Salicylic acid improves growth and essential oil accumulation in two *Nepeta cataria* chemotypes under water stress conditions

Hussein A.H. Said-Al Ahl, Ali S. Sabra, Mona H. Hegazy*

Abstract: *A pot experiment was carried out during two successive seasons to study the effects of three water stress treatments (pots irrigated to reach field capacity FC when soil moisture drops until 40, 60, and 80% of available soil moisture ASM, hereafter reported as 40 ASM, 60 ASM and 80 ASM) and salicylic acid (SA) foliar application (0 and 200 mg/L, hereafter 0 SA and 200 SA) on growth, essential oil percentage and composition of two types of* Nepeta *(*Nepeta cataria *and* Nepeta cataria *var.* citriodora*) under greenhouse conditions. Growth characteristics (plant height, number of branches and herb fresh weight/plant) and essential oil yield (mL/plant) in two cuts were significantly decreased with the rise in water stress levels, while essential oil percentage was stimulated in response to water stress. On the other hand, application of SA counteracted the adverse effects of water stress.* Nepeta cataria *var.* citriodora *gave higher values for all growth parameters (essential oil % and oil yield) than* Nepeta cataria*. The maximum growth and essential oil yield were obtained from plants treated with 80 ASM + 200 SA. On the contrary, 40 ASM + 200 SA gave the best result for the essential oil percentage in the two types of Nepeta. Geraniol, neral, geranial, nepetalactone and citronellol were the major marker compounds in* Nepeta cataria*, whereas, citronellol, geraniol, neral, geranial and myristicin were the major compounds in* Nepeta cataria *var.* citriodora *in the two cuts. The highest citronellol and geraniol percentages were recorded from 80 ASM and 200 SA treatment, while the highest neral percentage was recorded at 40 ASM + 200 SA in the first cut of* Nepeta cataria *and in the second cut of* Nepeta cataria *var.* citriodora*. In addition, geraniol % in the first cut was enhanced by 60 and 40 ASM + 200 SA in* Nepeta cataria *and* Nepeta cataria *var.* citriodora*, respectively. This study showed that the accumulation of essential oil constituents in* Nepeta *chemotypes is influenced by the level of water stress as well as its seasonal variations. In this context,* Nepeta cataria *var.* citriodora *showed superior performance for growth and essential oil production than Nepeta cataria. It can be concluded that the application of SA could be a practical approach for improving the performance and enhancing the accumulation of essential oil marker compounds in* Nepeta *plants under drought stress.*
Keywords: *essential oil,* Nepeta cataria, Nepeta cataria *var.* citriodora, *salicylic acid, water stress.*

Riassunto: *Un esperimento in vaso è stato effettuato nel corso di due stagioni consecutive per studiare gli effetti dei trattamenti di stress idrico (vasi riportati a capacità di campo quando l'umidità del terreno scende rispettivamente fino 40, 60, e 80% dell'acqua utile ASM, di seguito indicati come 40 ASM, 60 ASM e 80 ASM) e acido salicilico (SA) con applicazione fogliare (0 e 200 mg/L, qui di seguito indicati come 0 SA e 200 SA) sulla crescita, la percentuale di olio essenziale e la composizione di due tipi di Nepeta (*Nepeta cataria *e* Nepeta cataria *var.* citriodora*) in serra. Parametri di crescita (altezza della pianta, numero di steli e foglie fresche peso/pianta) e resa in olio essenziale (ml / pianta) in due tagli sono diminuiti significativamente all'aumentare dei livelli di stress idrico, mentre la percentuale di olio essenziale è stata stimolata in risposta a stress idrico. D'altra parte, l'applicazione di SA ha contrastato gli effetti negativi dello stress idrico.* Nepeta cataria *var.* citriodora *ha dato luogo a valori più alti per tutte le variabili studiate rispetto a* Nepeta cataria*. La crescita massima e la resa in olio essenziale sono state ottenute con 80 ASM + 200 SA. Al contrario, il trattamento 40 ASM + 200 SA hanno dato il miglior risultato per la percentuale di olio essenziale nei due tipi di Nepeta. Geraniolo, nerale, geraniale, nepetalactone e citronellolo sono stati i principali composti marcatori in Nepeta cataria, considerando citronellolo, geraniolo, nerale, geraniale e miristicina erano i principali composti in* Nepeta cataria *var.* citriodora *nei due tagli. Le più alte percentuali di citronellolo e geraniolo sono state registrate dal trattamento 80 ASM + 200 SA, mentre la percentuale più elevata di nerali è stata registrata a 40 ASM + 200 SA nel primo taglio di* Nepeta cataria *e nel secondo taglio di* Nepeta cataria *var.* citriodora*. Inoltre il geraniolo nel 1° taglio è stato migliorato da 60 e 40 ASM + 200 SA in* Nepeta cataria *e* Nepeta cataria *var.* citriodora *rispettivamente. Questo studio ha dimostrato che l'accumulo di oli essenziali in chemiotipi Nepeta è influenzata dal livello di stress idrico e le sue variazioni stagionali. In questo contesto,* Nepeta cataria *var.* citriodora *ha mostrato prestazioni superiori per la crescita e la produzione di olio essenziale rispetto a* Nepeta cataria*. Si può concludere da questo studio che l'applicazione di SA potrebbe essere un approccio pratico per migliorare le prestazioni e migliorare l'accumulo di composti essenziali precursori di oli essenziali negli impianti di* Nepeta *sottoposti a stress idrico.*
Parole chiave: *olio essenziale,* Nepeta cataria, Nepeta cataria *var.* citriodora, *acido salicilico, stress idrico.*

1. INTRODUCTION

Aromatic plants of *Lamiaceae* family are economically important due to their essential oils production. *Nepeta cataria* (catnip) and *Nepeta*

* Corresponding author's e-mail: hussein_saidalahl@yahoo.com
[1] Department of Medicinal and Aromatic Plants, National Research Centre, Dokki, Giza, Egypt.
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cataria var. *citriodora* (lemon catnip) are perennial herbs belonging to the family *Lamiaceae* and native to Asia, Europe, North Africa and North America (Klimek and Modnicki, 2005; Wójciak-Kosior *et al*., 2011). Traditionally, *Nepeta cataria* (catnip) has been used to treat insomnia, flatulence, and upset stomach due to its sedative, carminative, and antispasmodic properties. It has also been used to treat the common cold, flu, and fevers (Tucker and Tucke, 1988; Grognet, 1990). Lemon catnip is an aromatic herb with a lemon fragrance, resembles true catnip but is not attractive to cats and it has been used as digestive, sedative, relaxant, spasmolytic and tonic remedy (Klimek and Modnicki, 2005; Wójciak-Kosior *et al*., 2011). Nowadays it is extensively grown for the extraction of essential oil and for culinary purposes.

