Int. Agrophysics, 2010, 24, 183-188

INTERNATIONAL
Agrophysics
www.international-agrophysics.org

Response of different basil varieties to soil salinity

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Received January 28, 2009; accepted June 18, 2009

A b s t r a c t. A pot experiment was carried out during two successive seasons to study the effects of varieties of basil (*Ocimum basilicum* var. odoratus, *Ocimum basilicum* var. alba, *Ocimum basilicum* var. thyrsiflorum and *Ocimum basilicum* var. purpurascens) and soil salinity levels (0, 1 500, 3 000 and 4 500 mg kg^{-1}) on oil content and its composition. Essential oil (%) and oil yield (ml $plant^{-1}$) were significantly increased by using 1 500 mg kg^{-1} of soil salinity compared to control. On the contrary, there was significant decrease in this regard by using higher level of soil salinity, at 4 500 mg kg^{-1}. The major components of the essential oil of basil varieties were eugenol and linalool in the two cuts. Soil salinity treatments at 1 500 and 4 500 mg kg^{-1} levels increased the content of linalool and, on the contrary, there was a decrease in eugenol content by using 1 500 and 4 500 mg kg^{-1} of soil salinity in *Ocimum basilicum* var. purpurascens.

K e y w o r d s: basil, soil salinity, essential oil, eugenol, linalool

INTRODUCTION

Nowadays, medicinal and aromatic plants occupy a prominent economic position because of the continuous and increasing demand for their products. Basil is one of the most important plants in this concern. The oil is extensively employed in several countries for flavouring of food stuffs, confectionery goods, and condiments and in toiletry products. It also finds a prominent place in the flavouring of foods, and in perfumes industry. Various uses are attributed to different parts of the plant in indigenous system of medicine and homoeopathy. It is also recognized as a febrifuge and antimalarial plant. Basil is represented by the plant *Ocimum basilicum* L. belonging to the family *Lamiaceae*. *Ocimum basilicum* L. is the most important species being utilised as a source of essential oil. In view of the great diversity, the various species and varieties have been classified, in accordance with their chemical composition and geographical sources, into four major types as follows: European or sweet basil; Reunion basil; methyl cinnamate basil; and eugenol basil; it is distilled in Russia and some North African countries, including Egypt and Morocco, it is an oil rich in eugenol (Husain *et al.,* 1988).

In Egypt, saline water is used for irrigation in some areas. At the same time, under the arid climatic conditions prevailing in Egypt and associated with the perennial irrigation practices, imperfect drainage system, continuous increase of water table level and the relatively high salinity levels of water sources, particularly in the new reclaimed land, the salinization of Egyptian soils is rapidly becoming an acute problem. However, in our opinion, such goal may be achieved by introducing new species or varieties of basil to be cultivated for the first time in Egypt under soil salinity conditions, and clarifying to what extent the different species and varieties of basil can tolerate soil salinity and through selecting the superior tolerant species or varieties against soil salinity conditions.

This work aimed to evaluate the essential oil content and its composition of different varieties of basil cultivated in saline soil.

MATERIALS AND METHODS

The present work was carried out under the natural conditions of the greenhouse of the National Research Center, Dokki, Giza, Egypt, during the two successive seasons of 1995 and 1996.

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Four varieties of *Ocimum basilicum* L. viz., *Ocimum basilicum* var. odoratus (V1), *Ocimum basilicum* var.alba (V2), *Ocimum basilicum* var. thyrsiflorum (V3) and *Ocimum basilicum* var. purpurascens (V4) were introduced from Suadi Arabia. These four varieties were identified botanically by Herbarium Royal Botanic Gardens, Kew, Richmond, Surry TW9 3EA, England.

