Int. Agrophysics, 2006, 20, 289-296

INTERNATIONAL
Agrophysics
www.ipan.lublin.pl/int-agrophysics

Influence of water stress on growth, essential oil, and chemical composition of herbs (*Ocimum* sp.)

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Received March 31, 2006; accepted June 19, 2006

A b s t r a c t. This work investigates the influence of water stress on vegetative growth, essential oil, proline, total carbohydrates, N, P, K, and protein contents of two species of an herb plant *ie Ocimum basilicum* L. (sweet basil) and *Ocimum americanum* L. (american basil). Experiments were carried out during two successive seasons, 2004 and 2005, with experimental pots containing Typic Torrifluvents soil (a clay loam) in a growth chamber at the Experimental Farm, National Research Center (NRC), Giza, Egypt.

Seedlings were treated with different levels of water-stress, determined as a percentage of field water capacity (FWC) by weight (50, 75, 100, and 125%). Fresh and dry weights of the herbs were significantly influenced by water stress. For both species under water stress, essential oil percentage, the main constituents of essential oil, proline, and total carbohydrate content increased, and N, P, K, and protein content decreased. Seventy five percent field water capacity resulted in the highest yield of herb and essential oil for both species.

K e y w o r d s: *Ocimum* sp., water stress, vegetative growth, essential oil, chemical composition

INTRODUCTION

The genus *Ocimum* (family Labiatae) includes at least 60 species and numerous varieties (Sirvastava, 1982). It represents an important source of essential oil used in the food, perfumery, and cosmetics industries. Some *Ocimum* species are used in traditional medicine for different applications, especially in many Asian and African countries (Yusuf *et al.,* 1994). The recurring polymorphism determines a large number of subspecies that produce essential oils with varying chemical composition. Some have a high camphor content, while others contain citral, geraniol, methylchavicol, eugenol, and thymol (Lawrence *et al*., 1980).

Medicinal and aromatic plants are of prime economic importance because of the continuous and increasing demand for their products by local and foreign markets. Basil is one of the most important plants in this regard. Its essential oil is extensively employed in several European countries and the USA for flavouring and foodstuffs, confectionery goods, condiments, and toiletry products such as mouthwashes and dental creams. It also finds a prime place in the flavouring of foods such as spices, meats, sausages, tomato pastes, various kinds of sauces, fancy vinegars, pickles, ketchups, and beverages. In the perfume industry, the essential oil is used for compounding popular perfumes, notably jasmine blends. Different parts of the plants are used in endogenous cultures for medicine and homoeopathy. It is also used as a febrifuge and antimalarial plant. The plant infusions are taken for cephalalgia, gouty joints, and gargle for foul breath. The juice obtained from leaves relieves sore throats and earaches and counteracts ring worm. Seeds are used internally for constipation and piles (Husain *et al.,* 1988).

In aromatic plants, growth and essential oil production are influenced by various environmental factors, such as water stress (Burbott and Loomis, 1969). Solinas and Deiana (1996), reported that secondary products of plants can be altered by environmental factors and water stress is a major factor affecting the synthesis of natural products. Changes in essential oils extracted from aromatic plants and their composition were observed with water stress (Sabih *et al.,* 1999). Water stress resulted in significant reduction of fresh and dry matter, nutrient content, and essential oil yield of Japanese mint plants (Mirsa and Strivastava, 2000). Fresh and dry weights of *Ocimum basilicum* L. were decreased as plant water deficit increased (Simon *et al.,* 1992). The

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linalool and methyl chavicol contents of sweet basil, as percentage of total essential oil, increased as water stress increased (Simon *et al.,* 1992). Essential oil and proline contents of sweet basil increased in response to water stress but plant growth was decreased with increasing water stress. The essential oil yield of basil was increased by subjecting plants to water stress just before harvesting (Baeck *et al*., 2001). Water stress reduced fresh and dry weights of *Satureja hortensis* L. (Savory) plants. Severe water stress increased essential oil content more than moderate water stress. The main constituents, such as carvacrol, increased under moderate water stress, while γ-terpinene content decreased under moderate and severe water stress of *Satureja hortensis* L. (Baher *et al*., 2002). Essential oil, total carbohydrate, and proline contents were pronouncedly increased with increasing stress levels of *Salvia officinalis* L. (Sage) plants (Hendawy and Khalid, 2005).