Essential oils are secondary metabolites that function as signaling compounds between different types of organisms and diverse biological systems. In addition, they are known for their fragrance and food flavoring properties. Due to their antimicrobial and anti-oxidative effects and medicinal activities, essential oils have various applications in agriculture, medicine, pharmaceutical industry and biotechnology. Changed consumer demands and increased interest in natural product compounds, especially essential oils, have formed the basis for initiating research on *Nepeta cataria* and *Nepeta cataria* var. *citriodora* to encourage its cultivation, processing, marketing and distribution as aromatic and medicinal plant. Lemon oil is used in perfumes, candies, and pharmaceuticals (Klimek and Modnicki, 2005). Efforts have been made to determine the factors, particularly abiotic stresses which, that affect the accumulation of essential oil in aromatic plants (Yamaura *et al*., 1989; Chalchat and Lamy 1997; Karousou *et al*., 1998; Hudaib *et al*., 2002; Said-Al Ahl *et al*., 2014).

Abiotic stresses are among the environmental stresses that most limit productivity and plants yield in arid and semi-arid zones. Water scarcity, for instance, is increasing due to increasing population pressure and intensive use of natural resources. The growth and biosynthesis of secondary metabolites in medicinal and aromatic plants are strongly influenced by abiotic factors, such as water stress. Water deficit in plants lead to reduction in transpiration and photosynthesis (Sarker *et al*., 2005) and may cause significant changes in yield and composition of essential oils in aromatic plants. For example, water deficit decreased oil yield of *Rosmarinus officinalis* L. (Singh and Ramesh, 2000), *Pimpinella anisum* L. (Zehtab-Salmasi *et al*., 2001) and *Origanum vulgare* (Said-Al Ahl and Abdou, 2009). By contrast, water stress caused a significant increase in oil yield of citronella grass (*Cymbopogen winterianus* Jowitt.) and this response varied based on cultivar and plant density (Fatima *et al*., 2000).

Salicylic acid plays a critical role in increasing plant resistance to environmental stress factors (Sticher *et al*., 1997, Klessig *et al*., 1998). Salicylic acid is a plant phenol that is involved in signal transduction pathways and defense mechanisms against a wide array of biotic and abiotic stresses. It has been recognized as an endogenous regulatory signal in plants mediating plant response to salinity (Shakirova *et al*., 2003) and drought (Singh and Usha, 2003). The ameliorative effects of SA on plant growth under abiotic stress conditions have been related to its role in nutrient uptake, membrane stability, water relations, stomatal regulation, photosynthesis and inhibition of ethylene biosynthesis (Srivastava and Dwivedi, 2000; Khan *et al*., 2003; Stevens *et al*., 2006; Arfan *et al*., 2007).

Little information is available about the adaptation of *N. cataria* and *Nepeta cataria* var. *citriodora* to the Egyptian environment. In addition, the qualitative and quantitative changes in essential oil in response to water stress and salicylic acid are not known. Therefore, the objective of this study was to investigate the effects of water stress levels and SA on growth, yield, essential oil percentage and composition of two chemotypes of *Nepeta* (*N. cataria* and *Nepeta cataria* var. *citriodora*). These changes might be relevant to the use of this plant in some pharmaceutical and cosmetic industries.

MATERIALS AND METHODS

Plant material and growing conditions

A pot experiment was carried out under greenhouse conditions at the National Research Center, Dokki, Giza, Egypt, during the two seasons of 2012/2013 and 2013/2014. Seeds of *Nepeta cataria* and *N. cataria* var. *citriodora* were obtained from the HEM ZADEN B.V, P.O. Box 4, 1606 ZG Venhuizen, The Netherlands. They were sown in the nursery on the first of November of both seasons. After 60 days, uniform seedlings were transplanted into pots of 30 cm diameter containing 10 kg of air-dried soil. The physical and chemical analyses of the soil were conducted according to Jackson 1973. The soil texture consisted of: 45.00% sand, 28.25% silt, 26.75% clay and 0.85% organic matter. Chemical analysis of the soil showed: pH = 8.40; E.C. = 0.79 dsm^{-1}; total nitrogen = 0.13%; available phosphorus = 2.18 mg/100 g; potassium = 0.02 mg/100 g. Field capacity (FC) and wilting point (WP) were determined following the methods of Black, 1965. Two-season means of field capacity, permanent wilting point, available soil moisture (ASM), and bulk density (BD) were 34.50%, 16.01%, 18.49% and 1.36 g/cm³, respectively. Salicylic acid (SA) as a foliar spray (0 and 200 mg/L, hereafter 0 SA and 200 SA) was applied after 60, 90, 150 and 180 days after transplanting. One month after transplantation, irrigation treatments were at 80%, 60% and 40% ASM (hereafter 80 ASM, 60 ASM and 40 ASM), which were equal to 30.80, 27.10 and 22.61 ASM, respectively. Pots were weighed daily. When percent of soil moisture reached the above mentioned levels, pots were irrigated to reach field capacity (34.50% soil moisture). The differences between the needed soil moisture for the previous treatments and field capacity were added to the pots in the different treatments. The experimental layout was factorial in a complete randomized design, with three replications. Each replicate contained 7 pots of 3 plants each. Meteorological data of Giza for the two growing seasons are shown in Tab. 1 (source: Central Laboratory for Agricultural Climate - CLAC).

