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GC-MS analyses of black cumin essential oil produces with sodium chloride

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Abstract

The black cumin essential oil and seeds are used in folk medicine, as a bread or cheese flavoring and as a spice in various kinds of meals. In arid and semi-arid regions, where water availability is a major limitation in crop production, using alternative water resources, such as saline water is one way to utilize these barren lands. In this study, we investigate the possible effect of saline irrigation water (SIW) on essential oil (EO) of black cumin an economically important aromatic plant in Egypt. Application of SIW caused a pronounced increment in both EO content and yield. The highest yields of EO (1.1 ml 100 plant^{-1}) obtained from the treatments of 3.1, 4.6 and 6.3 dSm^{-1}. The main constituents of *Nigella sativa* L seeds EO as detected by GC/MS were p-cymene, α-thujen, β-pinene, γ-terpinene, terpinen-4-ol, thymoquinone and carvacrol which increased as saline irrigation water level increase. Monoterpene hydrocarbons, oxygenated monoterpene and sesquiterpene hydrocarbons were increased as SIW level increase.

Introduction

Black cumin or *Nigella sativa* L *(N. sativa)* belongs to the family *Ranunculaceae* and is widely distributed and cultivated in Mediterranean countries, middle Europe and western Asia. The ancient Egyptians, Greeks and Romans were already aware of the therapeutic properties of *N. sativa*, the essential oil and seeds of which are still used in folk medicine, as a bread or cheese flavoring and as a spice in various kinds of meals (Wajs, 2008). Previous studies have shown monoterpenes, including p-cymene, α-thujene, γ-terpinene, carvacrol, α-pinene and β pinene, to be the main components of the essential oil from black cumin (Wajs, 2008). High saline sodic condition has become a major restrain adversely affects plants physiological processes and limits field and horticultural crops performance particularly in arid and semi-arid regions of the world. High salt occurrence in the soil solution is linked to create the high osmotic pressure in the rhizosphere and ultimately reduced availability of water and nutrients to plant. Salinity conditions are known to affect plants physiological and biochemical potential, which in turn affect crops primary and secondary metabolism (Hebbara *et al*., 2003; Hendawy and Khalid, 2005). The different results were dedicated from the effect of salinity stress on the essential oil. For instance, it was found that increasing of salinity stress decreased EO amount in Chamomile (Razmjoo *et al*., 2008) and EO yield in peppermint and lemon verbena (Tabatabaie and Nazari, 2007). Also, the effect of salinity parameter on EO quality in lemon balm showed the increased amount of geranial as salinity level was increased (Ozturk *et al*., 2004). Irrigation with SIW increased the calendula and lemon balm EO content and its main components (Khalid and Jaime, 2010; Khalid and Cai, 2011). Salinity significantly affects the EOs of rosemary compounds (Langroudi *et al*., 2013). The Egyptian climate is mostly arid and semi-arid, where water availability is a major problem for aromatic plants production. Nineteen percent of total agricultural lands of Egypt have salt in water or soil (Abou El-Fadl *et al*., 1990). In such conditions cultivation of resistant aromatic plants is one way to utilize these lands and therefore the selection of suitable crops, which could cope with these conditions, is a necessity. In arid and semi-arid regions, where water availability is a major limitation in crop production, using alternative water resources, such as saline water is one way to utilize these barren lands. The major challenge facing water management is the availability of water. Its amount is fixed, but its demand will continue to increase steadily into the foreseeable future. Reclamation of desert lands has been a top priority and challenge for the Egyptian

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government over the last few decades. In this study, we investigate the possible effect of saline irrigation water on EO of *N. sativa* an economically important aromatic plant in Egypt.

Materials and Methods

Experimental

The present study was carried out in the Experimental Farm, Faculty of Agriculture, Ain Shams University, located at Shubra El-Kheima, Kalubia, Egypt, during two successive seasons of 2006 / 2007 and 2007 / 2008. *N. sativa* seeds were obtained from the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt. In the first week of November during both seasons seeds were sown in plastic pots (30 cm diameter and 50 cm height), 10 seeds per pot. The viability of seeds was approximately 92%. In the third week of December during both seasons, the pots were transferred to a greenhouse adjusted to natural conditions. Each pot was filled with 10 kg of air-dried loamy clay soil. 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. Eight weeks after sowing the seedlings were thinned to three plants per pot. Pots were divided into five main groups were subjected to different levels of saline irrigation water, 0.4 (tap water as control), 1.6, 3.1, 4.6 and 6.3 dSm^{-1}. To prepare irrigation water with different salinity levels, highly soluble NaCl salt was used. All agricultural practices were conducted according to the main recommendations by the Egyptian Ministry of Agriculture.