For cultivation, earthenware pots were used. The pots used were painted with three layers of tar (bitumine) and their bottom holes were blocked to prevent water loss, then each pot was filled with 7 kg of air dried soil. Before sowing, physical and chemical properties of the soil of the experiment were determined by standard methods. The soil texture was sandy loam, having a physical composition as follows: 50.8% sand, 26% silt, 23.2% clay and 0.58% organic matter. The results of soil chemical analysis were as follows: pH – 8.4; total nitrogen – 0.05%; available phosphorus – 0.65 mg 100 g^{-1} and potassium – 18.92 mg 100 g^{-1}.

Four levels of artificial soil salinity were used by dissolving known mass of natural salt crust of sea water, which was obtained from El-Nasr for Saline's Company, in tap water. The levels of soil salinity applied were: tap water (control), 1 500, 3 000 and 4 500 mg kg^{-1} (based on air dry soil mass). For the salinization of the soil, the appropriate portion of the salt was dissolved in sufficient amount of tap water to ensure more uniform distribution of salt within the pot soil. This procedure was carried out prior to transplantation. The plants were continuously irrigated when needed to maintain soil moisture at 65-70% of field capacity.

The seeds of basil varieties were sown in the nursery on March 6th throughout the two successive seasons of 1995 and 1996. One and a half month after seed sowing, uniform seedlings were transplanted into pots. Each pot contained five plants.

The experimental design was factorial between varieties (four varieties) and soil salinity (four levels) in complete randomised design with three replicates. Each replicate contained seven pots. These treatments were symbolized as follows: S0 – tap water (control), S1 – 1 500, S2 – 3 000, and S3 – 4 500 mg kg^{-1}.

Essential oil percentage of the fresh leaves of each replicate at the first and second cuts after 60 and 120 days from transplanting, respectively, was determined according to the method described in the British Pharmacopoeia (2002) by using Clevenger apparatus and expressed as (ml 100 g^{-1} fresh herb), while essential oil yield per plant was expressed as ml $plant^{-1}$. The resulted essential oil of each treatment was collected and dehydrated over anhydrous sodium sulphate and kept in refrigerator until GLC analyses. The GLC analysis of the oil samples was carried out in the second season using G cv Pye-Unicam gas chromatograph equipped with dual flame ionisation detectors at the Central Laboratory of the Faculty of Agriculture, Cairo University. The chromatograph was fitted a coiled glass column (1.5 m x 4 mm) packed with diatomite C (100-120 mesh) and coated with (10%) PEGA. The oven temperature was programmed at 4°C min^{-1}, from 70 to 190°C, and was held at 190°C for 15 min. Detector and injector temperatures were 250 and 300°C, respectively. Gas flow rates for N_2, H_2 and air were 30, 33 and 330 ml min^{-1}, respectively. Main compounds of the essential oil were identified by matching their retention times with those of the authentic samples that were injected under the same conditions. The relative percentage of each compound was calculated from the peak area corresponding to each compound. Except for the constituents of the essential oils, the data of this experiment were statistically analysed using LSD at the 5% level.

RESULTS AND DISCUSSION

Data in Table 1 indicate that the oil % of *Ocimum basilicum* var. purpurascens (V4) was higher than in the other varieties, whereas *Ocimum basilicum* var. alba (V2) was lower in this respect in the two cuts during the two seasons. In the meantime, oil percentage of V4 was significantly increased compared to that of *Ocimum basilicum* var. alba (V2) at the two cuts in both years. In addition, there were significant differences between *Ocimum basilicum* var. alba (V2) and *Ocimum basilicum* var. odoratus (V1) or *Ocimum basilicum* var. thyrsiflorum (V3) in this regard in the first cut during the two seasons. Generally, oil percentage values recorded for basil varieties were as follows: V4 (0.2041-0.1966), V1 (0.1999-0.1812), V3 (0.1728-0.1770) and V2 (0.1583-0.1666) in the second cut during the two seasons, respectively. Table 2 shows that soil salinity at 1 500 mg kg^{-1} significantly increased oil %, but the higher level of 4 500 mg kg^{-1} significantly decreased oil % at the first cut of both seasons. At the second cut, levels at 1 500 and 3 000 mg kg^{-1} showed significant increases in this respect, Moreover, as salinity levels increased the oil % was decreased to reach its minimum at higher one of 4 500 mg kg^{-1}. The results were similar in both of the two seasons. An inhibitory effect of high level of salinity was also found by Abd El-Wahab (2006), Baghalian *et al.* (2008), Ozturk *et al.* (2004), Razmjoo *et al.* (2008), and Shalan *et al*. (2006). The increase in oil % due to lower levels of salinity was also found by Baher *et al.* (2002), Hendawy and Khalid (2005), and Tabatabale and Zari (2007). From data in Table 1 it can be concluded that the interaction treatments between varieties and salinity level at 1 500 mg kg^{-1} mostly increased oil %, whereas, those at 3 000 and 4 500 mg kg^{-1} decreased it compared with the control of every variety alone. The highest value of oil % was obtained by using V4 under soil salinity at 1 500 mg kg^{-1}.

