RESPONSE OF TWO SLENDER LEAF (CROTALARIA SPP) SPECIES TO WATERING REGIMES

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Abstract
Slender leaf is an indigenous vegetable in Kenya. Two main species of slender leaf are *Crotalaria brevidens* and *Crotalaria ochroleuca.* Despite their healthy benefits they contain alkaloids and oxalates which in high amounts, are toxic to human. They bind calcium in the body leading to kidney stones. It is necessary to define the best watering regime so as to use water efficiently, since climate change threatens water sustainability. Two pot experiments were conducted between January and December 2014 in a tunnel in JKUAT to evaluate the effects of watering regime on the growth and yield of two slender leaf species and on their alkaloid and oxalate content at two growth stages (vegetative and reproductive). The treatments were the two species and 4 watering regimes (40%, 60%, 80% and 100% of 1500ml). Agronomic data (leaf number, height, girth, flowers and branch number) was collected on tagged plants weekly and subjected to analyses of variance. Alkaloid and oxalates content in the harvested leaves were gravimetrically determined. There was a significant interaction ($p \leq 0.05$) between the water regime and variety with respect to leaf number and branches. Watering regime had a significant ($p \leq 0.05$) effect on alkaloid content in the two varieties. Water deficit (40%) reduces yield and increases alkaloids and oxalates concentration which increases with plant age. Oxalates and alkaloids are significantly more in C. *brevidens* than *C. ochroleuca.* Recommended water regime is 60% after 3 days*. C.ochroleuca can be consumed at both stages while* C. *brevidens* at vegetative stage.

Key words: Slender leaf, *Crotalaria brevidens, Crotalaria ochroleuca* watering regime, alkaloid, oxalates

1.0 Introduction

Slender leaf (*Crotalaria* spp) is an indigenous vegetable found in Kenya that is mainly grown for its young shoots and leaves. It is a nitrogen fixing legume (Schippers, 2002) that belongs to the family Fabacea. Slender leaf is highly nutritious it contains iron, vitamin A and C and 40 % of proteins when 100 grams of fresh weight are consumed. (Abukutsa-Onyango, 2007). It has also been reported to have medicinal applications such as treating stomach related ailments, swelling and malaria (Olembo et al, 1995, Abukutsa-Onyango, 2004). The two main species of slender leaf used as vegetables are *Crotalaria brevidens* and *Crotalaria ochroleuca.* The distinctive differences are that *Crotalaria brevidens* is bitter and shorter while *Crotalaria ochroleuca* is taller and has a mild taste. One of the factors can affect growth, yield and the content of alkaloids and oxalates is the amount of water available to the plant or the crops water use efficiency.

1.1 Water Use Efficiency

Water use efficiency (WUE) of a given species refers to its ability to economize on the water taken up and optimize dry matter production. It is here that it calls for looking into the extent to which withholding water might influence growth, development and yield of any given crop. (Salisbury and Ross, 1978). Very little has been reported on water use efficiency and the effects of watering regimes on growth and yield of slender leaf.

1.2 Alkaloids

Alkaloids include molecules like nicotine, atropine, solanidine and pyrrolizidine among others. (Hostege et al.1995). The bitter taste of slender leaf could be attributed to the presence of alkaloids and phenolic compounds (Schippers, 2002). The recommended daily intake of alkaloids is between 1-75 mg above this it can become toxic to the human body

1.3 Oxalates

Oxalates in plants are synthesized via incomplete oxidation of carbohydrates. In the body, oxalic acid combines with divalent metallic cations such as calcium (Ca^{2+}) and iron (II) (Fe^{2+}) to form crystals of the corresponding oxalates which are then excreted in urine as minute crystals. These oxalates can cause kidney obstruction. These compounds are enhanced under stress conditions like water deficit. (Jaleel et al., 2009)
The aim of experiments was to evaluate the effects of watering regimes on the growth, yield and water use efficiency of two slender leaf species and its effect on the alkaloid and oxalate content of the two species at two growth stages.