Morphological characteristics and essential oil production

During each growing season, selected plants were harvested twice at the full flowering stage (135 and 210 days after transplantation, respectively).

The plants were harvested at 5 cm above the soil, and some different vegetative parameters (plant height; number of branches and herb fresh weights) were recorded. The essential oil percentage of each replicate at the two cuts was determined in the fresh flowering herb according to Guenther (1961). Essential oil yield per plant was calculated by multiplying the oil percentage by its fresh weight and expressed as mL/plant. The extracted essential oil was dehydrated using anhydrous sodium sulfate and stored in a refrigerator until Gas chromatography/mass spectrometry (GC/MS) analysis. The GC/MS analysis of the essential oil samples was carried out for the second season.

Gas chromatography/mass spectrometry (GC-MS) analysis

The analysis of the essential oil was performed using a GC/MS system composed by a HP 5890 series II gas chromatograph and a HP 5973 mass detector. A TR-FAME (Thermo 260 M142 P) capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) was used with helium as the carrier gas, at a flow rate of 1.5 ml/min. GC oven temperature was programmed at an initial temperature of 40°C for 5 min, then heated up to 140°C at 5°C/min and held at 140°C for 5 min, then ramped up to 280°C at 10°C/min and held for 5 additional minutes. Injector and detector temperatures were 250°C. Diluted samples (1/100, v/v in heptane) of 1.0 µl were injected automatically. Mass spectrometry was run in the electron impact mode (EI) at 70 eV. Identification of components was based on the comparison of their GC retention times. Mass spectra interpretation was confirmed by mass spectral library search using the National Institute of Standards and Technology (NIST) database (Masada, 1976; Adams, 2007).

Statistical analysis

Except for the constituents of the essential oil, data from this study were analyzed using analysis of variance (ANOVA) by JMP 10 program (SAS Institute, NC, USA). The mean values of treatments were compared using Tukey's HSD test. Values accompanied by different letters are significantly different at p≤0.05.

Month	2012/2013 season				2013/2014 season			
	Tn (°C) Min.	Tx (°C) Max.	RH %	WS km/h	Tn(°C)	Tx (°C)	RH %	WS km/h
November	9.9	32	66	2	11	33.8	65	2.7
December	1.1	30.6	61	1.7	3.5	29.9	68	2.6
January	5.4	28.4	61	2.3	1.7	25.6	72	2.6
February	6.6	28.8	56	2.2	6.4	29.9	67	2.4
March	8.8	37.6	48	2.3	6.8	33.1	55	2.6
April	11.5	38.3	47	2.1	12.7	38.1	49	2.9
May	15.7	43.8	44	2	14.4	43	46	2.6
June	20.5	46.2	48	2	18.5	43.8	48	2.8
July	21.6	38.9	57	1.7	22.3	40	56	2.6
August	22.2	40.1	55	2	23.1	39.2	56	2.5

Source: Meteorological data of Giza (CLAC, Egypt), average values; Tx and Tn are monthly averages of maximum and minimum temperatures; RH is monthly average relative humidity; WS is monthly average wind speed

Tab. 1 - Meteorological data of Giza (CLAC, Egypt) during the two growing seasons.
Tab. 1 - Dati meteorologici registrati a Giza (CLAC, Egitto) durante le due stagioni di crescita.

Treatments	First season (1st)				Second season (2nd)			
	N. cataria		*N. cataria* var. *citriodora*		*N. cataria*		*N. cataria* var. *citriodora*	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
40% ASM*	30.0±0.6k***	41.3±0.6hi	32.3 ±1.1j	43.6±1.0gh	31.6±0.6q	42.7±1.2m-o	33.7±0.6pq	41.7±0.8j-m
40% ASM+ SA**	38.1±0.7j	42.4±1.1gh	39.7 ±0.5ij	45.3±0.3ef	38.0±0.5pq	43.3±0.6i-m	40.4±0.4n-p	43.4±0.4g-k
60% ASM	41.1±0.2ij	45.6±0.5ef	41.9±0.1gh	49.9±0.2de	40.6±1.0op	44.4±0.9g-j	41.7±0.1l-o	48.0±0.5e-g
60% ASM+SA	43.4±0.4gh	46.2±0.8de	44.3±0.9fg	52.0±1.4bc	42.5±2.0k-n	46.4±1.2d-g	43.4±0.7h-m	52.1±2.0b-f
80% ASM	44.4±0.4fg	51.0±0.3cd	47.8±0.2ef	57.8±1.1ab	44.1±0.6g-l	50.4±0.6c-e	45.6±0.4g-i	56.3±0.6a-c
80% ASM+SA	45.2±0.6ef	57.7±0.7ab	49.4±0.9cd	61.9±0.4a	46.2±0.6f-h	53.7±1.1ab	49.3±0.4d-f	60.7±0.6a

*ASM (available soil moisture); ** SA (salicylic acid); *** numbers with different letters within the season are significantly different at p≤0.05 by Tukey's HSD test

Tab. 2 - Plant height (cm) of *Nepeta cataria* and *N. cataria* var. *citriodora* as affected by water stress and SA application. The two cuts in the first season and the second season are represented.
Tab. 2 - Altezza delle piante (cm) di Nepeta cataria *e* N. cataria *var.* citriodora *in relazione all'effetto dello stress idrico e dell'applicazione di SA. I due raccolti nella prima e seconda stagione sono riportati in tabella.*

RESULTS AND DISCUSSION

Growth parameters and yield

Tab. (2-4) indicate that decreasing irrigation water significantly decreased plant height, number of branches and fresh weight, in the two cuts of both seasons. Irrigation at 80 ASM resulted in the highest values of these parameters, while 40 ASM treatment resulted in the lowest values. Increasing water stress reduced growth parameters, which may be due to reduction in photosynthesis, stomatal and mesophyll conductance and plant biomass. The pronounced effect of increased irrigation on growth may be attributed to the availability of sufficient moisture throughout the root system (Singh *et al*., 1997). Similar findings have been reported by other researchers (Baher *et al*., 2002; Moeini Alishah *et al*., 2006; Said-Al Ahl and Hussein, 2010).

