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Lemongrass Growth, Essential Oil, and Active Substances as Affected by Water Deficit

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Abstract: Environmental stress has a major influence on the growth and quality of medicinal plants. More than half of the agricultural land worldwide suffers from a lack of water. In this study, we estimated the effect of different irrigation intervals on growth, yield, and essential oil content as well as their effect on the main compounds of the essential oil of lemongrass, *Cymbopogon citratus*. The major objective was to test how much irrigation consumption can be lowered without a significant impact on yield and quality properties. Water deficit led to significant decreases in growth characteristics including the number of tillers as well as fresh and dry herb yield. In addition, the relative leaf greenness decreased under water deficit, especially in plants irrigated every 20 days. In contrast, proline content increased with increasing water deficit, especially in plants irrigated every 15 and 20 days. Essential oil percentage also increased under a water deficit condition, and the highest essential oil percentage was observed in plants irrigated every 15 and 20 days. However, the yield of essential oil per plant significantly decreased due to decreasing the herb yield. GC-MS analysis identified 31 compounds, mainly geranial and neral. Geranial and neral percentage decreased under a water deficit of 10-day irrigation intervals but increased with increasing the water deficit severity at irrigation intervals of 15 and 20 days. These results suggest that the lemongrass plant was sensitive to drought. Nevertheless, the quality represented by essential oil percentage and the main active substances improved with prolonging the irrigation intervals. This study recommends increasing irrigation intervals to 10 days to maintain small decreases in the yield with higher quality. In addition, it is recommended to conduct more studies to improve the growth of lemongrass under water shortage conditions.

Keywords: *Cymbopogon citratus*; citral; geranial; neral; water deficit; essential oil

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1. Introduction

Environmental stresses are the most important challenges facing food security, as they directly affect crop growth, development, yield, and quality [1]. Environmental stresses include heat stress, drought, flooding, salinity, and nutrient excess or deficit. Among these, drought remains the main limiting factor affecting plant growth and productivity around the world with arid and semi-arid areas accounting for more than fifty percent of agricultural land. In addition, it is expected that the severity of the drought problem will increase due to the global warming phenomenon [2]. Drought is one of the most crucial environmental stresses that affect sustainable production [3]. Plant growth and development are severely reduced under drought conditions [4,5]. In addition, productivity and quality of crops are negatively affected under drought stress [6,7]. Growth and development of plants are dependent on cell division, elongation, and differentiation. Drought causes loss of turgor, disordering of enzyme activities, and a decreasing energy supply from photosynthesis [4,8,9]. Drought stress reduces availability and uptake of nutrients, which lead to reduction of root development, transpiration, photosynthetic rates, and stomatal conductance leading to desiccation stress [10]. The water-deficit stress is mainly dependent on the following factors: the severity of stress and the duration and timing of the stress [7]. Fresh biomass of non-woody plants includes about 80–95% water which plays a key role in plant growth, development, and metabolism [11,12]. Several factors affect plant response to water deficit including genetic factors, age, and developmental stage as well as the length and severity of the water deficiency [13,14]. Plants have the ability to resist the stress by developing protective mechanisms under such conditions [15]. The plant can tolerate water deficit by maintaining cell water homeostasis under drought conditions by decreasing water loss and increasing water intake by the cells, leading to normal cell functions [12,13]. Water deficit resulted in negative impacts on plants' morphological, physiological, biochemical, and molecular properties [16–18]. Although drought stress has shifting effects on the primary and secondary metabolism of plants, insufficient reports regarding drought influence on plant metabolism have been documented [19].

Cymbopogon is a genus of aromatic grasses belonging to the Gramineae family, a popular genus of medicinal plants used by the native people of Africa, Asia, and America in folklore medicine. The main active substance in this genus is the essential oil that is readily involved in many industrial branches. The potential therapeutic uses of *Cymbopogon* essential oils and their active substances such as citral, citronellal, citronellol, and eugenol have been extensively documented [20]. Last decade, reports of essential oils of *Cymbopogon* have proven their hypocholesterolemic-hypoglycemic [21], neurobehavioral [22], acaricidal [23], antimycotic [24], anthelmintic [25], anti-allergic [26], gastroprotective [27], and antiseptic [28] properties. Other beneficial properties of *Cymbopogon* plants included treatment of cough, emesis, depression, fever, gastro-urinary disorders, spasms, tension, stress, and typhoid [22].

