IMPACT OF WATER DEFICIT STRESS ON GROWTH AND ALKALOID CONTENT OF ORGANS OF *SPIGELIA ANTHELMIA* (L.)

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ABSTRACT

Experiments were conducted to study the effect of water deficit stress on the growth and alkaloid content of different organs of *Spigelia anthelmia* (L), a medicinal plant used locally as an anthelminthic. Plants were subjected to 6 days drought at the early (EV plants) and late (LV plants) vegetative stages (30-35 and 52-57 days after planting respectively). Water stress caused a reduction in height, leaf area, root biomass, whole plant biomass, leaf area ratio and relative growth rate of stressed plants but the impact was more intense in EV plants. Minimal differences in alkaloid content (21.5-22.8 mg g^{-1}) occurred among organs (fruit, leaves, stem, and roots) of plants subjected to stress at both vegetative stages and the control. Thus, water deficit stress at the vegetative stage of *Spigelia anthelmia* caused reductions in growth but did not affect the concentration of alkaloid in the plant organs.

Keywords: Water Deficit, Alkaloids, Growth, *Spigelia anthelmia*

INTRODUCTION

Alkaloids are secondary metabolites synthesized and accumulated by numerous higher plants. About 12,000 alkaloids have been found to occur in approximately 20% of flowering plants, mostly herbaceous dicots. A few alkaloids may be found in several genera or even families but most species display their own unique, genetically determined patterns (Hopkins and Hüner, 2004). Furthermore, individual alkaloids may be restricted to particular organs, such as roots, leaves, cortex, young fruits and seeds, in parenchyma tissue or in cells. The same plant may accumulate both similar and different alkaloids. During the vegetative period, alkaloid concentration undergoes changes, the peak coinciding with flowering. At the end of vegetative period, alkaloids accumulate in seeds and roots (Hopkins and Hüner, 2004; Hondelmann, 1984).

Though the physiological roles of these metabolites have not been clearly demonstrated, alkaloids accumulated in the underground parts of a plant have been shown to participate in metabolic processes, induce root growth and make a barrier to microorganisms as leachates (Peneva, 2006). Alkaloids are said to protect plants from pests which are put off grazing by the acidic taste (Wink and Hartmann, 1982). They have wide use in medicine. By 1985, ten out of the twelve commercially most important plant-derived drugs were alkaloids. Alkaloids generate varying degrees of physiological and psychological responses in humans, often by interfering with neurotransmitters (Hopkins and Hüner, 2004).

The environment of the soil influences alkaloid concentration in plants. Water stress deficit reduces indole alkaloid and increases ajmalicine content in *Catharanthus roseus* (Jaleel *et al.,* 2007, 2008). Mercuric chloride, cadmium, manganese, nickel, lead and vanadium enhance the total alkaloids in *Catharanthus roseus* (Fathalla *et al.,* 2011, Srivastava and Srivastava, 2010, Tallevi and Cosma, 1988) while changes in temperature do not affect total alkaloids in this plant. Insect damage of the roots of tobacco plants also induces an accumulation of alkaloids (Katoh *et al.,* 2005).

Spigelia anthelmia L. Ch. (pink root, wormweed) of the family Loganiaceae, is a common weed of wasteland, cleared areas and roadsides. It is a very toxic plant with leaves and roots that have local medicinal use as an anthelminthic (Oliver, 1960). It produces an alkaloid, spigeline (Claus and Tyler, 1965). *Spigelia* is known to grow very well in loamy soil and averagely in other soil types (Olorode, 1979; Akobundu, 1987). It also shows phenotypic plasticity specific to populations, in time of germination, formation of various organs (Umebese and Omolokun, 1998).

In Nigeria, the leaves of *Spigelia* are picked from roadsides and wasteland and nothing is known about the possible effect of environmental variables on the alkaloid content. The main objective of this study is to determine the changes that occur in growth and organ alkaloid content of *Spigelia* subjected to water deficit stress at the early

and late vegetative stages of development.

MATERIALS AND METHODS

Fruits of *Spigelia anthelmia* L. Ch. were harvested from mature plants growing at the nearby bushes of the University of Lagos. They were stored in polythene bags for two weeks to release the seeds by explosive mechanism.

