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Growth and Essential Oil Yield of African Basil, *Ocimum gratissimum*, under Light and Water Stress

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Abstract

The growth and essential oil yield of African basil, *Ocimum gratissimum* L., grown under watered and water-stressed field conditions in full sunlight and natural shade (26.7-44.2% full sunlight) conditions were investigated. Seedlings, 42 days old and growing in a prepared mix contained in plastic bags, were randomly placed in one of four adjoining field plots, one in full sunlight with daily watering, one in full sunlight watered at five day intervals, one in natural shade with daily watering, and one in natural shade watered at five day intervals. Plant height and total leaf area of the African basil were decreased more by water stress than by the light or shade condition. Shade enhanced essential oil content and water stress boosted essential oil content under shade, but reduced oil content under full sunlight. The effects of water stress were only observed in plants in full sunlight. The results demonstrated that African basil will produce relatively high essential oil yields per plant when grown under natural shade, irrespective of water stress and despite poor vegetative growth.

Introduction

African basil, *Ocimum gratissimum* L., is a perennial herbaceous, drought tolerant plant with lime-green pubescent leaves, a characteristically strong fragrance, and a slight pungency. In Nigeria and several other countries, the plant plays an important role in traditional medicine preparations (Gill, 1992), including use as a stomachic and for treatment sunstroke, headache and influenza. In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever, and diarrhea (Effraim, et al., 2003), while in the Savannah areas leaf decoctions are used to treat mental illness (Akinmoladun, et al., 2007). Other uses include the treatment of fungal infections, fevers, colds, and catarrh (Ijeh et al., 2005). A number of other traditional uses of African basil have also been reported (Freire,, et al., 2006; Ilori et al., 1996; Nadkarni, 1999; Ngassoum et al., 2003; Okigbo and Igwe, 2007). The plant is known to contain phenolic compounds with therapeutic potential (Vierra and Simon, 2000).

Light and water stresses are environmental factors that can affect crop growth and yield. Reduced light can limit photosynthesis and alter plant development (Whitelam and Halliday, 2007). Water deficits result in lowered water potential, reducing the water flow and cell turgor pressure needed to maintain plant structure and promote growth (Hopkins, 1995). In the case of aromatic crops, reduced light levels and water deficits have been demonstrated to alter essential oil levels and constituency (Radusiene et al., 2011; Sabih et al., 1999). Studies with sweet basil (*Ocimum bacilicum*) have corroborated these effects for both light (Loughrin and Kasperbauer, 2003; Shiga et al, 2009) and water (Mirsa and Strivastov, 2000; Simon *et al*., 1992).

The deficit of a single environmental resource on plant growth and productivity are frequently investigated, but these studies cannot accurately predicate plant responses to two or more environmental deficits. No reports on the combined effects of natural sunlight and natural shade on basil plants under adequate water conditions and under water-stressed conditions are available. The objective of this project was to observe the effect of light, shade, and water stress on the growth and the essential oil content and yield of African basil in the natural environment.

Materials and Methods

Plant material. The African basil, *Ocimum gratissimum* L., was used in this study. The plants were started from mature seeds collected from within a population of African basil plants growing in the Botanic Garden at the University of Lagos, Nigeria. For reference purposes, a voucher specimen of the plants was deposited in the Herbarium at the Botanic Garden. The collected seeds were subsequently germinated in soil (loam:clay:chicken manure, 9:9:2 v:v) contained in plastic bowls (55 cm in diameter x 15 cm deep). The bowls with seeds were placed in a greenhouse for seed germination and initial plant growth.

Experimental. At 42 days after seeding, the developing seedlings were randomly transplanted into open-topped polyethylene bags (19 cm in diameter and 36.5 cm deep) filled with the above mixture of soil and manure used for seed germination to insure that all plants had the same root environment. Bags containing the plants were then randomly selected and placed 15 cm apart in four plantings of 5 rows x 10 plants each for a total of 50 plants in each planting. Of the plantings, two were under full natural sunlight and two were under natural shade conditions (26.7-44.2% full sunlight from daybreak to sunset) imposed by a large, 30+ year old *Plumeria alba* tree with wide stretched branches that hovered over the shaded plantings.

The plants in one planting under natural sunlight and the plants in one planting under natural shade each received by hand 400 mL of tap water daily to limit any water stress on the plants. The second set of plantings, one in natural sunlight and one under natural shade, were watered by-hand with 400 mL of tap water once every five days to induce a water stress on the plants. All plots were weeded regularly by hand.

Beginning the second week after placing the plants in the experimental plantings, the height of two plants under each treatment was measured from the soil surface to the plant tip using a meter rule. These plants were then carefully uprooted from the polyethylene bags and the soil was rinsed from the roots with water. The surface of the washed plant roots were immediately blotted dry with tissue and the fresh weight of the entire plant determined using a balance. The leaves from each plant were subsequently removed and the outline of each leaf traced on graph paper for use in determining the leaf area. To determine plant dry weights, all the parts from each plant were collectively placed in a labeled paper bag for drying in a mechanical oven at 80^{o}C until constant weights were reached at 72 h. The dry weight of each plant was determined using a balance.