Lemongrass, *Cymbopogon citratus* (DC.) Stapf, is a widely distributed perennial grass belonging to the *Cymbopogon* genus [29–31]. Lemongrass includes 1–2% essential oil in its herb. The main active compounds of lemongrass' essential oil are citral A (geranial) and citral B (neral) [14,32]. In this study, we estimated the effect of water deficit using different irrigation intervals on growth, yield, essential oil content, and the main compounds of the essential oil of lemongrass. The main objective was to test how much irrigation can be reduced without significantly reducing yield and quality properties.

2. Materials and Methods

This study was conducted during two successive seasons (2018 and 2019) at the Agricultural Experimental Farm, Faculty of Agriculture and Natural Resources, Aswan University, Sahary, Aswan, Egypt. The experiment was designed in completely randomized blocks that incorporated four replicates. The treatments included four irrigation intervals: 5 as control, 10, 15, and 20 days. Homogenous stalks of lemongrass were planted in terraces (width of 60 cm and length of 3 m) on the 20th of March in each year 2018 and 2019. Organic compost was used in the preparation period by adding and mixing 10 m^3 of compost per hectare in soils before planting. The stalks were cultivated on both sides of terraces, and distance between plants was 30 cm with total of 40 plants per plot. After two months of planting and/or cutting, the plants were irrigated according to irrigation intervals with a rate of 12 L/plant. Soil samples were obtained from a depth of 30 cm from the used soil surface in this study, and some physical and chemical properties of the soil were recorded according to the methods described by Jackson [33] and Black et al. [34] as shown in Table 1.

Table 1. Physical and chemical properties of the experimental soil.

1—Physical Analysis			
Sand %		94.67	
Silt %		2.27	
Clay %		3.07	
Soil Texture		Sandy	
2—Chemical Analysis			
pH	8.25	E.C. (ds/m)	0.25
Soluble Cations meq/L		Soluble Anions meq/L	
Na^{+}	17.74	CO^{-3}	0.00
K^{+}	7.51	HCO^{-3}	4.67
Ca^{++}	2.08	Cl^{-}	2.33
Mg^{++}	0.53		

2.1. *Vegetative Growth Characters*

Plants were harvested twice each season by cutting the shoots 10 cm above ground. The harvest was after two months of irrigation treatments. At harvest time, number of tillers and fresh herb weight per plant were recorded. Then the herb was air-dried for a week, and the dry herb weight was recorded. Fresh and dry herb yield per hectare (ton) was calculated by multiplying shoot fresh weight per plant and plant number per hectare.

2.2. *Relative Leaf Greenness*

The relative leaf greenness (level of chlorophyll) of lemongrass leaves was measured using chlorophyll meter reading (SPAD-502plus, Konica Minolta, Inc., Osaka, Japan). The SPAD values of fully expanded leaves of lemongrass were measured on the middle part of the leaf blade. The average of at least three readings was recorded in each experimental plot.

2.3. *Proline Content*

Free proline was determined on the basis of dry biomass according to the methods previously described by Soliman and El-Shaieny [35]. About 200 mg of dried leaves was homogenized for 10 min in 10 mL of 3% aqueous sulfosalicylic acid. After filtration, two milliliters of the filtrate, glacial acetic acid, and acid ninhydrin was mixed, and the mixture was heated for an hour in a water bath. Four milliliters of toluene was used to extract the developed color, and the extraction was colorimetrically measured at 520 nm against toluene using SPECTROstar Nano (BMG LABTECH GmbH, Ortenberg, Germany). A standard curve with proline was used to calculate the final concentrations.