As shown in Tab. (2-4), SA significantly increased all growth parameters when compared to unsprayed treatment. Maximum values of growth parameters were observed from plants sprayed with 200 SA. These results are in agreement with previous reports on *Satureja hortensis* (Yazdanpanah *et al*., 2011), *Cucurbita pepo* (Sure *et al*., 2011), and *Coriandrum sativum* (Said-Al Ahl *et al*., 2014), which indicated the importance of SA in ameliorating the adverse effects of abiotic stresses on plants. The beneficial effects of SA on plant growth may be attributed to its role in various biochemical processes, such as signal transduction pathways, growth development and alteration on antioxidant enzyme activities (Kang, 2003; Senaranta *et al*., 2000; Krantev *et al*., 2008).

Interaction between irrigation treatments and SA resulted in a significant increase of growth in both seasons. Maximum values in the two cuts of both seasons were recorded from the treatment 80 ASM + 200 SA (Tab. 2-3), while the minimum values resulted from the treatment 40 ASM + 0 SA. This finding is in agreement with a previous study, where water stress

Treatments	First season (1St)				Second season (2nd)			
	N. cataria		*N. cataria* var. *citriodora*		*N. cataria*		*N. cataria* var. *citriodora*	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
40% ASM	7.9±0.16q	8.82±0.24n-g	8.0±0.1pq	8.93±0.20k-o	7.64±0.32r	8.57±0.23op	7.7±0.3qr	9.2±0.2mn
40% ASM+SA	10.8±0.16o-q	11.4±0.38l-n	10.6±0.5m-p	11.8±0.18j-m	9.75±0.2pq	11.7±0.28lm	10.8±0.2no	12.0±0.03kl
60% ASM	12.1±0.28l-n	14.0±0.02g-j	12.6±0.3i-l	14.0±0.02f-i	11.87±0.13mn	13.9±0.12ij	12.2±0.2kl	14.2±0.16hi
60% ASM+SA	13.4±0.29h-k	14.3±0.39d-i	14.6±0.3f-j	17.6±0.59b-e	13.52±0.49jk	14.2±0.23gh	14.2±0.2ij	16.9±0.68de
80% ASM	14.5±0.29e-j	16.2±0.42b-f	15.2±0.2d-h	21.3±0.49a-c	14.30±0.35hi	17.2±0.42de	14.9±0.2fg	20.2±0.51bc
80% ASM+SA	15.6±0.48c-g	17.1±0.69ab	15.8±0.4a-d	21.9±0.70a	15.04±0.35ef	18.1±0.07b	16.2±0.4cd	21.7±1.03a

* ASM (available soil moisture); ** SA (salicylic acid); *** numbers with different letters within the season are significantly different at p ≤ 0.05 by Tukey's HSD test

Tab. 3 - Number of branches of *Nepeta cataria* and *N. cataria* var. *citriodora* as affected by water stress and SA application. The two cuts in the first season and the second season are represented.
Tab. 3 - Numero di ramificazioni di Nepeta cataria *e* N. cataria *var.* citriodora *in relazione all'effetto dello stress idrico e dell'applicazione di SA. I due raccolti nella prima e seconda stagione sono riportati in tabella.*

Treatments	First season (1St)				Second season (2nd)			
	N. cataria		*N. cataria*	var. *citriodora*	*N. cataria*		*N. cataria*	var. *citriodora*
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
40% ASM	23.9±1.0m	36.0±0.0j	24.7±1.1l	37.8±1.3hi	24.5±1.5p	33.6±0.5kl	26.8±1.1o	40.8±0.5j
40% ASM+SA	33.9±0.2l	42.2±1.5hi	35.2±0.7k	47.8±1.6fg	32.4±1.4n	43.3±2.3hi	34.6±1.7m	48.4±0.4fg
60% ASM	39.0±1.3k	48.0±1.2f	39.1±0.7ij	53.9±1.6e	37.0±0.2m	49.2±0.5ef	38.1±0.7l	51.6±0.6e
60% ASM+SA	40.7±1.0ij	50.0±1.1e	41.0±0.3h	61.5±1.8c	40.8±0.6jk	52.8±0.8d	41.5±0.3ij	54.1±1.5c
80% ASM	42.9±1.5gh	58.8±2.2cd	45.6±0.3f	68.4±1.3b	45.8±0.4gh	60.1±1.7c	46.2±0.6fg	62.5±1.4b
80% ASM+SA	44.4±1.4f	62.3±1.4b	50.4±0.6de	71.5±2.4a	48.3±1.2e	66.9±1.9a	51.2±0.8d	67.3±1.0a

* ASM (available soil moisture); ** SA (salicylic acid); *** numbers with different letters within the season are significantly different at $p \le 0.05$ by Tukey's HSD test

Tab. 4 - Fresh weight (g) of *Nepeta cataria* and *N. cataria* var.*citriodora* as affected by water stress and SA application. The two cuts in the first season and the second season are represented.
Tab. 4 - Peso fresco (g) di Nepeta cataria *e* N. cataria *var.* citriodora *in relazione all'effetto dello stress idrico e dell'applicazione di SA. I due raccolti nella prima e seconda stagione sono riportati in tabella.*

reduced growth parameters of lemongrass varieties, while the foliar application of SA restored growth processes (Idrees *et al.*, 2010).

Essential oil % and yield

Essential oil percentage significantly responded to irrigation water treatments in the two cuts of both seasons (Tab. 5). Increasing applied water quantity significantly decreased essential oil percentage to reach the highest value in plants irrigated with 40 ASM. On the other hand, essential oil yield (mg/plant) was significantly higher in plants that received 80 ASM than in plants that received 40 ASM (Tab. 6). Plants of the second cut nearly contained twice the oil yield of the first cut, which may be due to some environmental factors. These results indicate that the increment of oil yield with the increase in water stress was largely due to the adverse effect of stress on growth (Singh *et al.*, 1997). This is in agreement with the results of Idrees *et al.*, (2010) that reported a significant decrease of essential oil percentage and yield of lemongrass in water stressed plants. Similarly, Said-Al Ahl *et al.*, (2009) found a significant decrease in essential oil (% and yield) of *Melissa officinalis* as a result of water stress.