From data in Table 1, the results indicate that oil yield values recorded by basil varieties were as follows: V1 (0.0714-0.0608), V2 (0.0568-0.0604), V3 (0.0692-0.0637) and V4 (0.0849-0.0789) in the second cut during the two

T a b l e 1. Effect of varieties, soil salinity and their interaction treatments on oil content of basil at the two cuts (1st and 2nd) during the two seasons

Treatments		Oil (%)		Oil yield (ml plant^{-1})		Oil (%)		Oil yield (ml plant^{-1})	
		First season (1995)				Second season (1996)			
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
S0	V1	0.2000	0.1833	0.0642	0.0739	0.2080	0.1333	0.0496	0.0533
	V2	0.1830	0.1583	0.0491	0.0619	0.1750	0.1583	0.0431	0.0693
	V3	0.2333	0.1750	0.0740	0.0775	0.2160	0.1500	0.0635	0.0601
	V4	0.2000	0.1583	0.0585	0.0718	0.2000	0.1916	0.0637	0.0838
	Mean	0.2041	0.1687	0.0614	0.0712	0.1997	0.1583	0.0549	0.0666
S1	V1	0.2333	0.1833	0.0653	0.0670	0.2250	0.2000	0.0498	0.0749
	V2	0.2083	0.1666	0.0536	0.0610	0.2083	0.1833	0.0462	0.0682
	V3	0.2083	0.1833	0.0665	0.0782	0.2333	0.2166	0.0594	0.0856
	V4	0.3583	0.3250	0.1016	0.1452	0.3333	0.2783	0.0862	0.1147
	Mean	0.2520	0.2145	0.0717	0.0878	0.2499	0.2195	0.0604	0.0858
S2	V1	0.2583	0.2583	0.0441	0.0940	0.2083	0.2333	0.0326	0.0721
	V2	0.2000	0.1583	0.0408	0.0601	0.1666	0.1833	0.0242	0.0633
	V3	0.2500	0.1666	0.0506	0.0686	0.2250	0.1916	0.0398	0.0663
	V4	0.1833	0.1833	0.0420	0.0788	0.1833	0.1833	0.0266	0.0732
	Mean	0.2229	0.1916	0.0443	0.0753	0.1958	0.1978	0.0308	0.0687
S3	V1	0.1750	0.1750	0.0213	0.0510	0.1750	0.1583	0.0163	0.0431
	V2	0.1666	0.1500	0.0315	0.0445	0.1500	0.1416	0.0184	0.0408
	V3	0.1666	0.1666	0.0312	0.0526	0.1716	0.1500	0.0210	0.0431
	V4	0.1666	0.1500	0.0298	0.0438	0.1466	0.1333	0.0182	0.0441
	Mean	0.1687	0.1604	0.0284	0.0479	0.1608	0.1458	0.0184	0.0427
Mean	V1	0.2166	0.1999	0.0487	0.0714	0.2040	0.1812	0.0370	0.0608
	V2	0.1895	0.1583	0.0437	0.0568	0.1749	0.1666	0.0329	0.0604
	V3	0.2145	0.1728	0.0555	0.0692	0.2114	0.1770	0.0459	0.0637
	V4	0.2270	0.2041	0.0597	0.0849	0.2158	0.1966	0.0486	0.0789
Salinity*		0.0213	0.0204	0.0105	0.0094	0.0210	0.0158	0.0300	0.0087
Varieties*		0.2130	0.0204	0.0105	0.0094	0.0210	0.0158	0.0300	0.0087
Interaction*		0.0427	0.0408	0.0210	0.0189	0.0421	0.0316	0.0600	0.0174

