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EFFECT OF DIFFERENT WATER REGIMES ON THE GROWTH AND PHYTOCHEMICAL CONSTITUENTS OF *ACALYPHA WILKESIANA* HARVESTED AT 3AM AND 3PM

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ABSTRACT
The effect of water deficit on the growth and phytochemical composition of *Acalypha wilkesiana* was studied from 7 days of transplanting. Growth parameters such as plant height, number of leaves, leaf area, fresh weight and dry weight of the plant were analysed for six weeks. The quantitative analysis of tannins, alkaloids and flavonoids in leaves of *A.wilkesiana* harvested at 3am and 3pm were also carried out. Matured stem cuttings of bronze red *A.wilkesiana* were planted. The plants were divided into two sets; one set was watered daily and the other set was watered every three days. There was no marked difference in watering daily or watering at 3 days interval in terms of plant growth. However, daily watering boosted the phytochemicals investigated. The concentrations of total alkaloids and total tannins were significantly higher when leaves were harvested at 3am while the concentrations of total flavonoids were significantly higher when leaves were harvested at 3pm. The result shows that *A.wilkesiana* can withstand irrigation delay of up to 3-days interval in terms of growth. . However, daily watering will boost the accumulation of the phytochemicals investigated though the time of harvest is a predominant factor in determining the concentrations of these phytochemicals.

KEY WORDS: *Acalypha wilkesiana*, watering, time of harvest, tannin, alakaloid, flavonoid

INTRODUCTION

Water is vital to plant life for growth, development and productivity. It is an integral part of living systems. It is ecologically important because it is a major force in shaping climatic patterns and biochemically important because it is a necessary component in physiological processes (Brown, 1995). Water deficit is among the environmental constraints that affect crop growth and production worldwide (Ashraf and Foolad, 2007). Plants adapt to stresses by different mechanisms including changes in morphological, physiological and biochemical processes (Bohnert *et al*., 1995). These, include metabolic adjustments that lead to accumulation of organic solutes such as sugars, starches, lipids and proteins (Gill *et al.,* 2003). Water deficit is known to increase the secondary metabolites concentration in plant tissues. *Acalypha wilkesiana* is an evergreen shrub belonging to the family Euphorbiaceae. Its common names is Copper leaf (Burkhill, 1997). It is a tropical and subtropical plant. The leaves are coppery green with red splashes in colour. The leaves are large and broad with teeth around the edge. They can be flat or crinkled. The stem is erect with many branches. The branches have fine hairs. They have separate male and female flowers on the same plant. The male flowers are in long spikes which hang downwards while the female flowers are in short spikes. The flower stalks are 10-20cm long. It is a shrub of 1-5m high with pubescent to glabrous leaves which are broad and oval (Hutchinson and Dalziel, 1954). It does best in rich, moist but fast draining soil. It also does best in partial shade or sun but leaf colour develops best in bright light. It is damaged by both drought and frost. However, it must be maintained in warm, humid, bright environment. It has soil pH requirement of 5.6-7.5 (acidic to slightly alkaline). It is propagated using stem cuttings as it does not set seed that is: its flowers are sterile or plants will not come from true seeds (Sofowora, 2006) and can also be propagated by air layering. Oladunmoye (2006) reported that the mechanism of antimicrobial activities of *A. wilkesiana* is through the release of sodium and potassium ions which is probably affected by its extracts. This is further strengthened by the fact that *A. wilkesiana* contains flavonoids, alkaloids and tannins (Adekunle *et al*., 2011). The leaves are effective in treating *Pityriasis versicolor, Tinea pedia* and *Candida intetrigo* with 100% cure as well as *Candida albicans, Microsporum audonii* and *Aspergillus flavus* (Alade and Irobi, 1993). The leaf extract also has antimicrobial activity. It is used against methicilin resistant *Staphylococcus aureus* (Akinyemi *et al*., 2005). *Acalypha wilkesiana* is used for treating cutaneous diseases like dermatomycoses (*Adesina et al*., 2000). Adekunle *et al*. (2011) have shown that *A. wilkesiana*, leaves should be harvested at the early hours of the day at 3am to 9am, especially at 3am.to obtain high potency in the antifungal activity of *A. wilkesiana*. This is contrary to the opinions of traditional medicine practitioners who presume that 'plants sleep' at night and thus the leaves of *A. wilkesiana* will not be medicinally active between 7pm-5am (Adekunle's personal communications with practitioners, and Sofowora, 2006). Adekunle *et al*. (2011) further concluded that the time of harvest of *Acalypha wilkesiana* is definitely an important factor in getting maximum antifungal activity of this plant.