Application of SA to *N. cataria* and *N. cataria* var. *citriodora* plants attenuated the accumulation of essential oil in both seasons (Tab. 5 and 6). Spraying SA significantly increased essential oil percentage in the two cuts of both seasons. These results are in accordance with other reports, which showed an improvement in the essential oil percentage and yield of various plants, such as basil and marjoram (Gharib, 2006), Salvia (Rowshan *et al.*, 2010), and cumin (Rahimi *et al.*, 2013) in response to SA application. Idrees *et al.*, (2010) stated that improvement in the essential oil content by foliar application of SA might be due to the increase in growth, nutrients uptake or changes in leaf oil gland population and monoterpenes biosynthesis.

Treatments	First season (1St)				Second season (2nd)			
	N. cataria		*N. cataria*	var. *citriodora*	*N. cataria*		*N. cataria*	var. *citriodora*
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
40% ASM	0.113±0.003mn	0.257±0.003f	0.25±0.01gh	0.38±0.012ab	0.11±0.0ij	0.253±0.003e-g	0.24±0.010f-h	0.37±0.006ab
40% ASM+SA	0.127±0.003m	0.267±0.003e	0.26±0.00f	0.39±0.010a	0.12±0.006i	0.27±0.006d	0.263±0.012de	0.377±0.003a
60% ASM	0.107±0.003o	0.243±0.009gh	0.23±0.01i-k	0.353±0.007c	0.1±0.0jk	0.24±0.006e-h	0.233±0.009gh	0.343±0.003bc
60% ASM+SA	0.113±0.003n	0.253±0.007fg	0.24±0.01h-j	0.38±0.010b	0.11±0.006i	0.26±0.0d-f	0.25±0.010e-g	0.377±0.007a
80% ASM	0.093±0.003p	0.237±0.009jk	0.22±0.01l	0.343±0.009d	0.1±0.0k	0.23±0.010gh	0.223±0.003h	0.333±0.003c
80% ASM+SA	0.1±0.006o	0.243±0.003hi	0.22±0.01k	0.357±0.01c	0.107±0.007ij	0.25±0.006e-g	0.237±0.007f-h	0.367±0.003ab

*ASM (available soil moisture); ** SA (salicylic acid); *** numbers with different letters within the season are significantly different at $p \le 0.05$ by Tukey's HSD test

Tab. 5 - Essential oil % of *Nepeta cataria* and *N. cataria* var. *citriodora* as affected by water stress and SA application. The two cuts in the first season and the second season are represented.
Tab. 5 - Contenuto in oli essenziali di Nepeta cataria *e* N. cataria *var.* citriodora *in relazione all'effetto dello stress idrico e dell'applicazione di SA. I due raccolti nella prima e seconda stagione sono riportati in tabella.*

Treatments	First season (1 St)				Second season (2 nd)			
	N. cataria		*N. cataria* var. *citriodora*		*N. cataria*		*N. cataria* var. *citriodora*	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
40% ASM	0.027±0.002p	0.092±0.001i-k	0.061±0.005l	0.144±0.008d	0.027±0.002m	0.085±0.001hi	0.064±0.0j	0.151±0.004d
40% ASM+SA	0.043±0.001o	0.112±0.005gh	0.093±0.001k	0.186±0.004c	0.039±0.004l	0.117±0.009f	0.091±0.005i	0.182±0.001c
60% ASM	0.042±0.001o	0.117±0.002f-h	0.09±0.005jk	0.19±0.005c	0.037±0.0l	0.118±0.004fg	0.089±0.004i	0.177±0.001c
60% ASM+SA	0.046±0.002mn	0.126±0.002e	0.098±0.002hi	0.234±0.007ab	0.045±0.002k	0.137±0.002e	0.104±0.004gh	0.204±0.003b
80% ASM	0.04±0.001n	0.14±0.010ef	0.1±0.003h-j	0.235±0.002b	0.046±0.0k	0.138±0.003e	0.103±0.0gh	0.208±0.003b
80% ASM+SA	0.044±0.003lm	0.152±0.004d	0.113±0.004e-g	0.255±0.014a	0.052±0.005j	0.168±0.009d	0.121±0.004ef	0.247±0.005a

*ASM (available soil moisture); ** SA (salicylic acid); *** numbers with different letters within the season are significantly different at $p \le 0.05$ by Tukey's HSD test

Tab. 6 - Essential oil yield (ml/plant) of *Nepeta cataria* and *N. cataria* var.*citriodora* as affected by water stress and SA application. The two cuts in the first season and the second season are represented.

Tab. 6 - Produzione di oli essenziali (ml/plant) di Nepeta cataria *e* N. cataria *var.* citriodora *in relazione all'effetto dello stress idrico e dell'applicazione di SA. I due raccolti nella prima e seconda stagione sono riportati in tabella.*

Monoterpenes, a kind of terpenoid secondary metabolites, are induced by biotic and abiotic stresses and are thought to have essential ecological roles (Holopainen and Gershenzon 2010; Loreto and Schnitzler, 2010). Some signal transduction pathways are involved in the regulation of monoterpene biosynthesis in response to these stresses (Zhao *et al.*, 2006). It has been suggested that signaling molecules, such as SA, could be employed directly or indirectly in the production of plant secondary metabolites in response to various environmental stresses (Pieterse and van Loon, 1999; Zhao *et al.*, 2005; Hayat *et al.*, 2010). In recent years, the effect of SA on the accumulation of secondary metabolites in plants has received much attention. Ali *et al.*, (2006) and Shabani *et al.*, (2009) showed that SA induced the accumulation of triterpenoids, ginsenosides in ginseng and glycyrrhizin in licorice respectively. Production of sesquiterpenoids, such as bilobalide in *Ginkgo biloba* (Kang *et al.*, 2006), crepidiaside, deoxylactucin and sonchuside in chicory (Malarz *et al.*, 2007), and artemisinin in *Artemisia annua* (Pu *et al.*, 2009; Aftab *et al.*, 2010) were also stimulated by SA. There is little information about the effect of SA on monoterpenes biogenesis; however, the accumulation of diterpenes such as ginkgolides and taxol, which share the common non-mevalonate pathway in plastids with monoterpenes (Lichtenthaler, 1999) was enhanced by SA (Miao *et al.*, 2000; Kang *et al.*, 2006; Wang *et al.*, 2007). Moreover, monoterpene biosynthesis was suppressed in SA-deficient transgenic *Arabidopsis* plants (Munné-Bosch *et al.*, 2007). All of these results suggest that SA might be used as a potential enhancer to improve monoterpene production.