The major challenge facing water planners and managers is the availability of water. Its amount is fixed, but demand for it will continue to increase steadily into the foreseeable future. Reclamation of desert lands has been a top priority and challenge for the Egyptian government for the last few decades. In this study, we investigate the possible effect of water stress on the vegetative growth, essential oil content, and chemical content of *Ocimum basilicum* L. and *Ocimum americanum* L., that are two economically important plants in Egypt.

MATERIALS AND METHODS

Experiments were carried out at the Experimental Farm, National Research Centre (NRC), Giza, Egypt, during two successive seasons, 2004 and 2005. Physical and chemical properties of the soil used in this study were determined according to Jackson (1973) and Cottenie *et al*. (1982), and are presented in Table 1.

Seeds of *Ocimum basilicum* L. were kindly provided by the Department of Medicinal & Aromatic Plants, Ministry of Agriculture, Giza, Egypt, and seeds of *Ocimum americanum* L. were introduced from Cornell University, Ithaca, New York, USA, through scientific exchange, and were cultivated in Egypt for the propagation and adaptation to the environmental conditions of the country. Seeds of *Ocimum basilicum* L. and *Ocimum americanum* L. were sown in a nursery (in the field) in the first week of March in both seasons. After 45 days, the seedlings were transplanted into plastic pots (30 cm diameter and 50 cm in height); then the pots were transferred to a growth chamber adjusted to 35/24°C, 90/60% RH day/night and light intensity of approximately 3700 Lux for a period of 12 days. Each pot was filled with 10 kg of air-dried Typic Torrifluvents soil (USDA 1999), with a field water capacity of 43.5% based on the weight of the soil. Three weeks after transplanting, the seedlings were thinned to two plants per pot. Plants of both *Ocimum basilicum* L. and *Ocimum americanum* L. were subjected to different levels of water stress: 125, 100, 75, and 50% corresponding to the field water capacity (FWC) determined in the field (by weight). The first treatment (125% of field water capacity) was applied once at the beginning of the experiment; then the same amount of water was added to this treatment as was added to the 100% treatment, but drainage was prevented to maintain the 125% field water capacity level. A randomized factorial design with four replications was used in each season. Each replication contained eight treatments (2 *Ocimum* sp. x 4 water stress treatments). Each treatment had eight pots (2 plants per each). All agricultural practices, other than the experimental treatments, were done according to the recommendation of the Egyptian Ministry of Agriculture.

At full bloom, the plants were harvested three times (first, second, and third cuttings) during the growing seasons, by cutting the plants 5 cm above the soil surface. Total fresh and dry weights of the herbs (g plant^{-1}) were recorded.

The following chemical analyses were determined:

Fresh plants were collected from each treatment during the first, second, and third cuttings. They were dried by air and weighed to extract the essential oil. Dry plant material (300 g) from each replicate of all treatments was subjected to steam distillation for 3 h using a Clevenger type apparatus (Clevenger, 1928). The essential oil content was calculated as a percentage. In addition, total essential oil as g per plant was calculated by using the dry weight of the herbs. The essential oils extracted from *Ocimum basilicum* L. and *Ocimum americanum* L. were collected from the first, second, and third cuttings, then, the essential oils were collected from the treatments of 125, 100, 75 and 50% to identify the chemical constituents of the essential oil extracted from *Ocimum* sp.