2.4. *Essential Oil Extraction and GC Analysis*

About 300 g of lemongrass fresh leaves was hydro-distilled for three hours using Clevenger-type apparatus [36]. The essential oil concentration (%) basis of fresh biomass was recorded, and the total oil yield was calculated. The extracted essential oils were collected and dried using anhydrous sodium sulfate for chemical component identification.

The GC-MS analysis of the essential oil samples was carried out at the Department of Chemistry, Faculty of Science, Aswan University, Aswan, Egypt, in accordance with the specifications described by Hendawy et al. [37]. The instrument was Agilent 7890A Gas Chromatograph (Agilent Technologies, Inc., Wilmington, NC, USA) connected to a Thermo mass spectrometer detector (ISQ single quadrupole mass spectrometer). The system of GC-MS was equipped with a TG-WAX MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas with 1.0 mL/min flow rate and 1:10 split ratio. The following temperature program was used: 40 °C for one minute; an increase of 4.0 °C/min to 160 °C, held for six minutes; then an increase of 6 °C/min to 210 °C, and held again for one minute. Both injector and detector were held at 210 °C. About 0.2 µL of diluted samples (1:10 hexane, v/v) was injected. Mass spectra were obtained by electron ionization (EI) at 70 eV using an m/z 40–450 spectral range. Two analytical methods were used to identify the chemical compounds: (a) Kovats indices (KI), in reference to n-alkanes (C9-C22) (National Institute of Standards and Technology (NIST), 2009), and (b) mass spectra (authentic chemicals, Wiley spectral library collection and NIST library).

2.5. Statistical Analysis

The differences among irrigation interval treatments were tested for their significance by analysis of variance (ANOVA). The experiment was set up in a randomized block layout incorporating five replications. Data obtained were subjected to statistical analysis using "F" Test according to Snedecor and Cochran [38], in which the data for each harvest were analyzed separately. One factor was considered in this experiment, which was irrigation intervals. The differences among treatments were detected using Tukey's honest significant difference test according to Gomez and Gomez [39]. Statistical analysis was performed using Statistix 8.1 program.

3. Results and Discussion

In this study, irrigation intervals showed significant effects on the growth characteristics, number of tillers, fresh and dry herb weight per plant, and fresh and dry herb yield per hectare in the 1st and 2nd harvest of both 2018 and 2019 seasons (Table 2). All values of growth characteristics decreased significantly when irrigation intervals increased from 5 days to 10 days, and another significant decrease was observed in growth characteristics when irrigation intervals increased to 15 and 20 days, with no significant differences between intervals of 15 and 20 days. Relative leaf greenness showed significant decreases under drought stress (when irrigation intervals increased) compared to the 5-day interval (Table 2). The best greenness was observed under a 5-day irrigation interval, and the least greenness was observed under a 20-day irrigation interval in both harvests and both seasons. In contrast, proline content increased under drought stress in lemongrass leaves, as the highest proline content was observed in plants irrigated every 15 or 20 days, and the lowest proline content was observed under control in both harvests and both seasons (Table 2).

Table 2. Influence of irrigation intervals on the growth characteristics of lemongrass, number of tillers, fresh herb weight per plant, dry herb weight per plant, fresh herb yield per hectare, dry herb yield per hectare, leaf greenness, and proline content in the 1st and 2nd harvest of 2018 and 2019 seasons.