The aim of this experiment was to determine the effect of different water regimes on the growth and phytochemical constituents of *A. wilkesiana*. The experiment was further aimed at analyzing the phytochemical content of leaves of

A. wilkesiana at 3am and 3pm in order to ascertain the disparity in the antifungal activity of the leaves at early hours of the morning as against during full day.

METHODS

Mature stem cuttings of Bronze red *Acalypha wilkesiana* were obtained from the Botanical garden of the University of Lagos. The experimental set up was done inside the glass house in the botanical garden. Mature stem cuttings of *Acalypha wilkesiana* were cut into smaller pieces of about 21cm in length. Each stem cutting was placed in perforated polyethene bags filled with soil. The plants were placed in the nursery for 14 days after which they were transplanted to a glass house. The plants were separated into two sets; the first set of plants was watered daily and second sets of plants were watered every three days. Watering of the plants was done regularly. Harvesting of plant materials to measure growth parameters commenced after seven (7) days of transplanting and lasted for six (6) weeks). The rest of the plants were harvested for phytochemical analysis after eight (8) weeks of transplanting. Harvesting for phytochemical analysis took place at different times of day; 3am in the morning and 3pm in the afternoon.

GROWTH PARAMETERS

Plant height

The heights of plants were measured with a meter rule from each node.

Leaf Area

The leaf area was measured by tracing out fresh leaves on a graph sheet. The total number of boxes present in each traced leaf on the graph represents the area of the leaf.

Fresh Weight

After harvesting, the plants were placed in polyethene bags (to prevent them from drying up) and taken to the laboratory. The fresh weight of each plant was determined by placing fresh stalk with leaves on a weighing balance (Metter Pm 34 – k DELTA RANGE) and the readings were recorded.

Dry Weight

After weighing, the plants were kept in envelopes and placed in an oven set at 80^{o}C for 3 days after which the dry weights of the plants were determined.

PHYTOCHEMICAL ANALYSIS

The harvested plants were air dried for three weeks. The precise quantities of the investigated phytochemicals were determined using the following procedures. This was done in triplicates and their means calculated.

Tannin determination by Van-Burden and Robinson (1981) Method

500mg of the *A.wilkesiana* plant sample was weighed into a 50 ml plastic bottle. 50ml of distilled water was added and shaken for 1h in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. 5 ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1 NHCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120m within 10min.

Alkaloid Determination using Harborne (1973) Method

5g of the *A. wilkesiana* plant sample was weighed into a 250ml beaker and 200 ml of 10% acetic acid in ethanol was added. It was covered and allowed to stand for 4 hrs. This was filtrated and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

Flavonoid Determination by the Method of Bohm and Kocipai-Abyazan (1994)

10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible, evaporated to dryness over a water bath and weighed to a constant weight.

STATISTICAL ANALYSIS

The reading and analyses were done using triplicates from each group of *A. wilkesiana plant* harvested weekly. A one way analysis of variance (ANOVA) was used to analyse the growth data while a two way analysis of variance was used to analyse phytochemical constituents at a 5% level of significance (p=0.05).

RESULTS

The objective of this work was to determine the effect of different water regimes on the growth and phytochemical constituents of *Acalypha wilkesiana* plants. The plants were divided into two (2) sets. One set received daily watering and the other set was watered every three days.

Figure 1 shows the means of various growth parameters of *A. wilkesiana* plants subjected to two water regimes. Plants watered daily had higher plant height than those watered every three days throughout the period of analysis (A). However, the differences were only significant (p=0.05) at 28 and 35 days of treatment. Plants watered daily had larger leaf areas than those watered every three days throughout the period of analysis (B). However, the differences were only significant (p=0.05) at days 14 and 28 days of treatment. The fresh weight of plants watered daily were significantly higher (p=0.05) than those plants watered every three days except at 21 and 28 days of treatment (C). Also, the dry weights of plants watered daily were higher than those watered every three days. However, the differences were only significant (p=0.05) at 7 and 14 days of treatment.