2.0 Material and Methods

Two pot experiments were conducted between January and December 2014 in a tunnel in JKUAT to evaluate the effects of watering regime on the growth, yield, and alkaloid and oxalate content of two species of slender leaf at two growth stages. The media was prepared by mixing red soil, sand, manure (3:2:1) ratio of wheelbarrow. The media was put in 5litre pots. Meanwhile water holding capacity of the media was determined gravimetrically. Seeds were obtained from RPE JKUAT Slender leaf project. A total of 32 pots were arranged in a complete randomized design with eight treatment combinations. The two factors in the experiment were four (4) watering regimes W1= 100 %(control at Field capacity) W2= 80% Field capacity W3= 60% Field capacity W4= 40% Field capacity, two (2) species; two harvesting stages (vegetative and reproductive) replicated four times. Fifteen seeds per pot were sown and after seedling emergence and establishment, they were thinned to four plants per pot. Pots were kept at water holding capacity (Field capacity) until the first six true leaves developed when treatments commenced.

Weekly measurements for plant height, number of leaves, number of branches, stem diameter (girth) and flowers were taken for five weeks.

2.1 Laboratory Analysis

2.1.1 Alkaloid Determination

The alkaloid content for two growth stages (vegetative and reproductive) was determined gravimetrically. 5 grams of each oven dried sample was weighed using a weighing balance and dispersed into 10% acetic acid solution in ethanol. The mixture was shaken and allowed to stand for about four hours before it was filtered the filtrate was then evaporated to one quarter of its original volume on hot plate. Concentrated ammonia was then added drop wise in order to precipitate the alkaloids. A pre-weighed filter paper was used to filter off the precipitate and it was then washed with 1% ammonium hydroxide solution. The filter paper containing the precipitate was then dried in an oven at 60^0c for thirty minutes, transferred into desiccators to cool and then reweighed until a constant weight was obtained. Weight of the alkaloid = filter paper weigh/weight of sample *100%. The experiment was repeated thrice for each slender leaf variety and the readings recorded as the average of three replicates. (Harbone, 1973).

2.1.2 Oxalate Determination

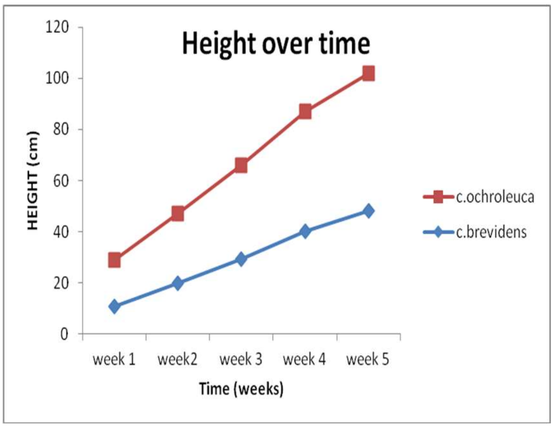
Two (2) grams of the oven dried sample were digested with 10 ml 6 M HCL for one hour and made up to 250 ml in a volumetric flask. The pH of the filtrate was adjusted with concentrated Ammonium solution changes from salmon pink to a faint yellow color. Thereafter, the filtrate was treated with 10 ml of 5% calcium chloride solution to precipitate the insoluble oxalate. The suspension was then centrifuged at 2500 rpm, after which the supernatant was decanted and precipitate completely dissolved in 10 ml of 20% Sulphuric acid. The total resultant filtrate from the dissolution in Sulphuric acid was made up to 300ml.An aliquot of 125 ml was heated until near boiling point and then titrated against 0.05 M of standardized KMnO4 solution to a faint pink color which persisted for about 30 s after which the burette reading was taken. The oxalate content was evaluated from the titre value. (OKE, 1966)

The data was analyzed by performing Analysis of Variance (ANOVA) using Genstat (14th Edition) software. The means were separated at LSD 5% Level to determine the interactions.

