70	0.0001
Coeff var = 38.03663					
Leaf water	Model	7	27.2281746	19.91	0.0001
	Error	10	1.3678889		
	Replicate	5	1.7805556	1.30	0.3370
	Treatment	2	90.8472222	66.41	0.0001
Coeff var = 37.39293					
Shoot height	Model	7	635.609127	32.63	0.0001
	Error	10	19.480556		
	Replicate	5	36.980556	1.90	0.1816
	Treatment	2	2132.180556	109.45	0.0001
Coeff var = 23.47008					

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Shoot dry wt	Model	7	0.09319603	3.32	0.0423
	Error	10	0.02803889		
	Replicate	5	0.02960556	1.06	0.4382
	Treatment	2	0.05602222	8.99	0.0058
Coeff var = 75.16377					
Root dry wt	Model	7	0.06668889	0.87	0.5623
	Error	10	0.07695556		
	Replicate	5	0.07095556	0.92	0.5053
	Treatment	2	0.05602222	0.73	0.5068
Coeff var = 125.4612					
Soil moisture	Model	7	0.16239524	6.37	0.0048
	Error	10	0.02550333		
	Replicate	5	0.01234667	0.48	0.7809
	Treatment	2	0.53751667	21.08	0.0003
Coeff var = 16.98911					
Chlorophyll a	Model	7	0.00043758	7.51	0.0026
	Error	10	0.00005826		
	Replicate	5	0.00005028	0.86	0.5377
	Treatment	2	0.00140582	24.13	0.0001
Coeff var = 29.82284					
Chlorophyll b	Model	7	0.01396797	12.99	0.0003
	Error	10	0.00107520		
	Replicate	5	0.00249026	2.322	0.1210
	Treatment	2	0.04266224	39.68	0.0001
Coeff var = 9.220524					

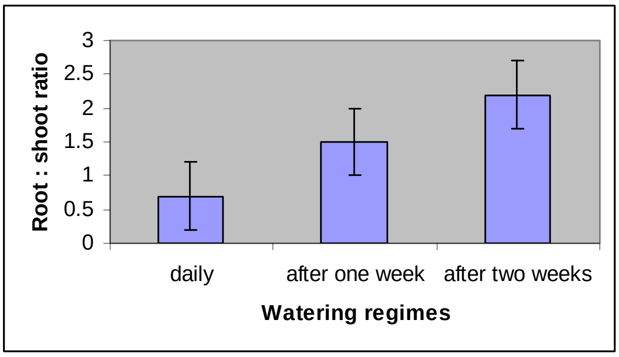

Figure-1. A graph showing the effect of watering regimes on root: shoot ratio of African nightshades after 35 days of water treatment. Means of six replicates ±S.E.

DISCUSSIONS

The results of the study indicate that water deficit decreased leaf number, leaf area, and leaf water content, shoot height, shoot dry weight, and chlorophyll concentration. However root dry weight is not significantly reduced. The results show that African nightshades adapts to soil water deficit stress mainly by regulating transpiration through reduction of leaf area and possibly stomatal conductance. Decreases in leaf number and leaf area are common occurrences in water deficit stressed plants (Luvaha *et al.*, 2008).

Reduction in leaf number under extreme water deficit may have been due to reduction in leaf formation. Reduction in number of leaves can be a phenomenon by the plants to reduce transpiration surface hence water loss. Similar results have been observed in mango rootstock seedlings, which show a decline in number of leaves due to drying or senescence of lower mature leaves (Luvaha *et al.*, 2008).

Leaf area development is directly related to the yield of African nightshades since the edible part is the leaf. Leaf area was greatly reduced by water deficit (Table-2). Water deficit reduces leaf growth by reducing rates of cell division and expansion due to turgor loss. It is well known that as soil water availability is limited, plant growth is usually decreased. This was previously considered to be due to turgor loss in the expanded cells. More recent studies, however, show that stem and leaf growth may be inhibited at low water potential despite complete maintenance of turgor in the growing regions as

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a result of osmotic adjustment (sharp, 1996). This suggests that the growth inhibition may be metabolically regulated possibly serving an adaptive role by restricting the development of transpiring leaf area in water stressed plants.

Root growth was less inhibited than shoot growth under water stress (Table-3). This is in agreement with studies conducted by Kirnak *et al.,* (2001) who reported that some roots continue to elongate at low water potentials that completely inhibited shoot growth. In plants growing in drying soil, the development of the root system is usually less inhibited than shoot growth and may even be promoted. In addition, roots growing at low water potential become thinner and this change in morphology is believed to be adaptive such that roots can further concentrate their use of resources (Sharp *et al.,* 2004). Maintenance of root growth during water deficit is an obvious benefit to maintain adequate plant water supply, and is under genetic control.

The reduction in shoot growth under extreme water deficit (Table-3) may have been due to reduced turgor, which affected cell division and expansion. However cell division has been reported to be less sensitive to water deficit than cell expansion or enlargement. Severe water deficit reduced stem elongation in mango seedling (Luvaha *et al.,* 2008), as in this study with African nightshades seedlings.

Chlorophyll concentration reduced with increase in water deficit. This could be attributed to an increase in oxidative stress. Under more prolonged water deficit, dehydration of plant tissue can result in an increase in oxidative stress, which causes deterioration in chloroplast structure and an associated loss of chlorophyll. This leads to a decrease in the photosynthetic activity (Jafar *et al*., 2004). This leads to a decrease in the photosynthetic activity (Jafar *et al*., 2004). The losses in chloroplast activity include decreases in the electron transport and photo-phosphorylation, and are associated with changes in conformation of the thylakoids and of coupling factor (ATP synthetase, a sub-unit of the thylakoids), and decreased substrate binding by coupling factor (Musyimi, 2005). The decrease in chlorophyll a and chlorophyll b under water deficit suggests that the chlorophyll pigments in these leaves were not resistant to dehydration.

Reduction in chlorophyll concentration in water stressed plants could indirectly lead to a decrease in photosynthetic activity. Increase in chlorophyll concentration in well watered plants, could have in turn led to increased protein synthesis and this has a direct consequence on the plant growth and photosynthesis hence increase in shoot dry weight.

Root- shoot dry weight ratio increased with increase in water deficit (Figure-1). This can be due to differential sensitivity of the root and shoot biomass production to soil water deficit. This differential sensitivity has also been reported in spider plant (*Gynandropsis gynandra*) (Masinde *et al.,* 2005). This is an adaptation to water deficit in most plants growing in arid conditions to increase the surface area for water absorption while reducing transpiration.

CONCLUSIONS

From the study it can be concluded that different watering regimes affect the growth of African nightshades seedlings. The African nightshades were found to be sensitive to water stress especially if watering is delayed up to two weeks. Water deficit reduced the number of leaves, leaf area, shoot height and chlorophyll concentration, which consequently reduced biomass production. Root: shoot ratio increased with increase in water deficit. There was no significant reduction in root growth in the water stressed plants. Maintenance of root growth during water deficit is an obvious benefit to maintain adequate plant water supply. Water deficit significantly affected the growth of African nightshades seedlings. Watering the seedlings once per week and once after two weeks demonstrated that these plants are very sensitive to drought; hence for good growth they need to be watered at least every week.

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