Planting Procedure

Seeds were planted in three batches of 12 planting pots, each containing loamy soil using a Randomized Complete Block Design. Watering was done daily and plants were thinned to 4 plants per pot after 3 weeks. The first batch of plants was subjected to 6 days water stress at the early vegetative stage (30^{th}-35^{th} days after planting, DAP). A second batch was subjected to water stress at the late vegetative stage (52^{nd}-57^{th} DAP) while the third batch was watered daily, serving as the control. Pots were kept at the greenhouse of the Botanic garden at the University of Lagos.

Growth Parameters

Samples of plants were harvested on the 35^{th} day after planting (DAP) for the determination of leaf area and dry biomass of whole plant and plant parts (leaves, stem, roots and fruits). Leaf area was measured as outlined by Eze (1965). Final harvest was done on the 85^{th} DAP, when the fruits were fully mature and the leaf area and dry biomass were again determined and the number of leaves and plant height were also recorded. Leaf area ratio (LAR), net assimilation rate (NAR) and relative growth rate (RGR) were computed from the leaf area and plant dry biomass values, as outlined by Noggle and Fritz (1976).

Extraction and Measurement of Alkaloid Content

Alkaloids were extracted from dry powdered whole plant and plant parts using the method of Harbone (1960) at the International Institute for Tropical Agriculture (IITA), Ibadan. 0.5 mg of each powdered sample was mixed with 20 ml 10% acetic acid in ethanol and left to stand for 5 hours. The alkaloid was precipitated by dropwise addition of concentrated ammonium hydroxide (NH_4OH). This was further centrifuged and washed with 1% NH_4OH. The residue was then dissolved in a few drops of chloroform. The extract was chromatographed using silica gel G plates in methanol**:** concentrated ammonium hydroxide (200:3) and the presence of alkaloids on the plate was detected by fluorescence in UV light. Quantitative determination was done on fresh alkaloid extract by scanning at a maximal value range of 220-320 nm using a UV spectrophotometer, Bechman automated scanner model 990 equipped with a computerized graphic analyzer.

Statistical Analysis

Means of three replicates were quoted with their standard error. The level of significance between means at $p<0.05$ based on Analysis of Variance (ANOVA), was determined using the New Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Spigelia anthelmia plants subjected to 6 days water deficit stress at the early and late vegetative stages (EV and LV plants respectively) showed differences in growth parameters but not in alkaloid concentration. Water stress caused significant reduction ($p<0.05$) in height and leaf area of EV plants while the impact was only slight in LV plants (Fig. 1). Generally, stress treatment caused reduction in fruit, root, shoot and whole plant biomass (Fig.2). Water stress has been shown to affect the phenology, growth, yield and quality of plants (Adejare and Umebese, 1998; 2008). The root biomass and whole plant biomass were significantly reduced in all treated plants but the reduction in shoot biomass was only significant in LV plants. Thus, EV plants were more sensitive to water stress than LV plants. This corroborates earlier reports that the effect of water deficit varies with the growth stage of the plant; the vegetative stage being more sensitive to water deficit than the reproductive stage, considering plant biomass (Forbes and Watson, 1992; Adejare and Umebese, 2007; Umebese *et al.*, 2009).

Leaf area ratio (LAR) is a measure of the proportion of the plant which is engaged in photosynthetic processes; net assimilation rate (NAR) is a measure of the amount of photosynthetic product going into plant material while both components contribute to the relative growth rate (Noggle and Fritz, 1976). Water stress caused significant decreases in LAR and RGR of stressed plants while the NAR appeared to be similar (Fig. 3). Water stress causes low leaf water potential accompanied by leaf stomatal resistance and the resulting effect is reduction in carbon