Irrigation Intervals	First Season (2018)		Second Season (2019)	
	1st Cut	2nd Cut	1st Cut	2nd Cut
	number of tillers			
5 days	88.0 ± 1.73 ^{a}	147.0 ± 3.46 ^{a}	28.0 ± 1.15 ^{a}	70.0 ± 2.89 ^{a}
10 days	76.5 ± 3.75 ^{b}	101.5 ± 4.91 ^{b}	20.0 ± 1.15 ^{b}	45.0 ± 2.89 ^{b}
15 days	61.5 ± 0.87 ^{c}	85.0 ± 5.20 ^{c}	17.0 ± 1.73 ^{c}	36.3 ± 0.88 ^{c}
20 days	56.0 ± 0.00 ^{d}	81.0 ± 9.81 ^{c}	19.0 ± 1.73 ^{c}	33.0 ± 1.73 ^{d}
	fresh herb weight per plant (g)			
5 days	218.7 ± 7.7 ^{a}	307.6 ± 4.6 ^{a}	172.4 ± 5.5 ^{a}	419.7 ± 17.7 ^{a}
10 days	146.9 ± 22.8 ^{b}	229.4 ± 0.1 ^{b}	125.5 ± 10.3 ^{b}	249.9 ± 10.1 ^{b}
15 days	85.3 ± 8.9 ^{c}	169.9 ± 3.9	77.7 ± 2.8	78.3 ± 0.4
20 days	71.3 ± 6.1 ^{d}	163.1 ± 10.1	63.0 ± 1.7	76.7 ± 8.3
	dry herb weight per plant (g)			
5 days	42.5 ± 1.44 ^{a}	45.0 ± 0.26 ^{a}	42.9 ± 8.46 ^{a}	136.7 ± 6.99 ^{a}
10 days	30.9 ± 2.92 ^{b}	36.7 ± 1.13 ^{b}	43.6 ± 1.99 ^{a}	45.5 ± 1.93 ^{b}
15 days	27.6 ± 1.47 ^{c}	32.4 ± 1.04 ^{c}	27.6 ± 5.46 ^{c}	36.6 ± 0.92 ^{c}
20 days	21.8 ± 3.75 ^{d}	31.1 ± 0.81 ^{c}	22.3 ± 3.44 ^{c}	34.6 ± 2.45 ^{c}

Table 2. *Cont.*

Irrigation Intervals	First Season (2018)		Second Season (2019)	
	1st Cut	2nd Cut	1st Cut	2nd Cut
	fresh herb yield per hectare (ton)			
5 days	10.93 ± 0.38 [a]	15.38 ± 0.23 [a]	8.63 ± 0.27 [a]	20.98 ± 0.89 [a]
10 days	7.35 ± 1.14 [b]	11.48 ± 0.00 [b]	6.28 ± 0.52 [b]	12.50 ± 0.51 [b]
15 days	4.27 ± 0.44 [c]	8.49 ± 0.19 [c]	3.88 ± 0.14 [c]	3.92 ± 0.02 [c]
20 days	3.57 ± 0.31 [d]	8.15 ± 0.51 [c]	3.15 ± 0.09 [d]	3.83 ± 0.41 [c]
	dry herb yield per hectare (ton)			
5 days	2.13 ± 0.07 [a]	2.25 ± 0.01 [a]	2.15 ± 0.42 [a]	6.83 ± 0.35 [a]
10 days	1.55 ± 0.14 [b]	1.83 ± 0.05 [b]	2.18 ± 0.10 [a]	2.28 ± 0.10 [b]
15 days	1.38 ± 0.07 [c]	1.62 ± 0.05 [c]	1.38 ± 0.27 [b]	1.83 ± 0.04 [c]
20 days	1.10 ± 0.19 [d]	1.55 ± 0.04 [c]	1.12 ± 0.17 [b]	1.73 ± 0.12 [c]

Data represent means ± standard deviation. Different letters above represent significant differences.

Drought stress results in the generation of reactive oxygen species in cells, which are associated with osmotic stress in plants. Under various stresses, reactive oxygen species act as signaling molecules and activate signal transduction processes [40,41]. Excessive levels of reactive oxygen species can cause lipid peroxidation, protein oxidation, nucleic acid damage, enzyme inhibition, programmed cell death pathway activation, and cell death [42]. Under stress conditions such as water deficit and salinity stresses, compatible solutes accumulate in plant tissue as a resistance mechanism in order to mitigate osmotic stress [12]. Compatible solutes are characterized by low molecular weight and can accumulate at high concentrations without negative impacts on the components and metabolism of the cell [5]. Compatible solutes, such as proline, lead to a raised cellular osmotic pressure and water uptake maintaining the turgor pressure and water content of cells. Proline is an amino acid known as an osmoprotectant [43,44]. Under drought stress, the level of proline in a cell increases as a result of two different processes: increasing proline synthesis and/or decreasing proline degradation by inhibiting the activity of enzymes responsible for the degradation process. Proline accumulation is closely related to plant resistance to water deficit stress [5,45]. Many studies reported the important role of proline in osmotic adjustment. In addition, proline protects cell components against oxidative stress and plays important roles in maintaining energy balance between chloroplasts and mitochondria [12].