Constituents of essential oil were determined by gas-liquid chromatography. The chromatograph (Model Perkin Elmer 3920B) was equipped with a thermal conductivity detector and a 2 m x 0.3 cm column packed with 10% Carbwax 20 M on 80/100 Chromsorb WAW, and hydrogen was used as the carrier gas at 0.5 cm^3 s^{-1}. The

T a b l e 1. Physical and chemical properties of the clay loam soil

Sand	Silt	Clay	Ca CO_3	OM	pH	Soluble cations and anions (meq l^{-1})								
		(%)				EC	Na^+	Mg^{++}	Ca^{++}	K^+	HCO_3^{-1}	Co_3^+	Cl^{-1}	SO_4^{-2}
26	36	38	4.5	1.3	7.7	0.57	2.23	0.88	1.11	1.48	1.12	0.73	2.1	1.62

column temperature was 130°C and detector and injector temperatures were 200°C. Constituents were identified by retention times and in conjunction with known structures.

Total carbohydrate levels were determined from plant material collected at the first, second, and third harvests of each treatment. The method of Dubois *et al*. (1956) was used.

Proline was determined in fresh leaves in the first, second, and third harvests using the method of Bates *et al.* (1973). Proline content was calculated as the average of the first, second, and third harvests.

Total nitrogen, protein, phosphorus, and potassium in the plants collected at the first, second, and third harvests of each treatment were determined using the methods described by the Association of Official Agricultural Chemists (A.O.A.C.) (1970).

The averages of data from each season were statistically analysed using analysis of variance (ANOVA) and values of least significant difference (L.S.D) at 5% according to Snedecor and Cochran (1990).

RESULTS AND DISCUSSION

The total fresh and dry weights of the plants (g plant^{-1}) were significantly affected by changes in soil moisture (Table 2). The interaction among treatments was significant during the two seasons. Total fresh and dry weights of *Ocimum americanum* L. were greater than those of *Ocimum basilicum* L.; this data may be the result of genetic differences between the species and suggests that *Ocimum americanum* L. is more tolerant to drought and excessive water than *Ocimum basilicum* L. (El-Beltagy and Soliman, 1983). Fresh and dry weights decreased under the various water stress levels. Differences among water stress treatments and the two species were significant. Total fresh and dry weights of plants were decreased due to exposure to injurious levels of drought (50%) or excessive water (125%). This could be the result of a reduction in chlorophyll content and, consequently, photosynthesis efficiency, as a reported by Abdul-Hamid *et al*. (1990), Castonguay and Markhart (1991), Nunez-Barrious (1991), and Viera *et al.* (1991). For both species in each season, the highest values of total fresh and dry weights were obtained with the 75% field water capacity treatment.

As shown in Table 3, an increase in essential oil percentage was observed under two water stress levels: 50% (drought) and 125% (excessive water) of field water capacity. These results are in line with those of Baher *et al*., 2002. While the highest yield of essential oil (g plant^{-1}) was obtained with the 100 and 75% field water capacity treatments for both species, these results may be due to the increment in herb dry weight of these treatments. The essential oil percentage of *Ocimum americanum* L. was less

T a b l e 2. Effect of water stress treatments, *Ocimum* sp. and their interactions on the fresh and dry weights of *Ocimum* sp. during both seasons

Ocimum sp.	Water stress treatments (%)	Total herb fresh weight (g plant^{-1})		Total herb dry weight (g plant^{-1})	
		1st season	2nd season	1st season	2nd season
Ocimum basilicum L.	125	872	794	176	179
	100	2162	2172	432	438
	75	2773	2703	554	541
	50	1393	1381	279	276
Over all *Ocimum basilicum* L.		1800	1764	360	359
Ocimum americanum L.	125	1162	1201	233	240
	100	2650	2512	534	551
	75	3428	3572	686	730
	50	2109	2379	418	427
Over all *Ocimum americanum* L.		2337	2416	468	487
Over all water stress treatments	125	1017	998	205	210
	100	2406	2342	483	495
	75	3101	3138	620	635
	50	1751	1880	349	352
LSD at 0.05					
Ocimum sp.		37.89	41.23	22.32	18.56
Water stress treatments		50.36	56.2	25.1	19.25
Ocimum sp. x water stress		40.23	45.23	30.12	29.45