3.0 Results

3.1 Plant Height

There was no significant ($p>0.05$) interaction between watering regime and variety with respect to height. There was a significant ($p\leq0.01$) difference between varieties with respect to plant height as shown in fig.1 a. where *C. ochroleuca* was significantly taller than *C. brevidens.* Watering regime and varieties had significant ($p\leq0.01$) effect on plant height in experiment 2. It was observed that plant height increased with increasing watering regime as shown in fig.1b



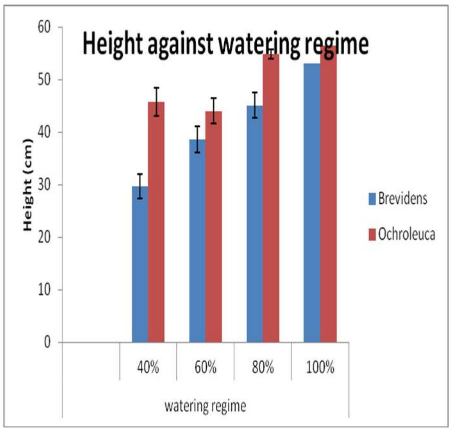


Fig.1 a *Fig.1b*

Fig. 1a: Effect of watering regime and variety on plant height overtime for experiment 1 after treatment

Fig. 1b: Effects of watering regime and variety on plant height at week 2 after treatment for experiment 2

3.2 Number of Leaves

There was significant (p≤0.05) interaction between watering regime and variety with respect to number of leaves. Watering regime had significant effect on the number of leaves with watering regime 60% recording the highest in *Ochroleuca* and watering regime of brevidens recording highest at 40% there was a significant difference between varieties at 1% level as shown in table 1a in experiment 1.

3.3 Girth

There was no significant (p>0.05) interaction between watering regime and variety with respect to girth. Watering regime had no significant (p>0.05) effect on girth of slender leaf. There was a significant difference between varieties at 1% level in the experiment 2 as shown in fig.2a.Girth was shown to increase over time.

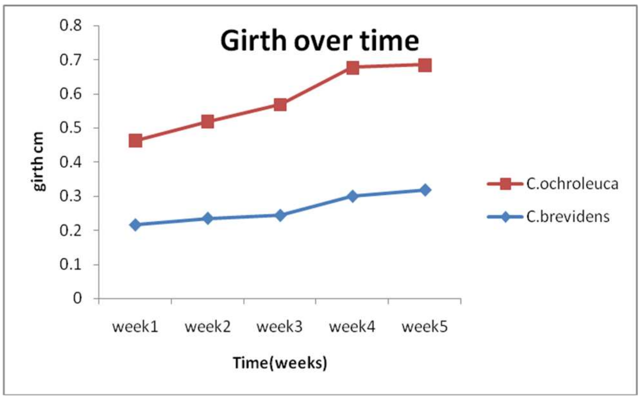


Fig. 2a: Effect of watering regime and variety on the girth over time in experiment

3.4 Branches

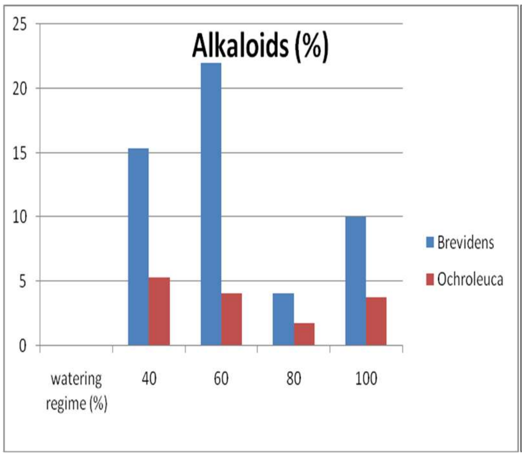
There was significant (p≤0.05) interaction between watering regime and variety with respect to branches. Watering regime had significant (p≤0.01) effect on the number of branches at 40% was highest in Brevidens similarly species were significantly different as shown in table 2a of experiment two.

3.5 Number of flowers

There was no significant (p≤0.05) interaction between watering regime and variety with respect to number of flowers. Watering regime had significant (p≤0.05) effect on the number of leaves with watering regime 60% recording the highest. There was a significant (p≤0.01) difference between varieties as shown in table 3a this was similar in experiment two.