Studies on the essential oil composition of lemongrass, *Cymbopogon citratus*, showed its richness in phenolic compounds, flavonoids, and tannins [46]. The major bioactive substances of the essential oil include geranial (citral A), neral (citral B), and myrcene [14], as well as other minor bioactive substances such as citronellol, geraniol, geranyl acetate, limonene, linalool, nerol, eugenol, etc. [29,47]. Drought stress had significant impacts not only on essential oil content but also on its compositions. In this study, the essential oil percentage (%) of fresh herb increased significantly under drought stress when irrigation intervals increased, with the highest percentage observed in plants irrigated every 15 and 20 days compared to control in the first and second harvest of both seasons. In contrast, the essential oil yield per plant and per hectare decreased significantly under wide irrigation intervals, and the lowest oil yield was observed under irrigation intervals of 15 and 20 days compared to irrigation intervals of 5 and 10 days (Table 3). The plant also showed significant differences in its essential oil composition under different irrigation intervals. GC-MS analysis showed about 31 compound identifications (Table 4). The major compounds were geranial (citral A) and neral (citral B). The highest geranial concentration was observed in plants irrigated every 5 days (35.01%), followed by a significant decrease in geranial concentration in plants irrigated every 10 days (28.45%), and then a gradual increase at irrigation intervals of 15 and 20 days (30.94% and 33.09%, respectively). The highest neral concentration was observed in plants irrigated every 5 days (31.48%), followed by a significant decrease in neral concentration in plants irrigated every 10 days (28.78%), and then a gradual increase at irrigation intervals of 15 and 20 days (30.44% and 31.20%, respectively). The total citral (geranial + neral) ranged between 57.23% and 66.49%. The highest citral concentration was observed in plants under control (5-day irrigation interval), while the lowest citral concentration was observed in plants under 10-day irrigation intervals. Meanwhile, plants irrigated every 15 and 20 days showed average values (Table 4).

Table 3. Influence of irrigation intervals on essential oil (%), essential oil content per plant, and essential oil yield per hectare in the 1st and 2nd harvest of 2018 and 2019 seasons.

Irrigation Intervals	First Season (2018)		Second Season (2019)	
	1st Cut	2nd Cut	1st Cut	2nd Cut
	Essential oil (%)			
5 days	0.67 ± 0.05 [a]	1.13 ± 0.07 [a]	0.82 ± 0.02 [a]	1.03 ± 0.12 [a]
10 days	0.90 ± 0.06 [b]	1.27 ± 0.07 [b]	1.11 ± 0.16 [b]	1.00 ± 0.12 [a]
15 days	1.10 ± 0.12 [c]	1.23 ± 0.03 [b]	1.40 ± 0.12 [c]	1.03 ± 0.07 [a]
20 days	0.89 ± 0.14 [b]	1.03 ± 0.09 [a]	1.47 ± 0.13 [c]	1.83 ± 0.62 [b]
	Essential oil content per plant (mL)			
5 days	1.46 ± 0.14 [a]	3.49 ± 0.25 [a]	1.41 ± 0.02 [a]	4.37 ± 0.63 [a]
10 days	1.31 ± 0.19 [ab]	2.90 ± 0.15 [b]	1.37 ± 0.16 [a]	2.51 ± 0.36 [b]
15 days	0.93 ± 0.03 [b]	2.10 ± 0.10 [c]	1.10 ± 0.13 [b]	0.81 ± 0.05 [d]
20 days	0.64 ± 0.14 [b]	1.70 ± 0.25 [d]	0.93 ± 0.09 [c]	1.44 ± 0.50 [c]
	Essential oil yield per hectare (L)			
5 days	73.3 ± 6.66 [a]	174.5 ± 12.45 [a]	70.5 ± 1.15 [a]	218.3 ± 31.44 [a]
10 days	65.4 ± 9.28 [ab]	145.2 ± 7.42 [b]	68.5 ± 7.91 [a]	125.6 ± 18.08 [b]
15 days	46.2 ± 1.60 [b]	104.8 ± 5.11 [c]	54.8 ± 6.42 [b]	40.4 ± 2.46 [d]
20 days	32.1 ± 7.02 [b]	85.1 ± 12.47 [d]	46.2 ± 4.43 [c]	71.9 ± 25.12 [c]

Data represent means ± standard deviation. Different letters above represent significant differences.