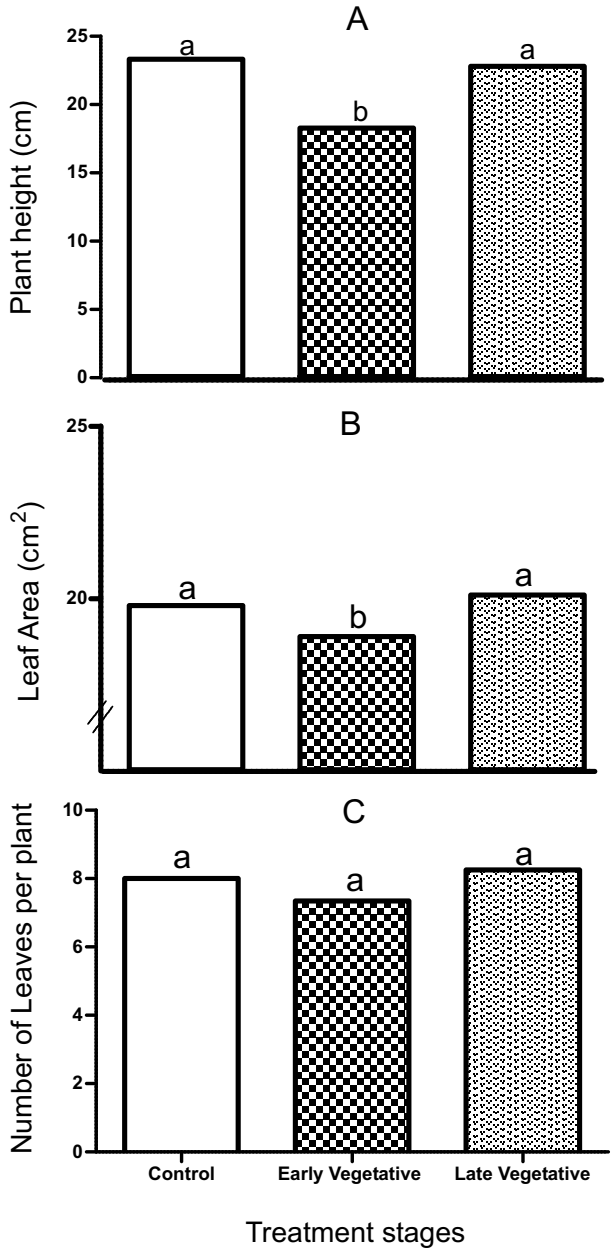

Fig. 1: Height (A), Leaf area (B) and Leaf number (C) of *Spigelia* plants subjected to water stress at the early and late vegetative stages. Bars with similar letters are not significantly different at $P < 0.05$ using the New Duncan's multiple range test

