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# Responses of slenderleaf rattlebox (*Crotalaria ochroleuca*) to water deficit

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**Key words:** Water deficit, *Crotalaria ochroleuca*, malnutrition, food security.

**Abstract**

Water deficit affects plant growth and development thus affecting crop productivity. It is the major a biotic stress experienced in the vast semi arid and arid regions of Kenya and the world as a whole. Water deficit mainly causes reduction in plant growth and development. Crotalaria ochroleuca is an important indigenous leafy vegetable which is a legume grown and consumed in Kenya and Tanzania and other parts of Africa. The slender leaf rattlebox is very rich in protein, calcium, iron, Beta carotene and ascorbic acid and therefore can contribute to good human health therefore increased production of this vegetable should be used to solve the problem of malnutrition in the country and also act as food security. A study was conducted to evaluate the responses of slender leaf rattlebox to Water deficit. The study as carried out at the University Botanic Garden. Twenty seeds of slender leaf rattlebox were arranged in a randomized complete block design (RCBD) with six treatments and four replications. After 15 days of germination, the plants were thinned up to four plants per pot and subjected to six treatments viz: watering daily (U), watering after 3 days (V), watering after 6 days (W), watering after 9 days (X), watering after 12 days (Y) and watering after 15 days (Z). Data on growth parameters (shoot height, leaf length, leaf width and number of leaves per plant) were collected on weekly basis. Leaf area, shoot/root fresh and dry weights, leaf chlorophyll concentration at different water regimes was also determined. Data collected was subjected to Analysis of variance (ANOVA) using CO-STAT statistical package and treatment means were separated and compared using least significance difference (LSD). The results show that Crotalaria ochroleuca seedlings are very sensitive to water deficit conditions. Water deficit significantly ($P \leq 0.05$) decreased the shoot heights, leaf number, leaf area, shoot and root fresh and dry weights and chlorophyll content in the Crotalaria ochroleuca seedlings. The study indicated 55 % reduction in shoot height, 90 % reduction in leaf area, 71% in fresh weight and 90% reduction in dry weights at water deficit treatments compared to the control plants. It may be recommended that for proper growth and development of Crotalaria ochroleuca plants, daily watering or at least once in three days should be carried out.

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**Introduction**

Among various types of vegetables, leafy vegetables are the most commonly consumed in Kenyan daily diet. The importance of leafy vegetables in the developing countries has been recognized due to their nutritional and medicinal value (Prasad *et al.,* 2008). The genus *crotalaria* includes about 500 species of herbs and shrubs and Africa is by far richest with approximately 400 species (Polhill, 1982), *Crotalaria* is mainly found in damp grassland, especially in floodplains, depressions and along edges of swamps and rivers, but also in deciduous bush land, roadsides and fields. It grows in open localities with adequate sunshine at 300–2000 m altitude. It is favored by warm conditions, and after the crop is well established and has formed long taproots and long lateral roots, it can tolerate rather dry conditions. The two African species used as a vegetable are *Crotalaria ochroleuca* and *Crotalaria brevidens*. *Crotalaria ochroleuca* is closely related to *Crotalaria brevidens* and information cannot always be attributed to either one of them with certainty (Abukutsa-Onyango, 2007). *Crotalaria ochroleuca* can best be distinguished by the color of the flower which is pale yellow while bright yellow in *Crotalaria brevidens*, the calyx (glabrous vs. often puberulous), and the fruit diameter ((1–) 1.5–2 cm vs. 0.5–1 cm). Another distinguishing factor is taste whereby *Crotalaria ochroleuca* has a mild taste whereas *Crotalaria brevidens* has a bitter taste, but both species are commonly called rattle pod, rattle box, sunnhemp or slenderleaf (Abukutsa-Onyango, 2007). *Crotalaria ochroleuca* is found almost throughout tropical Africa except the north-eastern and most southern parts and most islands of the Indian Ocean. Outside Africa, it has become naturalized in Brazil, the United States (Florida), Australia, New Guinea and China. It is used as a wild or cultivated vegetable in several African countries: Senegal, Nigeria, Cameroon, Congo, DR Congo, southern Sudan, western Kenya, Uganda and north -western Tanzania (Sarwatt and Mkiwa, 1987).