Harvesting

At fruiting stage, the plants of the two seasons were harvested; then, seed yield (g plant $^{-1}$) was recorded.

EO isolation

Ripening seeds were collected from each treatment during the first and second seasons; air dried and weighed to extract the EO, then 100 g from each replicate of all treatments were subjected to hydro-distillation (HD) for 3 h using a Clevenger-type apparatus (Clevenger, 1928). The EO content was calculated as a relative percentage (v/w). In addition, total EOs (ml 100 $plant^{-1}$) were calculated by using the dry seeds. EOs extracted from *N. sativa* seeds were collected during the first and second seasons from each treatment and dried over anhydrous sodium sulphate to identify the chemical constituents.

Table 1. Physical and chemical properties of the soil

Property	Value
Clay (%)	24
Silt (%)	9
Sand	67
Texture	Clay
Soluble cations (mg/100 soil)	
Ca	106
Mg	62
Na	41
K	40
Soluble anions (mg/100 soil)	
CO_3	-
HCO_3	2
Cl	5
SO_4	106
Organic matter (%)	1.4
Saturation Pecentage	32
$CaCO_3$ (%)	4.8
pH	7.2
Electronic Cnoductivity ($dS\ m^{-1}$)	1.8
NO_3 (ppm)	20.1
P (ppm)	`1.5
CO	-
Sodium Adsopation Ratio.	4.5

Gas chromatography (GC)

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a DB5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25µm). Oven temperature was held at 40°C for 5 min and then programmed until 250°C at a rate of 4°C/min. Injector and detector (FID) temperature were 260°C; helium was used as carrier gas with a linear velocity of 32 cm/s.

Gas chromatography-Mass spectrometry (GC-MS)

GC-MS analyses were carried out on a Varian 3400 system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.); Oven temperature was 40 to 240°C at a rate of 4°C/min, transfer line temperature 260°C, injector temperature 250°C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, flow rate 1.1 ml/ min, Ionization energy 70 eV; scan time 1 s ; mass range 40-350 amu.

Identification compounds

The components of the oils were identified by comparison of their mass-spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds. Kovat's indices (Adams, 1995) were determined by co-injection of the sample with a solution containing a homologous series of n-hydrocarbons, in a temperature run identical to that described above.

Statistical analysis

In this experiment, one factor was considered: SIW, 0.4, 1.6, 3.1, 4.6 and 6.3 dSm^{-1}. For each treatment there were 4 replicates, each of which had 8 pots; in each pot 3 individual plants were planted.

Table 2. effect of saline irrigation water on essential oil content

Salinity treatments (dSm^{-1})	Essential oil content			
	Percentage		Yield (mL 100 $Plant^{-1}$)	
	Mean	SD	Mean	SD
0.4	0.1	±0.0	0.4	±0.1
1.6	0.2	±0.1	0.8	±0.2
3.1	0.3	±0.1	1.1	±0.1
4.6	0.3	±0.1	1.1	±0.1
6.3	0.4	±0.1	1.1	±0.1
F ratio	4.9		17.8***	

* P ≤0.05 according to F-values (ANOVA-1).
** P < 0.01 according to F-values (ANOVA-1).
*** P < 0.001 according to F-values (ANOVA-1)

The experimental design followed a complete random block design. According to Snedecor and Chochran (1995), the averages data of two seasons were statistically analyzed using one-way analysis of variance (ANOVA-1). Significant values determined according the P values ($P \leq 0.05$ = significant, $P < 0.01$ = moderate significant and $P < 0.001$ = highly significant). The applications of that technique were according to the STAT-ITCF program (Foucart, 1982).

Results

EO content

Data presented in Table 2 indicated that saline irrigation water had a positive effect on EO content (%) and yield (ml 100 $plant^{-1}$). Application of SIW caused a pronounced increment in both EO content and yield compared with the control treatments. SIW water at 6.3 dSm^{-1} treatment resulted in the maximum mean values of EO content (0.4 %). The highest yields of EO (1.1 ml 100 $plant^{-1}$) obtained from the treatments of 3.1, 4.6 and 6.3 dSm^{-1}. The lowest EO content (0.1 %) and yield (0.4 ml 100 $plant^{-1}$) were recorded at control treatments. ANOVA indicated that the increases in EO (%) were insignificant for SIW treatments but the increases of EO yield (ml 100 $plant^{-1}$) were highly significant for SIW.