Essential oil analyses were done eight weeks after placing the plants in the experimental plantings. Fresh leaves from eight plants were harvested from within each treatment, thoroughly washed twice with distilled water, blotted dry, and then weighed before hydrodistilling for 3 h using a Clevenger apparatus and following the method outlined by Asawalam et al. (2008). The essential oils were collected in separate sterilized glass vials containing n-hexane (Aldrich HPLC grade).

Traces of water were removed from the oils by opening the collection vials in a closed container containing anhydrous sodium sulfate to absorb the water. The hexane was subsequently removed from the oil by distillation and the collected oil was stored in small amber-colored vials until weighed on a balance. The essential oil content was calculated based on the fresh weight of the extracted tissue and the essential oil yield was based on the fresh weight of the plant.

Statistical analysis. Experiments were done in triplicate and data analyzed are means ± SE subjected to one way ANOVA to determine the effects and interaction of light, shade, watered, and water-stressed conditions on the growth, development, and oil synthesis of African basil.

Results

No significant difference in plant height was observed during the first three weeks under the four treatments (Figure 1). By the fourth week, however, plants watered daily and under shade conditions had significantly longer stems than plants under the other treatments. By the sixth week, however, plants watered daily and in sunlight were the same height as plants watered daily and under shade. Ultimately, plants watered daily in shade or sunlight reached the same height at the time of harvest eight weeks after planting and were significantly taller than those plants watered every five days.

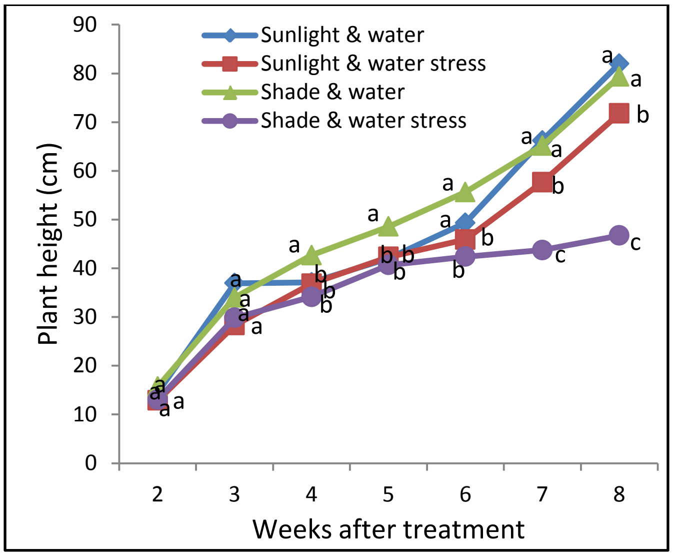

Figure 1. The effect of shade and water stress on plant height.

No significant differences in fresh weight of the plants under the different water and light treatments were observed during the first four weeks of treatments (Figure 2). After seven weeks growth, the fresh weight of plants subjected to water stress under both shade and light treatments was significantly less than the comparable shade and light treated plants not subjected to water stress. By the time of harvest, however, the fresh weight of the water stressed plants in light was significantly greater than both the watered and water stressed plants in the shade.

After six weeks treatment, plants receiving a daily watering every day under shade condition had a significantly higher dry weight than all the other water and light treatment conditions (Figure 3). By the seventh week of treatment, however, both the watered and water-stressed plants in the light had made rapid gains in tissue dry weight and by harvest time had accumulated were over twice the dry weight of plants in the shade irrespective of the watering regime.

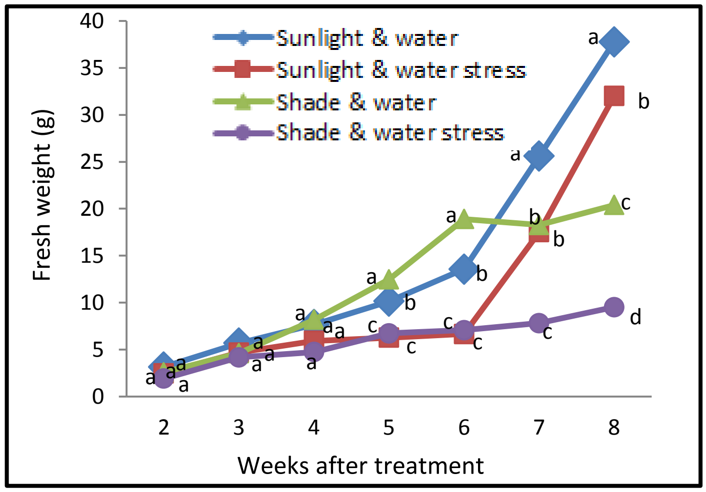

Figure 2. The effect of shade and water stress on plant fresh weight.