3.6 Alkaloids

There was a significant interaction between watering regime and variety. There was a difference in the percentage of alkaloids between the varieties at both stages. *C.brevidens* had higher alkaloid content compared to *C. ochroleuca.*



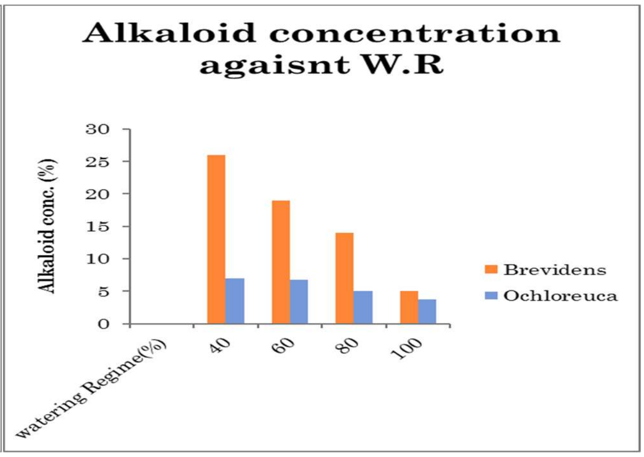


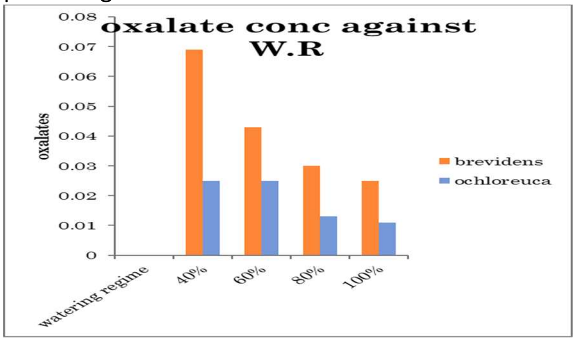
Fig. 3a *Fig. 3b*

Fig. 3a: Effect of watering regime and variety on the alkaloid content at vegetative stage in experiment 1

Fig. 3b: Effect of watering regime and variety on the alkaloid content at reproductive stage in experiment 2

3.7 Oxalates

There was a significant interaction between watering regime and variety. There was a difference in the percentage of oxalates between the varieties as shown in fig 4a in experiment 1 at vegetative stage.



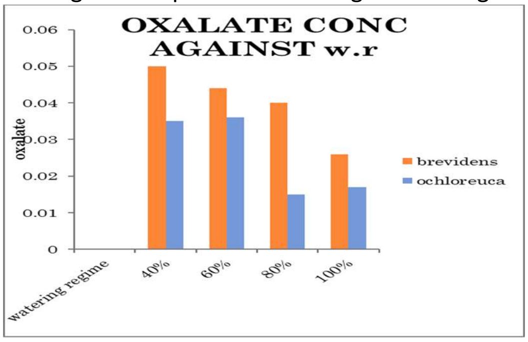


Fig.4a *Fig.4b*

Fig. 4b.1: Effect of watering regime and variety on the oxalate content at vegetative stage in experiment 2

Fig. 4b.2: Effect of watering regime and variety on the oxalate content at reproductive stage in experiment 2

4.0 Discussion

Water is one of the essential requirements of the plant growth it supports tissue elongation and cell division, water stress limits this processes. Water stress is characterized by a limited soil moisture content which drops water potential in the plant. (Chaves, Maroco, and Pereira, 2003). Difference in height is also morphologically *Crotalaria brevidens* grows to a height of 210 cm while *Crotalaria ochroleuca* grows up to 2.5 m tall. (Abukutsa-Onyango, 2007).