EO constituents

Sixteen constituents were identified in EO extracted from *N. sativa* seeds, accounting for 86.5 – 92.6 % of total constituents, and belong to four chemical main classes. Monoterpene hydrocarbons (MH) class was the major one (77.4% - 81.1%), the remaining fractions as oxygenated monoterpene (OM), sesquiterpene hydrocarbons (SH) and oxygenated sesquiterpene (OS) formed the minor classes (Table 3). The main constituents of *N. sativa* seeds EO as detected by GC/MS were p-cymene (59.5% - 60.3%), α-thujen (6.9% - 7.2%), β-pinene (2.4% – 2.6%), γ-terpinene (3.5% – 3.8%), terpinen-4-ol (2.1% - 2.5%), thymoquinone (3.0% - 3.3%) and carvacrol (2.4% - 2.7%) which increased as SIW level increase. 6.3 dSm^{-1} treatments resulted in higher values of main components compared with the control and other treatments. The chemical classes of *N. sativa* EO were changed in all treatments compared with control. MH, OM and SH were increased as SIW level increase compared with control. OS class was decreased with lowest levels of SIW (1.6 and 3.1 dSm^{-1}) but it was increased with the highest levels of SIW (4.6 and 6.3 dSm^{-1}) compared with control. ANOVA indicated that the changes in the values of myrcene, 1,8-cineol and p-cymen-8-ol were highly significant, while the changes in the values of sabinene, thymoquinone and longifoline were significant for salinity stress treatments. The changes in the values of other components were significant. On the other hand salinity treatments caused highly significant changes in MH and OS classes and caused a significant effect in OM but it caused insignificant changes in OS (Table 3).

Discussion

EOs are a mixture of various compounds, also known as secondary metabolites, with a peculiar taste, useful in modern industry including the pharmaceutical and food industries. For example, p-cymene found in *N. sativa* EO is a terpenoid compound originating in the mevalonic acid cycle (Aflatuni, 1982), which is a long biochemical pathway with many enzymes acting on it. Changes in enzymes activity modify the content of their substrate or product, improving EO quality (Maia *et al*., 2001). Research to determine the influence of saline solution on the EO content of *N. sativa* appears to be lacking. As shown in Table (2), the concentration of SIW exerted a greater influence on essential oil content. EO content was the highest in plants receiving the high levels of SIW (Table 2). Increasing levels of

Table 3. Effect of SIW on EO constituents

No.	Components (%)	RT	KI	SIW treatments (dSm^{-1})										F Ratio
				0.4		1.6		3.1		4.6		6.3		
				Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
1	α-Pinene	2.4	939	1.7	±0.2	1.5	±0.1	1.8	±0.3	1.4	±0.2	1.5	±0.3	2.1
2	Sabinene	3.5	976	0.9	±0.1	1.4	±0.1	1.6	±0.3	1.3	±0.3	1.8	±0.2	5.9*
3	β-Pinene	4.3	980	2.4	±0.4	2.5	±0.5	2.6	±0.3	2.5	±0.5	2.6	±0.2	0.1
4	Myrcene	4.6	991	0.1	±0.0	0.3	±0.1	0.5	±0.2	0.6	±0.1	0.9	±0.2	13.8***
5	α-Thujen	5.1	1005	6.9	±0.4	7.0	±0.5	7.0	±0.5	7.1	±0.1	7.2	±0.5	0.3
6	α-Terpinen	5.4	1018	1.0	±0.1	1.2	±0.2	1.4	±0.4	1.3	±0.3	1.5	±0.3	1.0
7	p-Cymene	6.3	1026	59.5	±0.5	60.1	±0.1	60.2	±0.2	60.2	±0.2	60.3	±0.5	0.8
8	Limonene	6.6	1031	1.4	±0.4	1.1	±0.1	1.3	±0.3	1.4	±0.4	1.5	±0.2	0.5
9	1,8-Cineol	7.7	1033	0.1	±0.0	0.1	±0.0	0.3	±0.1	0.7	±0.2	0.5	±0.3	11.0***
10	γ-Terpinene	7.9	1062	3.5	±0.5	3.6	±0.2	3.7	±0.2	3.7	±0.2	3.8	±0.5	0.4
11	Terpinen-4-ol	8.6	1177	2.1	±0.1	2.2	±0.2	2.3	±0.3	2.4	±0.7	2.5	±0.1	1.1
12	ρ-Cymen-8-ol	11.4	1183	0.2	±0.1	0.1	±0.0	0.4	±0.1	0.8	±0.1	0.6	±0.3	30.7***
13	Thymoquinone	11.6	1249	3.0	±1.0	3.1	±0.1	3.2	±0.2	3.1	±0.1	3.3	±0.2	3.7*
14	Carvacrol	13.5	1298	2.4	±0.4	2.5	±0.5	2.6	±0.1	2.6	±0.1	2.7	±0.1	0.4
15	Lohgifoline	19.5	1402	0.9	±0.1	1.1	±0.1	1.2	±0.2	1.3	±0.3	1.4	±0.1	3.5*
16	Thymohydroquinone	20.2	1509	0.4	±0.1	0.3	±0.1	0.3	±0.1	0.5	±0.1	0.5	±0.1	3.0
MH = Monoterpene hydrocarbons				77.4	±0.4	78.7	±0.2	80.1	±0.1	79.5	±0.5	81.1	±0.1	37.9***
OM = Oxygenated monoterpenes				7.8	±0.1	9.0	±0.1	8.8	±0.2	9.6	±0.3	9.6	±0.1	3.5*
SH = Sesquiterpene hydrocarbons				0.9	±0.1	1.1	±0.1	1.2	±0.1	1.3	±0.1	1.4	±0.1	3.0
OS = Oxygenated sesquiterpene				0.4	±0.5	0.3	±0.1	0.3	±0.4	0.5	±0.9	0.5	±0.6	41.4***
Total identified				86.5		88.1		90.4		90.9		92.6		