*Crotolaria ochroleuca* is an important indigenous leafy vegetable which is a legume grown and consumed in Kenya and Tanzania among other parts of Africa. It is known as 'marejea' in Tanzania (Mkiwa *et al.*, 1988) 'mitoo' in Kenya and 'alaju' in Uganda. *Crotalaria ochroleuca is* an erect much-branched annual or short-lived perennial herb; branches ascending, with short appressed hairs. It has bright green leaves, and grows to a height of 250cm. The flowers are pale yellow or creamish in colour and the seeds are normally but not always light yellow and the pods are wider in diameter and big. The slender leaf rattlebox is very rich in protein, calcium, iron, Beta carotene and ascorbic acid and therefore can contribute to good human health therefore increased production of this vegetable should be used to solve the problem of malnutrition in the country and also act as food security (Florence *et al.*, 2010). The nutritional value is comparable to other dark green leafy vegetables, except that the dry matter content is higher than average. Analyses in Kenya gave per 100 g fresh weight: protein 4.2–4.9 g, Ca 270 mg, Fe 4 mg, β-carotene 2.9–8.7 mg, ascorbic acid 115–129 mg (Schippers *et al.,* 2004). The bitterness in the leaves is caused by the presence of toxins such as pyrrolizidine alkaloids, diterpenes and phenolic compounds. The young shoots are harvested, but towards the end of the season also individual leaves are collected (Abayomi *et al.*, 2001).

Members of the pea/bean family are good soil builders because their roots support nitrogen-fixing bacteria which improve soil quality and help in maintenance and improvement of soil fertility (Daimon *et al.*, 1995), but they remain largely unexploited (Samba *et al.*, 2002). Like the common sun hemp (*Crotalaria juncea* L.) it is used as a green manure in crops or fallows, but this is not common. *Crotalaria ochroleuca* is also known to suppress nematode populations and is locally used by East African farmers either in crop rotations or as a companion crop with nematode-susceptible vegetables such as tomatoes besides being used as livestock fodder and seeds are fed to poultry (Nyalemegbe *et al.*, 2011). Furthermore cultivation of *Crotalaria ochroleuca* also leads to diversification in agricultural field. In areas with insufficient amount of rainfall, irrigation has to be done. Despite the numerous agronomic advantages of *Crotalaria ochroleuca* little research has been done on its response to water deficit and there is scarce documented literature on the response of *Crotalaria ochroleuca* to water deficit. However, In Kenya most regions are prone to drought and it is the major environmental factor limiting crop production in the arid and semiarid areas of Kenya and has contributed greatly to increased food insecurity (Florence *et al.*, 2010). The impact of drought stress on total plant surface and plant response to drought stress are very intricate, because it reflects combination of stress impacts and plant response in all essential levels of plant over time and place (Blum, 1996). Drought stress or lack of water is not only limited to arid or semi arid areas, but also sometimes, due to irregular distribution of rain. It's obvious that drought stress causes significant decrease of plant yield (Kumar and Singh, 1998). In areas with insufficient amount of rainfall, irrigation has to be done but the appropriate watering regimes and the amount of water required for proper growth and production of *Crotalaria ochroleuca* is not known. Water stress is an important environmental factor that affects photosynthesis, affecting plant growth (Chaves *et al.*, 2002; Akcay *et al.*, 2010). Water stress in plants is characterized by reduction of water content and decrease in cell enlargement and growth. Water stress, among other changes, has the ability to reduce the tissue concentrations of chlorophylls and carotenoids (Kiani *et al.,* 2008). It reduces plant growth by affecting various morphological and biochemical processes (Jaleel *et al.,* 2008; Farooq *et al.*, 2008). In plants, a better understanding of the morpho-anatomical and biochemical basis of changes in water deficit tolerance could be used to select or create new varieties of crops to obtain a better productivity under water stress conditions (Nam *et al.*, 2001; Martinez *et al.*, 2007). The reactions of plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of growth (Shao *et al.,* 2008; Jaleel *et al.*, 2008). Understanding plant responses to drought is of great importance and also a fundamental part for making the crops stress tolerant (Reddy *et al.*, 2004; Zhao *et al.,* 2008). Thus the research aimed to investigate the response of *Crotalaria ochroleuca* to water deficit by subjecting them to different watering regimes.