*LSD at 5%.

seasons, respectively. Moreover, V4 oil yield was significantly increased compared to that of V2. Also, the difference between V4 and V1 was significant at the two cuts in both years. It was noticed that V4 had the largest value of oil yield, whereas V2 had the lowest in this regard at the second cut during the two seasons. Furthermore, the difference between V4 and V3 was significant in the second cut during both seasons. Table 1 indicates that soil salinity treatments under 3 000 and 4 500 mg kg^{-1} in the first cut and that of 4 500 mg kg^{-1} in the second cut significantly decreased oil yield, while there was an increase in this regard by using the treatment of 1 500 mg kg^{-1} in the first cut (insignificant) and the second cut (significant) compared to that of control in both the two seasons.

However, increasing salinity levels increased the reduction in oil yield to reach its minimum at the higher one of 4 500 mg kg^{-1}. The results were similar in both of the two seasons. Similar results were found by Abd El-Wahab (2006), Baghalian *et al.* (2008), Ozturk *et al.* (2004), Razmjoo *et al.* (2008). An increase in oil yield due to lower levels of salinity was also found by Baghalian *et al.* (2008), Baher *et al.* (2002), Hendawy and Khalid (2005), Tabatabale and Zari (2007). The highest value of oil yield /plant was obtained by using V4 under soil salinity at 1 500 mg kg^{-1} level compared to the other interactions. Increasing the soil salinity under each variety decreased oil yield. But, under each level of salinity, V2 gave the lowest value in this respect in most cases. The interaction treatments between V3 and V2 and soil salinity decreased oil yield at the first and second cuts, respectively. At the first cut, there were increases in oil yield by using V1, V2 and V4 under salinity at 1 500 mg kg^{-1}, whereas, oil yield in these varieties (V1, V2 and V4) was decreased by using soil salinity at 3 000 mg kg^{-1}. At the second cut, using 1 500 mg kg^{-1} caused an increase in oil yield for the varieties of V3 and V4 also that of 3 000 mg kg^{-1} gave the same result by V1. Penka (1978) showed that the formation and accumulation of essential oil in plants was attributable to the action of environmental factors. It might be claimed that the formation and accumulation of essential oil was directly dependent on perfect growth and development of the plants producing oils. The decrease in oil production might be due to the decrease in plant anabolism. Morales *et al.* (1993) suggested that an increase in oil content in some of the salt stressed plants might be attributed to decline the primary metabolites due to the effects of salinity, causing intermediary products to become available for secondary metabolites synthesis. It is known that climatic conditions and water available in the soil can change the vegetal secondary metabolism and, consequently, alter the composition of essential oils, throughout the seasons of the year. Chemical variations in essential oils were associated with seasons for *Ocimum selloi* (Moraes *et al.,* 2002) and with time of day for *Ocimum gratissimum* (Vasconcelos Silva *et al.,* 1999). Also, Omer *et al*. (2008) who studied different basil species/varieties behaviour under soil salinity conditions found that essential oil content and their constituents were differed in different basil species/varieties studied.

Tables 2 and 3 show the data belonging to qualitative and quantitative constituents of essential oils distilled from the four basil varieties before flowering stage at both cuts during the season of 1996. According to analysis of essential oils, in both cuttings all identified compounds were detected in the oil of all varieties but at different percentages, 12 compounds were similarly identified. The known compounds were grouped into three items *ie* major compounds (more than 10%), minor compounds (less than 10% and more than 1%) and trace ones (less than 1%). In this respect, it is evident that linalool and eugenol exhibited as majors, 1, 8-cineol, methylchavicol, methyl eugenol and farnesol were represented as minors, and α – pinene, β – pinene, myrcene, ocimene, linalyl acetate and geraniol were considered as traces.