Table 4. Chemical compositions (%) of the lemongrass, *Cympobogon citratus*, essential oil as affected by irrigation intervals.

RT	Name	5 Days	10 Days	15 Days	20 Days
7.397	β-myrcene	7.31	6.97	7.22	5.95
8.498	trans-β-Ocimene	0.14	0.23	0.21	0.61
8.679	Benzeneacetaldehyde	—	0.1	0.06	—
8.767	β-ocimene	0.41	0.2	0.16	0.21
8.898	Geranyl isovalerate	0.1	0.11	0.09	0.1
10.093	1,6-octadien-3-ol,3,7-dimethyl-	1.63	1.95	1.76	1.77
10.255	photocitral B	0.18	0.31	0.25	0.26
10.524	4-methyl-3-(1-methylethylidene)-cyclohexene	0.32	0.39	0.26	0.3
11.281	epiphotocitral A	0.39	0.25	0.21	0.24
11.406	Geraniolene	0.46	0.6	0.49	0.57
11.575	photocitral A	0.35	0.49	0.63	0.71
11.976	Verbenol	1.33	2	1.71	1.51
12.476	pulegone	2.08	2.84	2.53	2.58
13.658	(R)-Citronellol	0.25	0.22	0.28	0.91
13.733	citronellol	0.45	0.7	0.6	0.56
14.096	Citral B (Neral)	31.48	28.78	30.44	31.2
14.371	Geraniol	3.2	4.56	4.06	3.88
14.816	Cital A (Geranial)	35.01	28.45	30.94	33.09
15.028	Epoxy-linalooloxide	0.7	0.31	0.29	0.59
15.166	2-undecanone	0.15	0.23	0.19	0.17
15.816	Neric acid	—	0.71	0.21	1.06
16.448	Geranic acid	0.46	0.68	0.78	0.94
16.967	3,7-dimethy-,(z)-3,7-dimethy-2,6-octadien-1-ol	2.7	2.75	3.48	2.77
17.656	2-ethylidene-6-methyl-3,5-Heptadienal	0.26	0.22	0.35	0.28

Table 4. *Cont.*

RT	Name	5 Days	10 Days	15 Days	20 Days
17.724	caryophyllene	0.91	0.23	0.71	0.15
17.962	α-Bergamotene	0.32	0.32	0.2	0.21
18.882	2-Tridecanone	0.42	0.5	0.34	0.29
19.832	Geranyl acetate	0.24	0.28	0.13	0.13
20.302	caryophyllene oxide	0.18	0.2	0.17	0.81
20.771	1H-cycloprop[e]azulene	0.73	0.74	0.67	0.54
21.309	γ-Selinene	0.31	0.13	0.12	0.1
	Total identifications	**92.47**	**86.45**	**89.54**	**92.49**

4. Conclusions

It is concluded from the results of this study that lemongrass was sensitive to water deficit. Water deficit has negative impacts on plant growth characteristics and relative greenness, but the essential oil percentage was affected positively under water deficit stress. Proline content increased under water deficit conditions which reflected its important role under such stress. In addition, the main compounds of essential oil—geranial and neral—increased under severe water deficit compared to low water deficit. Taking into account the water deficit challenges, especially in arid and semi-arid areas, it is recommended to increase the irrigation intervals to 10 days to decrease water consumption as well as to maintain small decreases in the herb and oil yield. The high quality of oil under stress will compensate for the decreases in yield economically by being sold at a high price. It is also recommended to conduct more research to increase the growth and quality of lemongrass under such stress conditions.

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