**Materials and methods**

The experimental study was conducted in the green house between February 2012 and August 2012 in the University Botanic garden, Maseno. The green house was naturally illuminated and the light, $CO_2$ concentration and temperature conditions were not controlled. Top soil was obtained from a portion of the garden. The garden soils have been classified as Acrisol, well drained and acidic with pH of 4.6 - 5.4 (Mwai, 2001).

*Experimental materials*

The seeds of *Crotalaria ochroleuca* were obtained from adaptability trials in the University Botanic garden, Maseno. About 3.5 kg of soil obtained was solarised and put in 5 litre pots. The pots were perforated at the bottom to prevent water logging. Eight viable seeds were planted in each pot and watered regularly for germination to occur. The plants received equal quantities of water for 10 days for uniform crop establishment. After 15 days, the plants were thinned to four plants per pot and the plants subjected to different watering regimes.

*Experimental design and treatments*

The treatment combinations consisted of six levels of watering regimes, viz; watering daily (U), watering after 3 days (V), watering after 6 days (W), watering after 9 days (X), watering after 12 days (Y) and watering after 15 days (Z)], 350 ml of tap water was added to every pot during the experimental period. The treatments were replicated four times and the pots arranged in a completely randomized block design. The weeds were controlled by hand pulling. Data collection commenced 15 days after sowing with the onset of the water treatments and was collected on weekly basis.

Measurement of parameters

*Shoot height*

Shoot height was measured from the soil level at the stem base to the shoot apex of the seedling using a meter rule. Four plants per treatments were sampled for the measurement. The measurements were carried out weekly up to the end of the experiment.

*Number of leaves*

The number of fully expanded mature leaves of each of the four plants per treatments sampled for measurements were counted and recorded every week up to the end of the experiment.

*Leaf area*

Leaf area was determined on four plants per treatment using the formula $A_L = 0.73(L_L \times W_L)$, where $A_L$ is the leaf area, $L_L$ is the leaf length, $W_L$ is the maximum width measured for each leaf on each plant.

*Root and shoot fresh and dry weights*

At the end of the experiment, the whole plants from each treatment were carefully uprooted from the soil, rinsed, and weighed using an electronic weighing balance to obtain the fresh weights. The plants were packed separately in envelopes and dried at 72°C in an oven for 48 hours to a constant weight after which the dry weights were obtained using an electronic weighing balance (Wood and Roper, 2000).

*Leaf chlorophyll content*

Chlorophyll content (SPAD Index) of flag leaf of four plants per treatment for all the replicates was estimated non destructively using a portable chlorophyll meter (SPAD-502 Minolta Co. Japan). The fully expanded leaves of 4 plants per treatments were used for SPAD measurement. Triplicate readings were taken on one side of the midrib of each single leaf blade, midway between the leaf base and tip and then averaged.

*Data analysis*

Data obtained from the study was subjected to analysis of variance (ANOVA) using COSTAT statistical computer package (Version 6.4). The treatments means were separated and compared using Least Significance Difference (L.S.D.) test at 5% level.

**Results**

*Shoot height*

Shoot height was significantly ($P \leq 0.05$) reduced by water deficit treatment (Table 1). The control plants recorded significantly higher heights as compared to water deficit treatments. Plants that were watered after every 12 days and after every 15 days had the lowest heights.

*Root and shoot fresh weights*

Root and shoot fresh weights were significantly affected ($P \leq 0.05$) by the treatments (Table 1). The most stressed plants recorded the least fresh weights while plants watered daily recorded the highest fresh weight. Fresh weights of plants watered after every twelve days recorded about 71% reduction from the control.