T a b l e 2. Effect of basil varieties (V1, V2, V3, and V4) on the constituents of volatile oil percentage at the two cuts (1st and 2nd) in the second season

Compounds	V1 odoratus		V2 alba		V3 thyrsiflorum		V4 purpurascens	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
α-pinene	0.3402	0.3117	0.1517	0.1279	0.1216	0.2936	0.1532	0.3681
β-pinene	0.4301	0.2406	0.2317	0.3333	0.1318	0.2010	0.3024	0.5594
Myrcene	0.3872	0.4853	0.0553	0.3748	0.2749	0.3462	0.1296	0.1613
1,8-cineol	5.4382	4.9983	4.1441	2.4667	0.2729	4.2334	4.7222	7.3321
Ocimene	0.1246	0.1611	0.1384	0.0965	0.0674	0.1210	0.2705	0.1147
Linalool	36.8780	36.4172	32.4107	27.3000	39.7444	38.5207	29.9783	33.3005
Linalyl acetate	trace	0.2510	0.3107	trace	0.2555	0.4968	0.1131	0.5129
Methylchavicol	8.7356	6.9409	1.1024	1.4712	12.3986	1.9104	1.3114	1.0600
Geraniol	0.2564	0.1924	0.0840	0.2109	0.3189	trace	trace	0.1530
Methyl eugenol	2.8989	4.5861	7.7714	6.6123	3.5614	5.6785	7.3030	5.8532
Eugenol	38.8716	42.8655	51.6916	57.7952	38.3674	46.4960	52.4778	48.1674
Farnesol	2.6220	1.4854	1.6008	1.6643	1.8103	1.1799	1.9584	0.8717
Identified compounds	96.9828	98.9355	99.6928	98.4531	97.3251	99.4775	98.7199	98.4543
Unidentified compounds	3.0172	1.0645	0.3072	1.5469	2.6749	0.5225	1.2801	1.5457

T a b l e 3. Effect of soil salinity treatments on the constituents of the volatile oil percentage in *Ocimum basilicum* var. purpurascens at the two cuts in the second season (1996)

Compounds	S1		S3	
	1st	2nd	1st	2nd
α-pinene	0.3019	0.2542	0.2709	0.3140
β-pinene	0.4545	0.3038	0.4238	0.2726
Myrcene	0.4627	0.2204	0.5388	0.2964
1,8-cineol	8.2130	5.0445	7.6832	6.4773
Ocimene	0.1824	0.2088	0.1570	0.2622
Linalool	35.8102	36.9534	41.3289	38.5281
Linalyl acetate	0.4864	0.3133	0.3130	0.4083
Methylchavicol	4.1565	3.4094	9.1591	6.0108
Geraniol	0.2947	0.3438	0.4577	0.3772
Methyl eugenol	6.0209	5.3061	4.6544	5.1951
Eugenol	39.7472	43.6879	29.9262	37.3412
Farnesol	2.5765	1.7585	2.8142	2.5612
Identified compounds	98.7069	97.8041	97.7272	98.0444
Unidentified compounds	1.2931	2.1959	2.2728	1.9556