T a b l e 3. Effect of water stress treatments, *Ocimum* sp. and their interactions on the essential oil content of *Ocimum* sp. during both seasons

Ocimum sp.	Water stress treatments (%)	Essential oil percentage		Total yield of essential oil (g plant^{-1})	
		1st season	2nd season	1st season	2nd season
Ocimum basilicum L.	125	0.36	0.32	0.63	0.6
	100	0.24	0.29	1.04	1.2
	75	0.33	0.31	1.83	1.68
	50	0.38	0.35	1.10	0.97
Over all *Ocimum basilicum* L.		0.33	0.32	1.15	1.11
Ocimum americanum L.	125	0.29	0.27	0.68	0.65
	100	0.23	0.21	1.23	1.16
	75	0.25	0.22	1.72	1.61
	50	0.30	0.25	1.25	1.07
Over all *Ocimum americanum* L.		0.27	0.24	1.22	1.12
Over all water stress treatments	125	0.33	0.30	0.66	0.63
	100	0.24	0.25	1.14	1.18
	75	0.29	0.27	1.78	1.65
	50	0.34	0.30	1.18	1.02
LSD at 0.05					
Ocimum sp.		0.001	0.001	0.01	0.01
Water stress treatments		0.005	0.004	0.03	0.02
Ocimum sp. x water stress		0.008	0.007	0.04	0.03

than that of *Ocimum basilicum* L., which may be due to genetic differences between the species (El-Beltagy and Soliman, 1983). However, *Ocimum americanum* L. had a greater content of total essential oil (g plant^{-1}) than *Ocimum basilicum* L. during both seasons. These results may be due to the increment in herb dry weight per plant of *Ocimum americanum* L. compared with *Ocimum basilicum* L.

Tables 4 and 5 show the effect of soil moisture levels on the chemical composition of essential oil extracted from *Ocimum basilicum* L. and *Ocimum americanum* L. The main components were found to be methylchavicol, linalool, 1,8-cineol, and geraniol for *Ocimum basilicum* L. and eugenol, methyl chavicol, terpneol, and farnesene for *Ocimum americanum* L. The lowest components of *Ocimum basilicum* L. were camphene and sabenene with 125% treatment, camphene with 100 and 75% treatments, and α-pinene with 50% treatment. The lowest components of *Ocimum americanum* L. were myrcene with 125% treatment, β-pinene with 100 and 75% treatments, and α-pinene with 50% treatment.

Compared with the constituents obtained from 100% of field water capacity treatment we can indicate that:

– 125% of field water capacity treatment increased the constituents of α-pinene, camphene, myrcene, 1,8-cineol, ocimene, linalool, camphore, methylchavicol, geraniol, β-caryophyelene germacrene D, α-cadinol (for *Ocimum basilicum* L.), 1,8-cineol, camphore, farnesene, β-bisabolene, methyl-chavicol, terpneol and eugenol (for *Ocimum americanum* L.), while it decreased the constituents of sabenene, β-pinene, limonene, terpinolene, eugenol, methyl-eugenol and geranyl iso-butyrate (for *Ocimum basilicum* L), α-pinene, β-pinene, myrcene, α-terpinene, limonene, linalool, linalyl acetate, methyl-eugenol, iso-eugenol and farnesol (for *Ocimum americanum* L.);

– 75% of field water capacity treatment increased the components of camphene, β-pinene, 1,8-cineol, ocimene, terpinolene, linalool, camphore, methyl-chavicol, geraniol, methyl-eugenol, β-caryophyelene, germacrene D, geranyl iso-butyrate (for *Ocimum basilicum* L), 1,8-cineol, camphore, farnesene, β-bisabolene, methyl-chavicol, terpneol, and eugenol (for *Ocimum americanum* L.), however it decreased the constiuents of α-pinene, sabenene, myrcene, limonene, eugenol and α-cadinol (for *Ocimum basilicum* L), α-pinene, β-pinene, α-terpinene, limonene, linalool, myrcene, linalyl acetate, methyl-eugenol, iso-eugenol and farnesol (for *Ocimum americanum* L.);