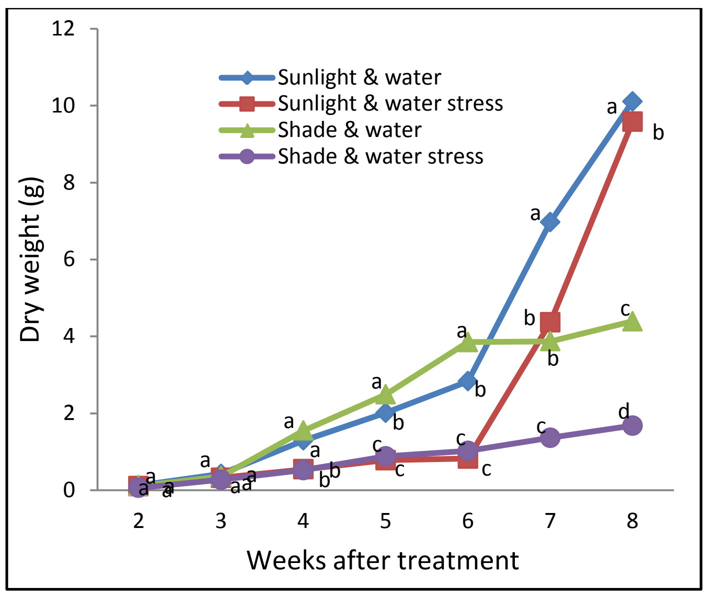

Figure 3. The effect of shade and water stress on plant dry weight.

Leaf area was affected by water stress (Figure 4). Plants watered daily under both light and shade conditions had more total leaf area than plants growing under a water stress. Water-stressed plants in the light, however, had over 60% more leaf area than water-stressed plants in the shade after eight weeks growth.

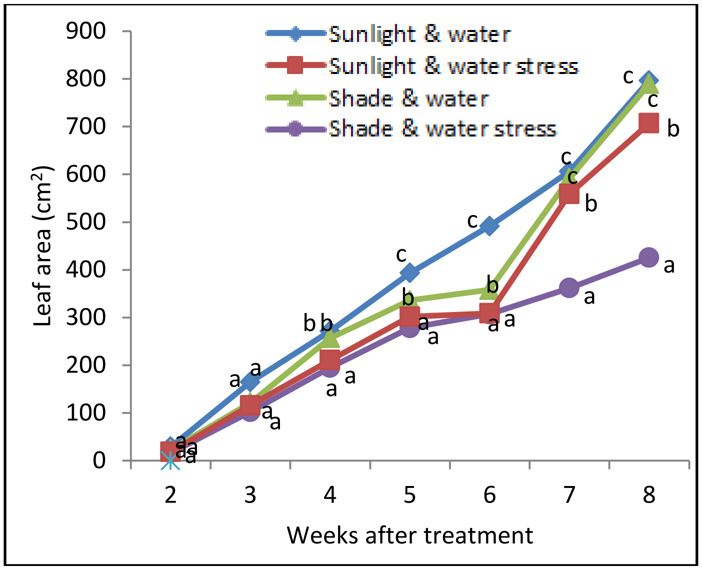

Figure 4. The effect of shade and water stress on leaf area.

The percent essential oil in the leaves of the basil plants growing in shade was higher than in plants growing in sunlight (Figure 5). Plants growing under simultaneous shade and water stress contained over 1% oil. In plants under natural sunlight, those under water stress had a higher essential oil content than those not under water stress (Figure 6).

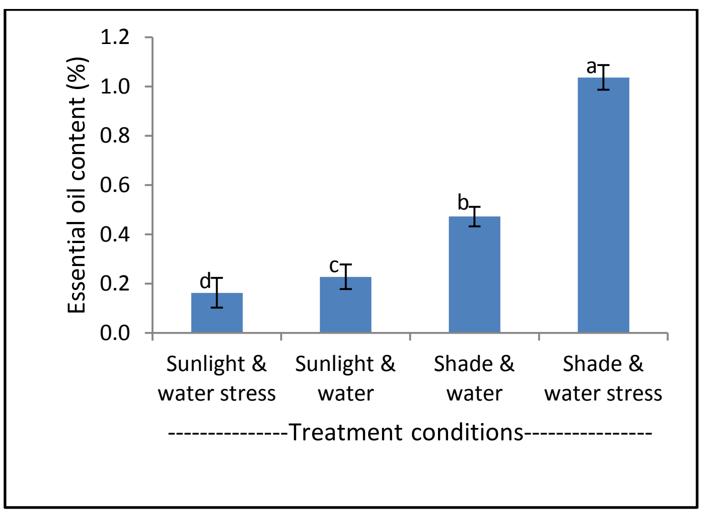

Figure 5. The effect of shade and water stress on essential oil content in fresh leaf tissues.