The interaction between irrigation water quantity and SA significantly affected essential oil percentage in *Nepeta* plants (Tab. 5). It also shows that the highest essential oil percentage in the two cuts of both seasons occurred in plants treated with 40 ASM + 200 SA, followed by plants that received 60 ASM + 200 SA. The lowest percentage of essential oil was from plants that received 80 ASM + 0 SA application in two cuts of both seasons. Regarding the essential oil yield (mg/plant), an opposite trend was obtained, where plants that received 80 ASM + 200 SA showed the highest oil production, while plants treated with 40 ASM showed the least oil production. Previous studies have shown that foliar application of SA ameliorates the adverse effects of water stress and enhance the accumulation of secondary metabolites as observed in *Simarouba glauca* (Awate and Gaikwad, 2014), *Nigella sativa* (Kabiri *et al.*, 2014) and *Matricaria aurea* (Behjou *et al.*, 2014).

GC-MS analysis

Tab. 7 and 8 show the qualitative and quantitative changes in the essential oils distilled from *Nepeta cataria* and *Nepeta cataria* var. *citriodora* at flowering stage for the two cuts of 2013. According to the essential oil analysis, the distribution of all identified compounds was affected by ASM level, SA application and time of cutting. The compounds identified were grouped into three types: 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 citronellol, geraniol, nepetalactone in *Nepeta cataria* and citronellol, and geraniol in *Nepeta cataria* var. *citriodora* were major compounds, while neral, geranial, caryophyllene, phorone, caryophyllene oxide, geranyl isobutyrate, cyclohexene,1-methyl-5-(1-methylethenyl), geranyl propionate, and 4-nitro-1,3-phenylenediamine in *Nepeta cataria* and citronellal, caryophyllene, neral, geranial, cyclopentane,1-methyl1-(2-methyl-2-propenyl), durohydroquinone, myristicin in *Nepeta cataria* var. *citriodora* were quantified as minors;

Compounds	First cut						Second cut					
	S0			S1			S0			S1		
	I1	I2	I3	I1	I2	I3	I1	I2	I3	I1	I2	I3
linalool	0.12	0.30	0.26	-	0.3 5	-	0.12	-	-	0.11	0.18	0.26
citronellal	0.32	0.40	-	0.46	-	0.32	-	0.21	0.31	-	-	-
caryophyllene	1.61	1.97	2.00	1.76	1.75	2.70	1.98	2.09	2.65	2.14	2.07	2.35
p-Menth-3-en-9-ol	-	-	0.26	0.30	0.19	-	-	0.16	-	-	-	0.23
citronellol	4.25	6.98	7.21	4.01	11 .76	11.94	9.63	9.99	9.21	8.32	9.03	7.84
hexahydroindole	-	0.62	-	0.40	-	0.52	-	0.71	0.41	-	-	0.39
phorone	-	-	1.25	0.89	-	0.62	0.46	-	-	0.74	1.11	1.01
geraniol	35.12	36.01	36.94	39.44	37.80	40.17	37.12	38.71	38.94	36.93	39.89	40.02
neral	4.92	4.20	3.77	5.96	4.00	5.30	5.32	3.72	4.34	5.45	4.60	4.50
geranial	6.00	5.52	4.97	6.00	7.09	5.61	6.60	4.00	5.40	6.51	4.40	4.23
caryophyllene oxide	0.75	1.20	0.85	0.81	0.39	0.74	1.12	1.09	0.71	0.92	1.58	1.03
nepetalactone	42.50	37.95	36.00	38.84	3 5.10	26.37	35.50	34.66	34.11	36.84	32.45	31.82
myristicin	0.13	0.20	-	0.25	0.21	0.28	-	0.26	-	0.04	0.10	-
ge ranyl propionate	0.40	1.21	1.34	1 .89	0.19	-	0.94	0. 61	0.22	-	0.23	-
geranyl isobutyrate	1.94	-	1.86	-	2.50	1.73	-	-	-	-	-	2.11
cyclohexene, 1-methyl -5-(1-methylethenyl)	2.08	1.36	-	2.39	3.14	2.21	-	-	2.20	-	-	-
4-Nitro-1,3-phenylenediamine	2.19	-	2.14	-	2.02	-	-	1.83	-	-	1.75	2.60
Total Identified	96.33	97.92	98.85	97.30	96.49	98.51	98.79	98.04	98.50	98.00	97.41	98.39

S = Salicylic acid, i.e. S0 = control, S1= 200 mg/L; I = irrigation, i.e. I1 = 40% available soil moisture, I2 = 60% available soil moisture, I3 = 80% available soil moisture.

Tab. 7 - Effect of water stress and/or salicylic acid on the % of essential oil constituents of *Nepeta cataria* plants during the two cuts in the second season.
Tab. 7 - Effetto dello stress idrico e/o dell'applicazione di acido salicilico sulla percentuale di costituenti di oli essenziali in piante di Nepeta cataria *durante i due tagli della seconda stagione di crescita.*

linalool, citronellal, p-Menth-3-en-9-ol, hexahydroindole, myristicin, geranyl propionate in *Nepeta cataria* and 5-Hepten-2-one,6-methyl, verbenol, 2,6-Octadiene,2,6-dimethyl, caryophyllene oxide, apiol in *Nepeta cataria* var. *citriodora* were considered as traces.