Table 4. Effect of water stress treatments on the chemical constituents of essential oil extracted from *Ocimum basilicum* L. herb during both seasons

Compounds		Water stress treatments *Ocimum basilicum* L.			
		125%	100%	75%	50%
1	α-Pinene	0.5	0.4	0.3	0.2
2	Camphene	0.2	0.1	0.2	0.3
3	Sabenene	0.2	0.3	0.2	0.4
4	β-Pinene	0.5	1.6	1.8	1.0
5	Myrcene	1.4	1.3	1.2	1.2
6	Limonene	1.2	1.3	1.2	1.1
7	1,8-Cineol	9.4	8.7	8.8	9.9
8	Ocimene	1.3	1.2	1.4	1.1
9	Terpinolene	1.1	1.2	1.3	1.1
10	Linalool	33.4	32.5	32.8	35.6
11	Camphore	1.6	1.5	1.8	1.9
12	Methyl-chavicol	35.0	34.0	34.6	34.5
13	Geraniol	5.8	5.1	5.6	6.0
14	Eugenol	1.5	2.7	2.6	1.0
15	Methyl-eugenol	1.5	3.2	3.9	1.0
16	β-Caryophyelene	1.8	1.6	1.8	0.7
17	Germacrene D	1.3	1.0	1.2	1.1
18	Geranyl iso-butyrate	0.9	1.0	1.1	1.0
19	α-Cadinol	1.4	13.0	1.2	0.9

Table 5. Effect of water stress treatments on the chemical constituents of essential oil extracted from *Ocimum americanum* L. herb during both seasons

Compounds		Water stress treatments *Ocimum americanum* L.			
		125%	100%	75%	50%
1	α-Pinene	1.09	1.27	1.07	0.10
2	β-Pinene	1.21	1.23	0.34	0.25
3	Myrcene	0.20	1.30	0.10	0.15
4	α-Terpinene	1.12	2.30	0.16	0.22
5	Limonene	1.00	1.55	0.12	0.12
6	1,8-Cineol	2.90	2.81	4.10	3.60
7	Camphore	2.00	1.96	2.30	2.20
8	Linalool	1.94	1.97	1.90	2.10
9	Linalyl acetate	1.78	1.85	0.75	0.66
10	Farnesene	10.60	9.74	13.20	12.40
11	β-Bisabolene	4.90	3.75	5.90	5.90
12	Methyl-chavicol	22.90	20.70	24.90	25.60
13	Terpneol	15.70	13.90	14.60	14.00
14	Methyl-eugenol	0.63	3.71	0.90	1.00
15	Eugenol	29.40	26.10	27.65	29.90
16	Iso-eugenol	1.74	3.11	1.14	1.00
17	Farnesol	0.89	2.75	0.87	0.80

T a b l e 6. Effect of water stress treatments, *Ocimum* sp. and their interactions on the chemical composition of *Ocimum* sp. during both seasons