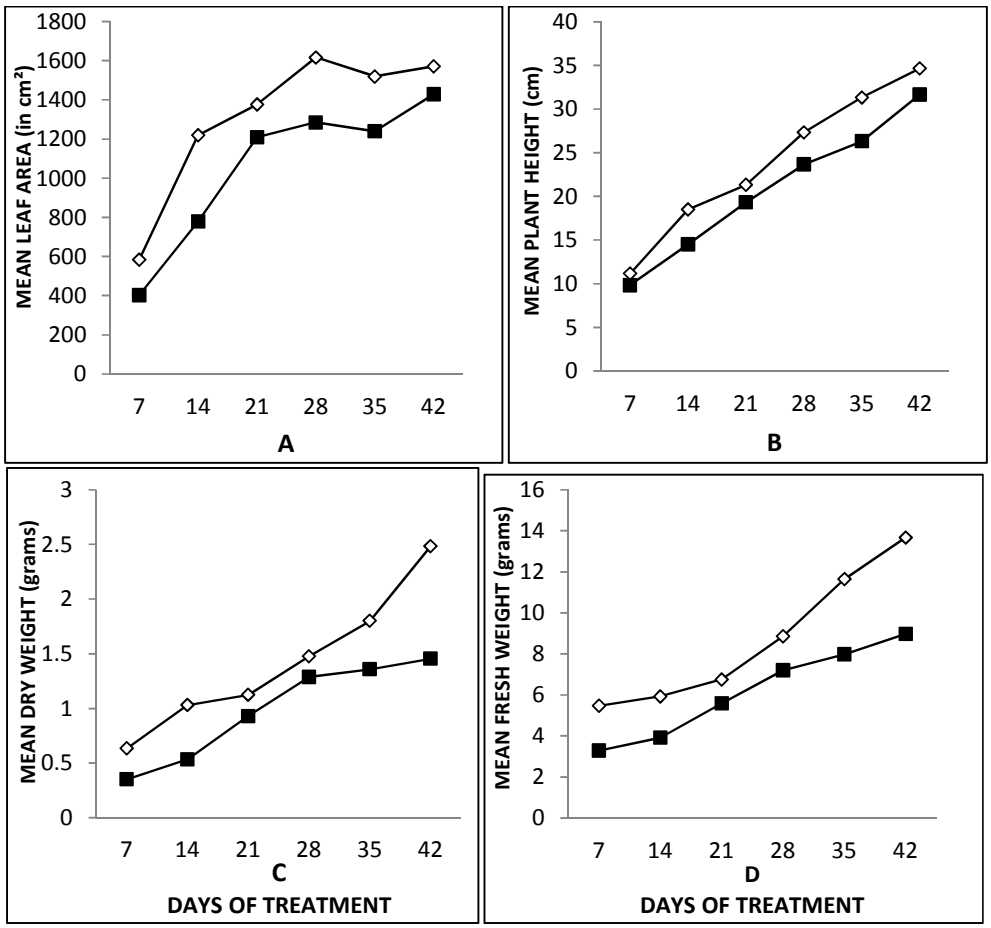

FIGURE 1: Means of various growth parameters of *Acalypha wilkesiana* plants over time subjected to daily (☐) and 3-days interval (■) water regimes.

The concentrations of tannins in leaves of plants watered daily were significantly higher in plants harvested at 3am than those harvested at 3pm (Table 1). Similar results were obtained in leaves of plants watered every three days. The mean concentrations of tannins in leaves of plants watered daily were significantly higher than those watered every three days, irrespective of harvest time.

TABLE 1: Concentrations of tannins in leaves of *Acalypha wilkesiana* subjected to different water regimes and harvested at 3am and 3pm. Means in the same column tagged with different letters are significantly different at p=0.05. Means tagged with asterisks are significantly higher than values on same row at p=0.05

	Watering Regime	
Time of harvest	Daily watering (mg/g)	Watering every three days (mg/g)
3am	121.55 b; *	105.07 b;
3pm	88.80 a; *	12.71 a;

The concentrations of alkaloids in leaves of plants watered daily were significantly higher in plants harvested at 3am than those harvested at 3pm (Table 2). Similar results were also obtained in plants watered every three days. The mean concentrations of alkaloids in leaves of plants watered daily were significantly higher than those watered every three days, irrespective of time of harvest.