The results in Tab. 7 and 8 show that *Nepeta cataria* gave the highest content of citral and caryophyllene oxide, whereas *Nepeta cataria* var. *citriodora* gave the highest content of citronellal, citronellol, caryophyllene, geraniol and myristicin. This indicates that each chemotype of *Nepeta* has its own distinctive chemical profile, which may be influenced by various environmental and agricultural conditions, such as location and agricultural practices in addition to genotype. Numerous studies have shown that variations in chemical composition of essential oil of *Nepeta* species are mainly attributed to the location and genetic background (Bourrel *et al*., 1993; Handjieva *et al*., 1996; Malizia *et al*., 1996; Chalchat and Lamy, 1997; Baranauskiene *et al*., 2003; Morteza-Semnani *et al*., 2004; Saeidnia *et al*., 2014). However, variation in morphological features of glandular trichomes, in which essential oil is synthesized, may also contribute to the variation in the essential oil profile and content (Bosabalidis, 2002). In this regard, Kolalite (1998) studied the morphology of glandular trichomes in different chemotypes of *N. cataria* and found that capitate hairs type II was characteristic to *N. cataria var. citriodora* with no analogue in *N. cataria.*

In the current study, geraniol, nepetalactone and citronellol, the three major compounds in *N. cataria,* showed quantitative changes in response to water quantity and SA application (Tab. 7). For example, the maximum nepetalactone (42.50%) was observed in plants that received 40 ASM, while the maximum geraniol (40.17%) and citronellol (11.94%) percentages in the first cut were obtained from plants that received 80 ASM + 200 SA. On the other hand, nepetalactone percentage was reduced in plants that received 80 ASM + 200 SA.

Compounds	First cut						Second cut					
	S0			S1			S0			S1		
	I1	I2	I3	I1	I2	I3	I1	I2	I3	I1	I2	I3
5 - Hepten - 2 - one, 6- methyl	0.51	-	0.12	-	0.20	0.43	0.23	-	0.45	0.49	-	-
citronellal	1.10	1.18	1.34	1.00	0.75	0.98	0.80	0.92	0.95	0.78	1.06	1.08
verbenol	0.45	-	0.60	-	0.23	0.15	0.19	-	-	0.24	-	-
caryophyllene	2.28	2.34	2.19	2.76	2.79	2.60	2.57	2.38	2.75	2.63	2.99	2.86
2,6 - Octadiene, 2,6 - dimethyl	0.37	-	-	-	0.32	0.12	-	-	0.22	-	-	0.06
citronellol	14. 72	17.38	18.45	17.35	13.98	20.56	18.34	17.05	19.02	17.55	17.11	16.89
geraniol	48. 92	50.89	52.98	49.86	54.95	54.98	50.83	50.85	52.55	51.77	53.86	53.76
neral	7.17	6.97	6.89	5.83	4.98	5.16	5.78	6.03	5.98	7.85	6.33	5.23
geranial	8.48	9.39	9.67	9.44	6.97	6.99	7.67	6.43	5.45	7.93	7.34	7.91
caryophyllene oxide	0.68	0.35	0.10	0.45	0.41	0.32	0.71	0.11	0.35	0.46	0.39	0.54
cyclopentane, 1 - methyl - 1 - (2 - methyl - 2 - propenyl)	-	0.96	0.37	1.53	1.50	1.34	0.86	1.50	-	0.99	0.79	-
durohydroquinone	2.69	1.64	-	2.72	2.36	-	1.97	1.51	1.79	1.44	-	2.17
myristicin	8.65	3.02	6.53	7.22	7.45	4.33	7.50	6.78	8.57	6.36	7.57	6.60
apiol	0.37	-	-	0.05	-	-	0.21	0.12	0.33	0.23	-	-
Total Identified	96.39	98.12	98.64	98.21	96.89	97.96	97.66	95.68	98.41	98.72	97.64	97.10

S = Salicylic acid, i.e. S0 = control, S1= 200 mg/L; I =irrigation, i.e. I1 = 40% available soil moisture, I2 = 60% available soil moisture, I3 = 80% available soil moisture.

Tab. 8 - Effect of water stress and/or salicylic acid on the % of essential oil constituents of *Nepeta cataria* var. *citriodora* plants during the two cuts in the second season.
Tab. 8 - Effetto dello stress idrico e/o dell'applicazione di acido salicilico sulla percentuale di costituenti di oli essenziali in piante di Nepeta cataria *var.* citriodora *durante i due tagli della seconda stagione di crescita.*

In *Nepeta cataria* var. *citriodora,* five main compounds were identified in the essential oil and geraniol and citronellol showed maximum percentages (54.98%) and (20.56%) with 80 ASM + 200 SA (Tab. 8), while percentages were reduced in drought-stressed plants. The maximum geranial and myristicin percentages were obtained from plants exposed to 40 ASM. On the contrary the percentage decreased for higher soil moisture. The chemical profile of major compounds identified in the current study is similar to some previous studies, which indicated that the main constituents of *Nepeta cataria* var. *citriodora* were citronellol, geraniol, geranial and neral (Héthelyi *et al*., 2000; Klimek *et al*., 2000; Smolik *et al*., 2008; Wójciak-Kosior *et al*., 2011).
Similar studies indicated that increasing soil moisture content up to 80 ASM increased some marker compounds, such as geranial in *Dracocephalum moldavica* essential oil (Said-Al Ahl and Abdou, (2009) and carvacrol in oregano essential oil (Said-Al Ahl and Hussein, 2010). On the other hand, increasing moisture levels reduced geraniol percentage in the first study and p-cymene and γ-terpinene in the second one.
Previous studies have shown that SA application affects the accumulation of certain compounds in the essential oil of aromatic plants. For example, α-pinene as well as γ-terpinene, limonene and decanal were decreased by SA application in coriander, but linalool, γ-terpinene and *p*-cymene percentages were increased (Said-Al Ahl *et al*., 2014). Gharib (2006) showed that SA increased the percentage of eugenol and sabinene and reduced *cis*-sabinene hydrate in the basil oil. Rowshan and Bahmanzadegan (2013) found that exogenous application of SA to *Achillea millefolium* increased 1,8-cineol, β-caryphyllen, ar-curcumen and spathulionl and decreased sabenin, comphore and α-bisabolen. Some compounds such as iso-spathulinol, γ-cadinene, β-himachalene, and trans-caryophyllen were detected only in plants treated with SA. Rahimi *et al*., (2013) found that γ-terpinene-7al was lower in the essential oil of SA-treated plants compared to control, while cumin aldehyde and γ-terpinene, ρ-cymene and β-pinene were increased. γ-terpinene-7-al increased with the application of 1mM of SA while other SA concentrations decreased it. All these results indicate that the effect of SA on the accumulation of essential oil is specie and rate dependent and it is influenced by compounds nature and structure.
The qualitative and quantitative changes in the essential oil constituents in *Nepeta cataria* and *Nepeta cataria* var. *citriodora* are presented in Tab. 7 and 8.