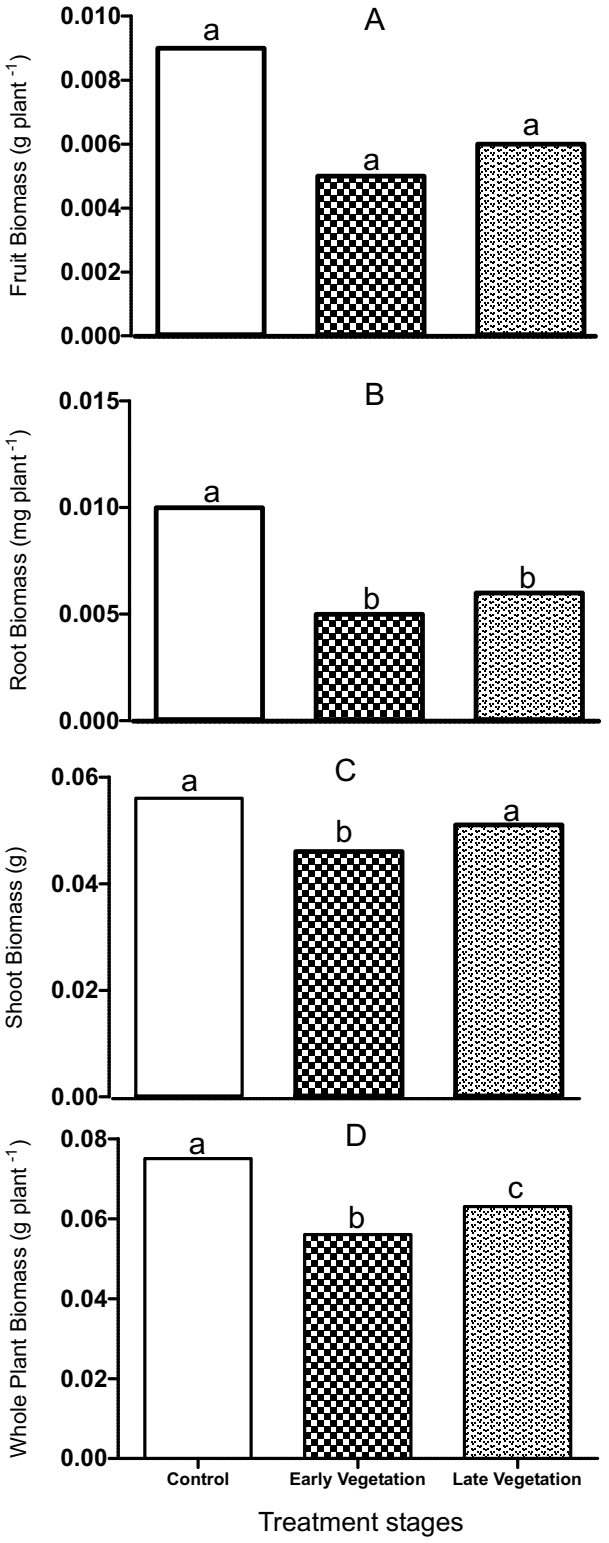

Fig. 2: Fruit (A), Root (B) Shoot (C) and Whole plant (D) biomass of *Spigelia* plants subjected to water stress at the early and late vegetative stages. Bars with similar letters are not significantly different at $P < 0.05$ using the New Duncan's multiple range tests