Discussion

Growth and development of the African basil were most limited by the lack of sunlight and the lack of water. Plant height, fresh and dry weight, and leaf area were the lowest under the combination of shade and water stress. Similar results have been observed in several plant species (Anjum, et al. 2011; Kramer, 1963; Shao, et al., 2008). The decrease in fresh and dry weights under water stress in *O. gratissimum* are similar to those observed in earlier studies with *O. bacilicum* (Simon et al., 1992) and Japanese mint (Mirsa and Strivastov, 2000). Although the watered plants subjected to shade were able to grow tall, the lack of fresh weight and dry weight, as compared with those in the sunlight, confirm that carbon fixation was quite limited by the lack of adequate light (Cavatte, 2012).

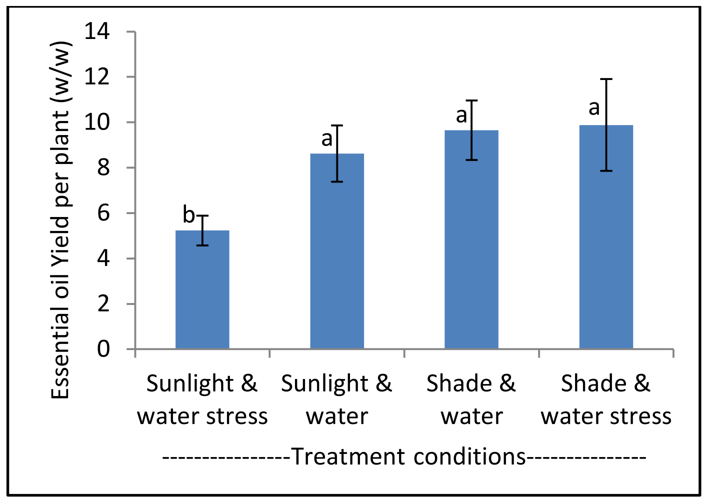

Figure 6. The effect of shade and water stress on essential oil yield per plant.

The shaded plants with water and shaded plants under water stress had significantly higher percentages of essential oil in the fresh tissue, but this had little effect on the essential oil yield of freshly harvested plants. The limited growth of the plants subjected to shade and water stress, as compared with the plants growing in full sunlight with daily watering, means lower oil yields per unit land area. Water stress has been reported by Bahlebi et al. (2008), Rajeswara (2002) and Singh (1999) to reduce essential oil yield by reducing vegetative growth.

Essential oil yield is a function of primary metabolite synthesis that leads to increased vegetative growth for increased oil production (Letchamo, et al., 1995; Sangwan, et al., 2001; Srivastava and Luthra, 1993, Bahlebi, et al, 2008). A comparison of dry weights of plants in light and shade indicated the shade caused over a 50% reduction in plant growth, while a water stress in the light caused a reduction in dry weight that was only a 5%, suggesting the light stress was more severe on African basil growth than the water stress.

In general, studies that have focused on the effect of shade on the production and quality of essential oils of medicinal and aromatic species indicate that each species responds differently with variations in essential oil yield and chemical composition, according to the environment (Li et al., 1996). High light conditions have been shown to increase essential oil yield in plants, such as *Thymus vulgaris* (Li et al., 1996) and *Matricaria chamomila* (Saleh, 1973), while plants such as *Anethum graveolens* (Halva, et al., 1992), *Salvia officinalis* (Li et al., 1996) and *Pothomorphe umbellate* (Mattana et al. 2010) had higher essential oil yield when cultivated under shade.

In this study, water stress coupled with high light condition reduced essential oil yield per plant. According to Sharafzadeh (2012), plant genotype, age, and environmental factors, such as temperature, water, nutrients, and geographical location, can interact with light conditions. The multitude of possible interactions may explain the different and sometimes contradictory results reported in the literature (Sharafzadeh, 2012).

The primary purpose of the current study was to observe the growth and oil production under field conditions that local growers in Nigeria frequently encounter. Exposing plant roots to the same environment ensured that light and water stress were the main environmental variables among the plantings. Except for the differences in watering, the conditions were not as fixed as in a number of other studies (Li, et al., 1996; Rajeswara, 2002) on the effects of light and water on plant development and oil production. For example, the level of shade provided by the *Plumeria alba* tree varied over the course of the day and growing period.

As a demonstration and education study on African basil, growers were able to see differences that light and watering among the plantings and recognize the benefits of light and watering on both plant and oil production. The African basil was able to produce a crop under shading, although the combination of shade and water stress severely limited growth and development. As with many other plants, providing African basil with natural sunlight and adequate water produces the best yields of foliage and essential oil.

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