TABLE 2: Concentrations of alkaloids in leaves of *Acalypha wilkesiana* subjected to different water regimes and harvested at 3am and 3pm. Means in the same column tagged with different letters are significantly different at p=0.05. Means tagged with asterisks are significantly higher than values on same row at p=0.05

	Watering Regime	
Time of harvest	Daily watering (mg/g)	Watering every three days (mg/g)
3am	895.73 b; *	522.85 b;
3pm	817.98 a; *	188.60 a;

The concentrations of flavonoids in leaves of plants watered daily were significantly higher in plants harvested at 3pm than those harvested at 3am (Table 3). Similar results were also obtained in leaves of *A. wilkesiana* watered every three days. The concentrations of flavonoids in leaves of plants watered daily were significantly higher than those watered every three days, irrespective of time of harvest.

TABLE 3: Concentrations of flavonoids in leaves of *Acalypha wilkesiana* subjected to different water regimes and harvested at 3am and 3pm. Means in the same column tagged with different letters are significantly different at p=0.05. Means tagged with asterisks are significantly higher than values on same row at p=0.05

	Watering Regime	
Time of harvest	Daily watering (mg/g)	Watering every three days (mg/g)
3am	199.50a; *	154.94a;
3pm	251.68b;	188.60b;

DISCUSSION

The effect of different water regimes on the growth and phytochemical constituents of *Acalypha wilkesiana* plants harvested at 3am and 3pm was investigated. The plants were divided into two (2) sets. One set received daily watering and the other set was watered every three days. From the results, the plant heights, number of leaves, total leaf area, fresh weights and dry weights of *A. wilkesiana* plants watered daily were numerically higher than those of plants watered every three days. However, the differences were not significant at p=0.05 most of the time and where differences were significant, the time period was not consistent, that is; beginning, middle or end of period of analysis across growth parameters measured. It therefore implies that daily watering or three days interval watering of *A. wilkesiana* did not affect plant growth significantly more than the other.

Tannin concentrations in leaves of *A. wilkesiana* were significantly higher in plants harvested at 3am than those harvested at 3pm irrespective of the watering condition. Daily watering boosted tannin concentration than watering at three days interval irrespective of time of harvest. The result shows that tannin concentration is higher in leaves harvested in the early hours of the day and even better when plants are watered daily. This is similar to the report of Ayet *et al.* (1997) who recorded higher concentrations of tannins in leaves of *Lens culinaris* watered daily and harvested in dark conditions than those harvested in light conditions. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sodipo *et al*., 1991). This investigation can be linked to that of Adekunle *et al*. (2011) who revealed that to obtain high potency in the antifungal activity of *Acalypha wilkesiana*, leaves should be harvested at the early hours of the day at 3am to 9am, especially at 3am. The presence of tannins in high quantities in the early hours of the morning could be responsible for the antifungal activity of this plant. Alkaloid concentrations in leaves of *A. wilkesiana* were significantly higher in plants harvested at 3am than those harvested at 3pm irrespective of the watering condition. Daily watering boosted alkaloid concentration than watering at three days interval irrespective of time of harvest. The result shows that alkaloid concentration is higher in leaves harvested in the early hours of the day and even better when plants are watered daily. Alkaloids are heterocyclic nitrogenous compounds that also act as antifungal agents. The presence of alkaloids in high quantities in the early hours of the morning could be responsible for the antifungal activity of this plant as reported by Adekunle *et al*. (2011).

Flavonoid concentrations in leaves of *A. wilkesiana* were significantly higher in plants harvested at 3pm than those harvested at 3am irrespective of the watering condition. Daily watering boosted flavonoid concentration than watering at three days interval irrespective of time of harvest. The result shows that flavonoid concentration is higher in leaves harvested in the afternoon and even better when plants are watered daily. This is similar to the report of Xie and Wang (2006) who recorded that flavonoid synthesis in leaves of *Ginkgo biloba* is absolutely dependent on light and its biosynthetic rate is related to light intensity and density. An activation of flavonoid biosynthesis by solar radiation was also detected in *Vaccinium myrtillus* (Witzell *et al.,* 2003).

The synthesis and accumulation of tannins and alkaloids in *Acalypha wilkesiana* leaves appear to be enhanced in the dark while that of flavonoids appear to be enhanced by light. In conclusion, *Acalypha wilkesiana* can withstand irrigation delay of up to 3-days interval in term of growth. However, daily watering will boost the accumulation of the phytochemicals investigated though the time of harvest is a predominant factor in determining the concentrations of these phytochemicals.

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