Ocimum sp.	Water stress treatments (%)	Total carbohydrates (%)		Proline (μm g^{-1})		N (%)		Protein (%)		P (%)		K (%)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Ocimum basilicum L.	125	9.53	10.00	6.97	7.27	2.27	2.50	14.19	15.63	0.39	0.44	2.70	2.71
	100	5.13	5.63	3.80	4.10	2.81	2.93	17.57	18.31	0.89	0.85	3.96	3.07
	75	6.30	6.80	4.67	5.00	2.51	2.47	15.70	15.43	0.71	0.64	3.04	2.74
	50	9.07	9.57	6.13	6.43	2.39	2.35	14.94	14.69	0.64	0.59	2.81	2.69
Over all *Ocimum basilicum* L.		7.51	8.00	5.40	5.70	2.49	2.60	15.60	16.00	0.66	0.63	3.13	2.80
Ocimum americanum L.	125	10.70	11.23	8.67	8.97	2.09	2.44	13.10	15.25	0.63	0.58	2.50	2.50
	100	5.50	5.97	4.10	4.40	2.72	2.72	17.00	17.00	0.74	0.73	3.29	2.96
	75	7.17	7.67	4.20	4.53	3.49	3.57	21.81	22.31	0.84	0.95	3.03	3.22
	50	9.77	10.27	6.20	6.50	2.31	2.41	14.43	15.07	0.66	0.79	2.74	2.83
Over all *Ocimum amer.* L.		8.30	8.80	5.80	6.10	2.65	2.79	16.59	17.41	0.72	0.76	2.89	2.88
Over all water stress treatments	125	10.10	10.60	7.80	8.10	2.18	2.47	13.65	15.44	0.51	0.51	2.60	2.61
	100	5.30	5.80	4.00	4.30	2.77	2.83	17.29	17.66	0.82	0.79	3.63	3.02
	75	6.70	7.20	4.40	4.80	3.00	3.02	18.76	18.87	0.78	0.80	3.04	2.98
	50	9.40	9.90	6.20	6.50	2.35	2.38	14.69	14.88	0.65	0.69	2.78	2.76
LSD at 0.05													
Ocimum sp.		0.26	0.26	0.14	0.14	0.05	0.03	0.52	0.20	0.03	0.02	0.03	0.07
Water stress treatments		0.32	0.31	0.17	0.17	0.06	0.04	0.63	0.24	0.03	0.02	0.04	0.08
Ocimum sp. x water stress		0.52	0.51	0.18	0.28	0.09	0.07	1.03	0.40	0.05	0.04	0.07	0.14

– 50% of field water capacity treatment increased the components of camphene, sabenene, 1,8-cineol, linalool, camphore, methyl-chavicol, geraniol, germacrene D, geranyl iso-butyrate (for *Ocimum basilicum* L), 1,8-cineol, camphore, linalool, methyl-chavicol, terpneol, farnesene, β-bisabolene and eugenol (for *Ocimum americanum* L.), while the constituents of α-pinene, β-pinene, myrcene, limonene, ocimene, terpinolene, eugenol, methyl-eugenol, β-caryophyelene, α-cadinol (for *Ocimum basilicum* L.), farnesol, α-pinene, β-pinene, myrcene, α-terpinene, limonene, linalyl acetate and methyl-eugenol (for *Ocimum americanum* L.) were decreased.

These results are in accordance with those obtained by Simon *et al.* (1992), and Vina and Murillo (2003). The effect of different treatments on essential oil and its constituents may be due to its effect on enzyme activity and metabolism (Burbott and Loomis, 1969).

Table 6 shows that total carbohydrate content was increased with water stress levels of 125 and 50% of field water capacity. However, the 100 and 75% field water capacity treatments caused a decrease in total carbohydrate of both species during each season. These findings are in accordance with those obtained by Khalid (2001) for *Nigella sativa* L. (Black cumin) plants and by Hendawy and Khalid, (2005), for *Salvia officinalis* L. plants. In the same trend, proline was increased with water stress levels (125 and 50% of field water capacity) and these results agree with Blum and Ebercon (1976), who indicated that proline is regarded as a source of energy, carbon, and nitrogen for recovering tissues, so it increased under water stress levels.

N, P, K, and protein contents were affected by excessive water treatment (125%) or drought (50%), which resulted in the lowest percentage of N, P, K, and protein content. The 75 and 100% field water capacity treatments gave the highest content of N, P, K, and protein content for both herbs in both seasons (Table 6). These results are confirmed by those of Mirsa and Strivastava (2000), for Japanese mint plants, Khalid (2001), for *Nigella sativa* L. plants, and Hendawy and Khalid (2005), for *Salvia officinalis* L. plants.

CONCLUSIONS

1. Water stress treatments increased essential oil percentage, main constituents of essential oil, proline, and total carbohydrate content,

2. Water stress treatment decreased N, P, K, and protein content.

3. Seventy five percent field water capacity resulted in the highest yield of herb and essential oil for both *Ocimum* species.

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