*Root and shoot dry weights*

The root and shoot dry weights were significantly ($P \leq 0.05$) affected by water deficit. Plants watered daily recorded the highest dry weights (Table 1). There was no significant difference in the dry weights of plants watered after nine days and those watered after twelve days. The dry weight was reduced by 90% in the most stressed plants compared to the control treatment.

*Leaf number*

The number of leaves per plant was significantly ($P \leq 0.05$) affected by the watering regimes (Table 2). There was no significant difference between plants watered daily and plants watered after every three days though they were significantly different from plants watered after every six days and nine days. The production of leaves decreased with increasing water deficit and the plants watered after every 15 days were the most affected.

*Leaf area*

Leaf area decreased significantly ($P \leq 0.05$) from the control among the treatments apart from the plants that were watered after every three days (Table 2). There was no significant difference ($P \leq 0.05$) in leaf area of the plants watered daily and the plants watered after every three days (Table 1). The leaf area was greatly reduced by about 90% in the highly stressed plants compared to the control.

*Leaf chlorophyll content*

There was significance difference ($P \leq 0.05$) in the leaf chlorophyll content as indicated by the SPAD units among the treatments (Table 3). The SPAD units were lowest in the plants watered after every fifteen days. Plants watered daily had significantly ($P \leq 0.05$) higher chlorophyll content as compared to water deficit treatments.

**Table 1.** Treatment means of shoot height, fresh weights and dry weights for Crotalaria ochroleuca grown at six levels of watering treatments (Means of three replicates).

| Treatments | Shoot height (cm) | Fresh weights (g) | Dry weights (g) |
|---|---|---|---|
| Watering daily | 8.85a | 2.36a | 0.19a |
| Watering after 3 days | 7.95b | 1.80b | 0.15b |
| Watering after 6 days | 7.03c | 1.32c | 0.11c |
| Watering after 9 days | 6.05d | 0.91d | 0.05d |
| Watering after 12 days | 4.98e | 0.68e | 0.05d |
| Watering after 15 days | 3.95f | 0.43f | 0.02e |
| LSD | 0.41 | 0.18 | 0.02 |

Means followed by common letters in column are not significantly different by LSD at P = 0.05

**Table 2.** Treatment means of leaf number and leaf area for Crotalaria ochroleuca grown at six levels of watering treatments (Means of three replicates).

| Treatments | Leaf number | Leaf area ($cm^2$) |
|---|---|---|
| Watering daily | 8.25a | 5.24a |
| Watering after 3 days | 8.50a | 4.92a |
| Watering after 6 days | 7.0b | 4.40b |
| Watering after 9 days | 6.5b | 2.15c |
| Watering after 12 days | 5.0c | 0.89d |
| Watering after 15 days | 4.25d | 0.50e |
| LSD | 0.58 | 0.29 |

Means followed by common letters down the column are not significantly different by LSD at P = 0.05.

**Table 3.** Treatment means of total chlorophyll content (SPAD units) for Crotalaria ochroleuca grown at six levels of watering treatments (Means of three replicates).

| Treatments | SPAD units |
|---|---|
| Watering daily | 35.78a |
| Watering after 3days | 34.23b |
| Watering after 6days | 33.0c |
| Watering after 9days | 29.85d |
| Watering after 12days | 24.25e |
| Watering after 15days | 23.21f |
| LSD | 0.75 |

Means followed by same letters down the column are not significantly different by LSD at P = 0.05

**Discussion**

The results from this study indicate that plant height was sensitive to water supply and water deficit significantly led to a decrease in the shoot heights of *Crotalaria ochroleuca* (Table 1). The first two watering regimes which were daily watering and watering after three days produced the tallest plants, while the shortest plants were produced at a higher water deficit (Watering after 15days). The results are consistent with the findings of Mafakheri *et al.* (2010) on chicken pea cultivars and with the results of Emmam *et al.* (2010) who reported decrease in plants height of two common bean cultivars with contrasting growth habits as a result of water deficit. The reduction in shoot growth under extreme water deficit may have been due to suppressed cell expansion and cell growth probably due to low turgor pressure (Jaleel *et al.,* 2009). The reduction in plant height may also have been caused by a reduction in the nutrient uptake by the plants under water deficit conditions since most of the elements are absorbed via roots. The decrease in shoot growth may constitute an adaptive response to water deficit and may be attributed to the reduction in plant cell turgor which may have affected cell division and expansion hence reduction of stem and leaf expansion. Water deficit causes deceleration of cell enlargement and therefore reducing stem lengths by inhibiting inter nodal elongation and also check the tillering capacity of plants (Ashraf and O'leary, 1996).