Nepeta cataria gave the highest citral, caryophyllene oxide percentages, while *Nepeta cataria* var. *citriodora* gave the same result for citronellal, caryophyllene, citronellol, geraniol, myristicin in the first and second cuts. On the other side, linalool, p-Menth-3-en-9-ol, hexahydroindole, phorone, nepetalactone, geranyl propionate, geranyl isobutyrate, cyclohexene,1-methyl-5-(1-methylethenyl), geranyl propionate and 4-Nitro-1,3-phenylenediamine were found in *Nepeta cataria*, while 5-Hepten-2-one, 6-methyl verbenol, 2,6 octadiene, 2,6-dimethyl cyclopentane, 1-methyl-1-(2-methyl-2-propenyl) durohydroquinone and apiol compounds existed only in *Nepeta cataria* var. *citriodora*.

It is clear that, citronellal and citral compounds had a higher percentage in *Nepeta cataria* and *Nepeta cataria* var. *citriodora* in the first cut, while caryophyllene, citronellol, geraniol and caryophyllene oxide percentages were higher in the second cut in both chemotypes. Also nepetalactone and geranial percentages in *Nepeta cataria;* neral and geranial in *Nepeta cataria* var. *citriodora* were higher in the first cut. This indicates that the distinctive chemical profile of essential oil in each taxon is reflected by the differences in genetic background as well as the seasonal variations of such compounds.

In *Nepeta cataria,* increasing water irrigation increased the contents of linalool, caryophyllene, citronellol, hexahydroindole, and geranyl isobutyrate (Tab. 7). While, citronellal, nepetalactone, geranyl propionate percentages were decreased by increasing water irrigation. On the contrary, lower and medium irrigation rates resulted in higher geraniol, caryophyllene oxide, myristicin, cyclohexene, 1-methyl-5-(1-methylethenyl) percentages than that observed with higher irrigation. However, SA spraying gave the highest percentages of linalool, caryo-phyllene, p-Menth-3-en-9-ol, citronellol, hexahydroindole, phorone, geraniol, neral, geranial, geranyl isobutyrate, myristicin, cyclohexene,1methyl-5-(1-methylethenyl) and 4-Nitro-1,3-phenylenediamine. On the contrary, higher citronellal, hexahydroindole, caryophyllene oxide, nepetalactone, geranyl propionate percentages were obtained without SA application. Caryophyllene, citronellol, hexahydroindole, phorone, geraniol, caryophyllene oxide percentages were higher in the first cut, while the other components were higher in the second cut.

In *Nepeta cataria* var. *citriodora*, increasing water irrigation increased citronellal, caryophyllene and geraniol percentages, while, 5-Hepten-2-one, 6-methyl, verbenol, citronellol, geranial, neral, caryophyllene oxide, durohydroquinone, myristicin and apiol were decreased by increasing water irrigation (Tab. 8). On the contrary, lower and medium irrigation rates were more appropriate for producing cyclopentane,1-methyl-1-(2-methyl-2-propenyl) than higher irrigation rate. However, spraying SA gave the highest percentages of citronellal, geraniol, geranial, neral, caryophyllene oxide and cyclopentane,1-methyl-1-(2-methyl-2-propenyl). On the other hand, higher percentages of 5-Hepten-2-one,6-methyl, verbenol, 2,6-Octadiene,2,6-dimethyl, citronellol, durohydroquinone and apiol were obtained without SA. When comparing the profile in the first and second cuts, 5-Hepten-2-one,6-methyl, caryophyllene, citronellol, geraniol, caryophyllene oxide, myristicin and apiol percentages were higher in the first cut, while the other components were higher in the second cut indicating that the accumulation of essential oil constituents was influenced by the seasonal variations.

CONCLUSIONS

Nepeta cataria var. *citriodora* recorded higher values of growth parameters and oil production compared to those of *Nepeta cataria.* Irrigation increased *Nepeta cataria* and *Nepeta cataria* var. *citriodora* production and the optimum irrigation level for the highest yields of fresh herb was 80 ASM. Whereas, essential oil % decreased with increasing irrigation levels and the optimum irrigation level for the highest essential oil % was 40 ASM. However, for the essential oil yield (mL/plant), plants that received 80 ASM produced more essential oil yield than plants received 60 or 40 ASM. Salicylic acid increased growth, herb fresh yield and essential oil production not only under well-watered conditions (80 ASM) but also both under moderate-watered conditions (60 ASM) and under water deficit conditions (40 ASM). Supplying plants with a water level at 80 ASM + 200 SA gave the best result for herb fresh yield and essential oil production. It can be concluded that application of SA could be a practical approach for enhancing the essential oil accumulation in *Nepeta* chemotypes. The current study provided important information about the qualitative and quantitative changes in the essential oil of two chemotypes of *Nepeta* in relation to SA application under water stress conditions; however, changes in other important classes of secondary metabolites merit further consideration.

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