RT = Retention time
*KI = Confirmed by comparison with Kovats indices on DB5 column (Adams, 1995).
* P ≤0.05 according to F-values (ANOVA-1).
** P < 0.01 according to F-values (ANOVA-1).
*** P < 0.001 according to F-values (ANOVA-1)

SIW resulted in a higher oil concentration (% or per 100 plant). These results suggest that a high rate of SIW suppresses EO biosynthesis in *N. sativa* L. The high NaCl concentration solution has a positive impact on the oil content. In some vegetables, such as tomatoes, enhanced fruit quality is the result of the increased sugar content, which is correlated with the dry matter content (Adams, 1991). However, organic constituents involve both enzymatic and metabolite changes caused by ion concentrations and/or osmotic effects (Greenway and Munns, 1980; Staple and Toenniessen, 1980). One explanation for the increased EO concentration in the high SIW levels could be the accumulation of dry matter in the plants grown in the increased SIW concentration. The increase in EO yield due to higher SIW concentration depended not only on the increase in seed yield, but also on the increase in seed EO concentration, presumably indicating an enhancement in EO biosynthesis (Sangwan *et al*., 2001). Additionally, the plants might produce secondary metabolites to cope with stressful conditions (Taiz and Zeiger, 2002). Seeds produced by plants grown at higher levels of SIW may have a higher oil gland density as a result of a stress induced reduction in seed area. Such a change in gland frequency could provide a partial explanation for the observed high EO concentration per unit. Alternatively, higher level of SIW may increase the absolute number of glands produced prior to seed emergence (Charles *et al*., 1990). Higher SIW levels may also have affected EO accumulation indirectly through its effects on either net assimilation or the partitioning of assimilates among growth and differentiation processes. The reduction in growth induced by lower osmotic potential may have resulted in a new pattern of resource partitioning, perhaps providing additional carbon skeletons for terpene biosynthesis and accumulation. On the other hand, the benefit of increased EO concentration because of the increased SIW concentrations would be offset by any reduction in biomass production in high SIW concentrations treated plants. Although EO concentration was affected due to higher SIW levels (6.3 dS m^{-1}), a significant adverse effect of high SIW concentration was observed when data for EO concentration were transformed into per plant fresh weight. Therefore, total yield of oil in higher SIW concentrations fell due to the production of the reduced fresh weight. A high reduction in total yield of oil of these crops could imply maintaining the SIW concentrations of solution at an appropriate level. In *N. sativa* seeds, the site of terpene biosynthesis has been localized in the secretary cells of the glandular trichomes, which are mainly located on the seed surfaces of Mentha spicata (Gershenzon *et al*., 1989). The bulk of the monoterpenes of *N. sativa* EO is produced by and stored in the peltate glandular trichomes (Turner *et al*., 2000).

Conclusion

Application of SIW caused a pronounced increment in both EO content and yield. The main constituents of *N. sativa* seeds EO monoterpene hydrocarbons, OM and SH were increased as SIW level increase.

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References

Abou El-Fadl, I. A., Abd-Ella, M. K. and Hussein, E. H. 1990. Effect of irrigation by saline water on the growth and some principal compounds of peppermint and spearmint in two types of soil. Journal of Agricultural Research, Tanta University 16: 276 – 295.

Adams, P. 1991. Effect of increasing salinity of the nutrient solution with major nutrient or sodium chloride on the yield, quality and composition of tomato grown in rock. World Journal of Horticultural Sciences 66: 201-207.