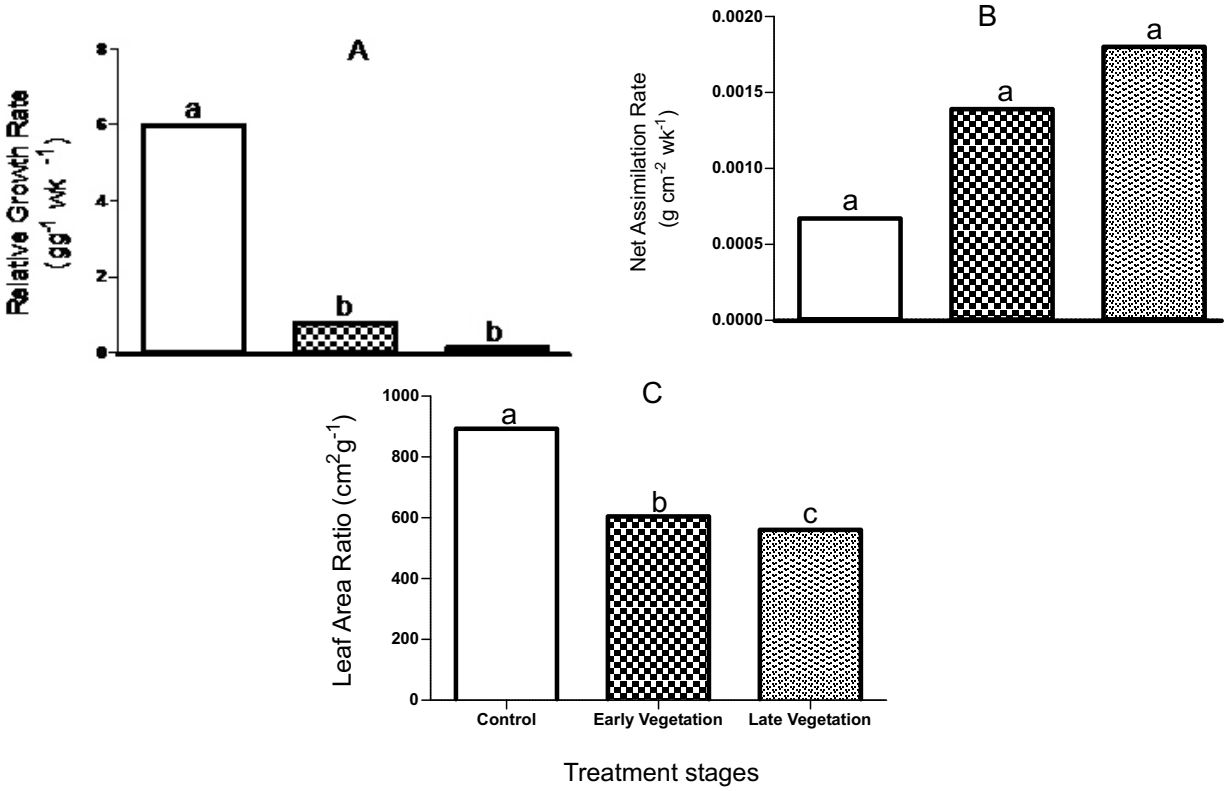

Fig. 3: Relative Growth rate (A), Net assimilation Rate (B) and Leaf Area Ratio(C) of *Spigelia* plants subjected to water stress, at the early and late vegetative stages. Bars with similar letters are not significantly different at $P < 0.05$ using the New Duncan's multiple range tests

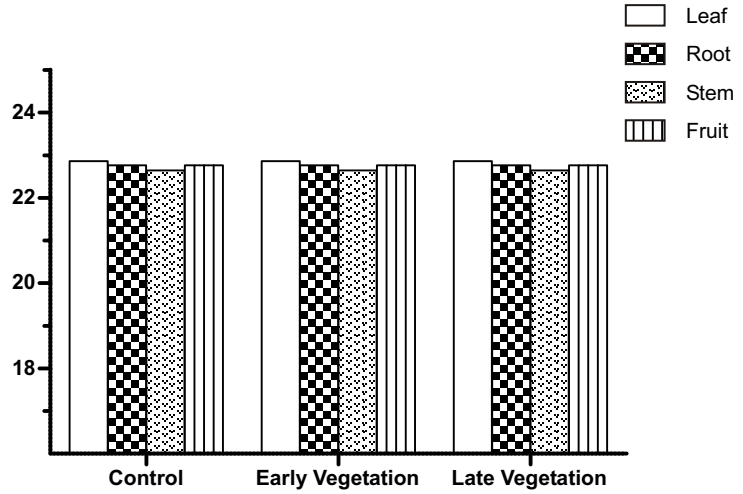

Fig. 4: Alkaloid concentration of various organs of *Spigelia* plants subjected to water stress at the early and late vegetative stages. Bars with similar letters are not significantly different at $P < 0.05$ using the Duncan's multiple range tests

assimilation and subsequent biomass production (Adejare and Umebese, 2007). During environmental stress such as drought, reactive oxygen species (ROS) which include oxygen ions, free radicals and peroxides, increase dramatically resulting in oxidative damage to proteins, DNA and lipids (Apel and Hart, 2004). This was corroborated by the observed reduction in root and whole plant biomass, LAR and RGR in stressed plants.

Alkaloid concentration was not affected by the stress treatments given at both early and late vegetative stages and it was almost evenly distributed among all plant organs: fruit, leaves, stem, and roots (Fig. 4). Thus, moderate water stress at the vegetative stage caused reductions in plant biomass but it did not translate to reduction in concentration of alkaloid. Many plant alkaloids are antioxidants. Antioxidants protect plants exposed to environmental stress from damage.

The lack of impact of water stress on alkaloid concentration in *Spigelia anthelmia* plants despite the significant reduction in biomass and relative growth rate, may suggest that spigeline, the alkaloid present in *Spigelia*, does not have antioxidant activity.

Environmental stress, such as water stress, affects the concentration of different alkaloids in some medicinal plants. The environment of the soil influences alkaloid concentration in plants. Water stress deficit reduces indole alkaloid and increases ajmalicine content in *Catharanthus roseus* (Jaleel *et al.,* 2007; 2008). In the case of *Spigelia anthelmia* water stress during the vegetative period did not affect the alkaloid concentration.

CONCLUSION

While it is true that environmental stress, such as water stress, affects the concentration of alkaloids in some medicinal plants, when *Spigelia anthelmia* plants were subjected to water stress at the early and late vegetative stages of growth, the concentration of alkaloid in all organs of the plant remained the same. The significant reduction in growth in stressed plants did not affect the alkaloid concentration in plant parts.

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