The results in Table 2 show that V4 and V2 gave the highest content of eugenol, methyl eugenol and linalyl acetate, and that V4 and V1 gave the same result of 1, 8-cineol, α – pinene, β – pinene and ocimene in the first and second cuts, respectively. However, V3 followed by V1 gave the highest content of linalool, methylchavicol and geraniol. Also, V1 and V2 gave the same result of farnesol and myrcene. These results were recorded in the two cuts. According to major compounds, it was obviously clear that linalool was decreased and eugenol was increased at the second cut, comparing to that of the first cut *ie* V1, V2 and V3, whereas, linalool in V4 was increased and eugenol was decreased. With regard to the minors, it was evident that methylchavicol and farnesol were increased in V2, whereas both compounds were decreased in V1, V3 and V4 at the second cut compared to that of the first cut. On the other hand, α – pinene and 1,8-cineol behaved at the same trend - both compounds were increased in V3 and V4, whereas, they were increased in V1 and V2 at the second cut comparing to that of the first cut. Also, methyl eugenol and ocimene were increased in V1 and V3 and decreased in V2 and V4 at the second cut comparing to that of the first cut.

Basil essential oil could be classified into four chemotypes: linalool and methylchavicol type; methylchavicol type; methyl cinnamate type and eugenol type (Vernin *et al.,* 1984). Hegnuaer (1966) reported that these were four distinct chemotypes of *O. basilicum eg* methylchavicol/ linalool; camphor; methyl cinnamate and eugenol types. Sobti and Pushpangadan (1982) reported that, in addition to methylchavical and linalool containing oils, they found the following: (1) geraniol (40-50%) and eugenol (20-30%), (2) eugenol (20-40%), (3) camphor (10-15%), (4) methyl cinnamate (60-65%), (5) geraniol (20-35%), linalool (30-35%) and eugenol (20-30%). According to the chemical composition and geographical origin, Lawrence (1988, 1989) and Lawrence *et al.* (1980) observed that the chemical composition and morphological characters of *O. basilicum* varieties very variable and established four essential oil chemotypes *ie* methylchavicol; linalool; methyleugenol and methylcinnamate and also numerous subtypes. According to the biosynthetic origins of major compounds, he classified them as chemotypes with single or double biosynthetic pathways.

Data in Table 3 show that there was a considerable change in the contents of compounds from V4 at the two cuts under soil salinity levels at 1 500 and 4 500 mg kg^{-1}. Soil salinity treatments caused an increase in the contents of linalool, methylchavicol, farnesol, geraniol and myrcene compared to control, at the two cuts. On the contrary, soil salinity treatments caused a decrease in the contents of eugenol and methyl eugenol as compared to control at the two cuts. Also, ocimene, 1, 8-cineol, α – pinene, β – pinene and linalyl acetate were decreased by using soil salinity levels at 1 500 and 4 500 mg kg^{-1} at the first and second cuts, respectively. Furthermore, the highest content of linalool, methyl-chavicol, farnesol, ocimene and geraniol were increased to record its maximum by using that of 4 500 mg kg^{-1} at the first cut.

The present study was in agreement with that by El-Keltawi and Croteau (1987) on spearmint and marjoram who indicated that irrigation of both plants with saline solution consisting of $CaCl_2$ and NaCl reduced essential oil. They added that under salinity in spearmint the content of limonene was increased and carvone was concomitantly decreased relative to control treatments irrigated with water only. In the case of marjoram, salt stress led to an increase in the content of sabinene, which was accompanied by a decrease in the content of sabinene hydrate. Hendawy and Khalid (2005), in a study on *Salvia officinalis*, reported that treatment of 2 500 mg kg^{-1} soil salinity increased α – thujone, camphor and 1,8-cineol but it decreased the component of β – thujone compared with the control treatment.

CONCLUSIONS

1. *Ocimum basilicum* var. purpurascens (V4), followed by *Ocimum basilicum* var. thyrsiflorum (V3), recorded higher values of oil production compared to those of the other ones of *Ocimum basilicum* var. alba (V2) then *Ocimum basilicum* var. odoratus (V1).

2. The treatment of soil salinity caused an increase in oil percentage up to 3 000 mg kg^{-1} and treatment at 4 500 mg kg^{-1} decreased the oil percentage, but soil salinity treatments caused a decrease in oil yield.

3. *Ocimum basilicum* var. purpurascens (V4), under soil salinity at 1 500 mg kg^{-1} treatment, recorded an increase in oil production compared to the other interactions in most cases.

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