Adams, R. P. 1995. Identification of essential oil components by gas chromatography/mass spectrometry, Allured, Carol Stream, Illinois, USA.

Aflatuni, A.1982. The yield and essential oil content of mint (*Mentha* sp.) in northern Ostrobothnia. Journal of Essential Oil Research 14: 243-246.

Charles, D. J., Joly, R. J. and Simon, J. E. 1990. Effect of osmotic stress on the essential oil content and composition of peppermint. Phytochemistry 29: 2837-2840.

Cottenie, A., Verloo, M., Kiekens, L., Velghe, G. and Camerlynck, R. 1990. Chemical analysis of plant and soil, Laboratory of Analytical and Agro chemistry, State Univ. Ghent. Belgium.

Clevenger, J. F. 1928. Apparatus for determination of essential oil. Journal of American Pharmaceutical Association 17: 346 – 349.

Foucart, T. 1982. Analyse factorielle, programmatiol sur micro-ordinateur. Masson, ITCF, Paris.

Gershenzon, J., Maffei, M. and Croteau, R. 1989. Biochemical and histochemical localization of monoterpene biosynthesis in the glandular trichomes of spearmint *(Mentha spicata)*. Plant Physiology 89: 1351-1357.

Greenway, H. and Munns, R. 1980. Mechanism of salt tolerance in non-halophytes. Plant Physiology 32: 149-190.

Hebbara, M., Rajakumari, G.R., Ravishankari, G. and Raghavaiah, C. V. 2003. Effect of salinity stress on seed yield through physiological parameters in sunflower genotypes. Helia 26: 155-160.

Hendawy, S. F. and Khalid. K. A. 2005. Response of sage (*Salvia officinalis* L) plants to zinc application under different salinity levels. Journal of Applied Science Research 1: 147-155.

Jackson, M. L. 1973. Soil chemical analysis. Published by prentice Hall Indian Private Limited. M.97, Connght Citrus, New Delhi, India.

Khalid, A. K. and Cai, W. 2011. The effects of mannitol and salinity stresses on growth and biochemical accumulations in lemon balm. Acta Ecologca Sinica 31:112-120.

Khalid, A. K. and Jaime, A. T. S. 2010. Yield, essential oil and pigment content of *Calendula officinalis* L. flower heads cultivated under salt stress conditions. Scientia Hortculturea 126: 297–305.

Langroudi, M. E, Sedaghathoor, S. and Bidarigh, S. 2013. Effect of different salinity levels on the composition of rosemary *(Rosmarinus officinalis)* essential oils. American-Eurasian Journal of Agricultural & Environmental sciences 13: 68-71.

Maia, N. B., Bovi, O. A. and Newton, M. M. 2001. Essential oil production and quality of *Mentha arvensis* grown in nutrient solution. Acta Horticultureae 548: 181-187.

Ozturk, A., Unlukara, A., Ipek, A. and Gurbuz, B. 2004. Effects of salt stress and water deficit on plant growth and essential oil content of lemon balm (*Melissa officialis* L). Pakistan Journal of Botany 36: 787-792.

Razmjoo, K. P., Heydarizadeh, A. S. and Sabzalian, M. R. 2008. Effect of salinity and drought stresses on growth parameters and essential oil content of *Matricaria chamomila.* International Journal Agriculture and Biology 10: 451-454.

Sangwan, N. S., Farooqi, A. H., Shabih, A. F. and Sangwan, R. S. 2001. Regulation of essential oil production in plants. Plant Growth Regulators 34: 3-21.

Snedecor, G. W. and Chochran, W. G. 1991. Statistical Methods. 11th Ed. Iowa State Univ., Press. Ames, Iowa, USA.

Staple, R. C. and Toenniessen G. H. 1984. Salinity Tolerance in Plants. John Wiley and Sons, Toronto, Canada.

Tabatabaie, J. and Nazari, J. 2007. Influence of nutrient concentrations and NaCl salinity on the growth, photosynthesis and essential oil content of peppermint and lemon verbena. Turkish Journal of Agriculture and Forestry 31: 245-253.

Taiz, L. and Zeiger, E. 2002. Plant Physiology. Sinaur Associates, Sunderland, MA, USA.

Turner, G.W., Gershenzon, J. and Croteau, R. B. 2000. Distribution of peltate glandular trichomes on developing leaves of peppermint. Plant Physiology 24: 655-663.

Wajs, A. 2008. Radoslaw Bonikowski and danuta kalemba. Composition of essential oil from seeds of *Nigella sativa* L cultivated in Poland. Flavor and Fragrance 23: 126–132.