Crotalaria brevidens can be said to be more drought tolerant than C.ochroleuca since most leaves were produced at 40%. This can be attributed to the fact that under drought stress, plants physiological characteristics are affected such as transpiration and photosynthesis which in turn affects growth and yield (Cornic and Massacci, 1996). This leads to the reduction in leaf formation it is attributed to the plants effort to reduce the transpiration surface so as to conserve water. (Luvaha, Netondo, and Auma, 2008). Plant growth and development is significantly affected by water deficient which in turn can lead to reduced yields from the plants according to (Sikuku, Musyimi, Kariuki, and Okello, 2013) as observed in *ochroleuca.*

Girth increased with increased water regime,This is because there is increased cell enlargement and elongation during the growth period. Girth forms the shoot part of the slender leaf. The *C.ochroleuca* is taller than *C.brevidens* therefore it may need more support to prevent bending as shown in table3a and 3b Water is important to maintain plant turgidity and thus leaves exposure to sunight and eventually influence the process of photosynthesis.

Brevidens significantly branched at 40% this attributed to the branching characteristic of the brevidens variety(Abukutsa-Onyango, 2007).*Brevidens* has good water use efficiency since it had the highest branching at water regime 40%.water stress enhances branching.

Crotalaria brevidens is an early flowering variety as compared to *crotalaria ochroleuca.* Flowering is a reproductive stage in the growth and development of a plant they form the basis of seed formation. Water stress limits moisture content. A variety of physiological and biochemical changes are developed due to this phenomenon some of them are triggered by the changing water status of the tissues while others are brought up by plant hormones (Chaves, Maroco, and Pereira, 2003).

Alkaloid concentration increases with water stress. These results are in consonance with those of Briske and Camp, who did a research on thread leaf groundsel which was investigated in a controlled environment by monitoring leaf xylem water potential and found out that water deficit increased alkaloid concentration (Briske and Camp, 1982). According to Sofo et.al in response to water stress, plants typically accumulate a wide range of antioxidants including enzymatic anti-oxidants and non-enzymatic antioxidants to quench the reactive oxidative species induced by the stress. Some other plants release antioxidants as a self defense mechanism against pests and other rodents.

Crotalaria brevidens had a higher amount of alkaloids and oxalates as compared to *crotalaria ochroleuca* .This can be attributed to the fact that *Crotalaria brevidens* is the bitter variety the bitterness is attributed to the high amount alkaloid and oxalates present in this slender leaf variety. (Abukutsa-Onyango, 2007). The interaction between physiological age was significant thus they also increase with physiolgical aging.The results of the experiment are consonace with (Nandi, 1996) who reported maximum accumulation of alkaloids in the leaves of Di. Innoxia during flowering stage.

5.0 Conclusion

Interactions between watering regime and variety were significant ($p \leq 0.05$) for number of leaves and branches. There was a significant ($p \leq 0.05$) effect of watering regime on growth and yield of the two slender leaf species.

Interactions between watering regime and variety was significant at the two physiological stages. Alkaloid and oxalate concentration was higher at reproductive than vegetative stage. *Brevidens* had significantly higher alkaloid and oxalate content than ochroleuca.

The recommended water use is at 80% field capacity . *Crotalaria ochroleuca* is recommended for consumption since it has less amount of alkaloid and oxalate concentration.For *Crotalaria brevidens* the recommended harvesting stage is the vegetative one before the alkaloid and oxalate concentration reaches the toxic level.

Table 1a: Effect of watering regime and variety on number of leaves at week 1 treatment in experiment 1

Variety	Watering regime (%)	40	60	80	100	Mean
Brevidens		6.25	5	5	5.5	5.44
Ochroleuca		8	9	7.5	8.75	8.31
Mean		7.13	7	6.25	7.13	
Lsd $_{5\%}$		0.481				

Table 1b: Effect of watering regime and variety on the number of branches at week 1 after treatment in experiment 2

	Watering regime (%)	40	60	80	100	Means
Brevidens		4.25	0.25	2.75	3.75	3.667
Ochroleuca		1.25	0.11	0.25	1	0.6525
LSD (%)	0.706					

Table 3a: Effect of watering regime and variety on the number of flowers at week 4 after treatment in experiment 1

	Water_regime (%)	40	60	80	100	Means
Brevidens		3.5	4.25	2.5	4.5	3.68755
Ochroleuca		1.5	0	2	2	1.375
LSD (5%)	2.152					

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