Whole plant fresh and dry weight significantly declined with moisture deficit (Table 1). This finding is in agreement with the results reported by Emmam *et al.* (2010) and Rosales-Serna *et al.* (2004), who reported significant difference in shoot biomass accumulation among dry bean varieties grown under water deficit conditions. Water deficit may have influenced the height and leaf area per plant which ultimately influenced the shoot dry matter of plants. Reduced biomass production could be associated with reduced rate of leaf production and hence less number of leaves. A reduction of photosynthetic surface by water deficit leads to a substantial drop in the supply of assimilates to the growing parts of the plant hence decreases the ability of plants to produce dry matter. Water enhances cell division and promotes secondary wall formation (Potikha *et al.*, 1997). More recent studies have however shown that stem and leaf growth may be inhibited at low soil moisture content despite complete maintenance of turgor in the growing regions as a result of osmotic adjustment (Bimpong *et al.*, 2011). This suggests that growth inhibition may be metabolically regulated possibly serving as an adaptive role by restricting the development of transpiring leaf area in the water stressed plants.

Leaf number and leaf area were significantly affected by water deficit (Table 2). The amount of leaf production in *Crotalaria ochroleuca* was reduced with an increase in water deficit. The results are in agreement with the findings of Vurayai *et al.* (2011) on bambara groundnuts and Muthomi and Musyimi (2009) who reported a reduction in leaf number and leaf area under water deficit conditions in African nightshade. Reduced number of leaves on a plant greatly reduces the rate of water loss through transpiration, thus the plant under water deficit have reduced leaf formation. The number of leaves and leaf area is directly related to the potential yield of *Crotalaria ochroleuca*, since they are the edible part. Increase in water deficit greatly reduced the leaf area of *Crotalaria ochroleuca*. Reduction in leaf area with increasing water deficit has also been observed in soya bean (Zhang *et al.*, 2004) and many other species (Farooq *et al.*, 2009). The development of optimum leaf area is important to photosynthesis and dry matter yield. The decrease in total leaf number and leaf area under water deficit conditions may be due to reduced turgor in the cells thus less cell division, expansion and extension in the leaves (Vurayai *et al.*, 2011; Deblonde and Ledent, 2001). Leaf expansion during vegetative stage is very sensitive to water stress and according to Yawson *et al.* (2011) higher amount of water is required for rapid canopy expansion during crops development stage. The decrease in leaf area reduces the intercepted solar radiation (Salisbury and Ross, 1992). Controlled changes in leaf structure or anatomy in response to gradually induced drought conditions may increase plant resistance by favouring $CO_2$ assimilation with minimal water loss.

Chlorophyll content in the *Crotalaria ochroleuca* seedlings were significantly decreased by water deficit (Table 3). The results are in agreement with the results of Sikuku *et al.* (2012) on rice plants and of

Nadler and Bruvia (1998) who reported a significant decline in chlorophyll content with increasing water deficit in potato leaves. The significant decrease in total chlorophyll content might be attributed to the increased degradation of chlorophyll pigments due to stress induced metabolic imbalance (Long *et al.*, 1994).

## Conclusions

The results from the study demonstrated that different watering regimes affect the growth of Crotalaria *ochroleuca* seedlings. The growth is sensitive to water deficit especially if watering is delayed up to 15 days and can result to crop loss. Water deficit decreased the shoot heights, leaf number, leaf area, shoot and root fresh and dry weights and chlorophyll content in the *Crotalaria ochroleuca* seedlings. Therefore water deficit has a significant negative impact on the growth of *Crotalaria ochroleuca*.

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