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THE INFLUENCE OF NaCl-INDUCED STRESS ON THE GROWTH AND VOLATILE PROFILE OF *CURCUMA LONGA* L. LEAVES

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Abstract. *Herein, for the first time, the influence of salt-induced stress on the vegetative growth and the volatile profile of* Curcuma longa *L. leaves was investigated.* C. longa *was grown in a quarter-strength Hoagland's solution to which NaCl was added to give four final concentrations: 0 (control), 25, 50 or 75 mM NaCl. In the case of the plants grown in the 25 mM NaCl medium, leaf biomass production was the same as in the control experiment, but it decreased significantly at higher salinities (50 mM and 75 mM NaCl). The volatile constituents of the leaves were isolated by hydrodistillation and analyzed by GC and GC/MS. The essential-oil yield (calculated on the basis of dry weight) was 2.0% for the control plants, and increased at low-to-medium NaCl concentrations (2.5% and 2.8% for the 25 and 50 mM NaCl media, respectively). Contrary to that, the essential-oil yield decreased (1.6%) in the case of plants grown in the 75 mM NaCl medium. The major volatile constituents of* C. longa *leaves were identified as: α-phellandrene (38.3-42.4%; more than one third of the total oil), terpinene-4-ol (5.6-10.5%), geraniol (5.6-7.9%), p-cymene (5.2-9.6%), α-thujene (4.5-7.3%), β-sesquiphellandrene (4.8-6.8%), β-myrcene (2.6-3.8%) and α-bisabolol (1.5-2.7%).*

Key words: *Curcuma longa* L., *essential oil, NaCl, α-phellandrene*

1. INTRODUCTION

Plant genus *Curcuma* belongs to the Family Zingiberaceae. It is a genus of about 70 species of rhizomatous herbs distributed in India, Thailand, Archipelago and northern

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Australia. About 30 species occur in India, of which a few are of economic importance. *Curcuma longa* L. syn. *C. domestica* (turmeric), a perennial herb native in southern Asia, is cultivated extensively throughout the warmer parts of the world. It is grown on a large scale in India and China. In India it is cultivated in almost all states, particularly in Tamilnadu, Maharashtra and Bengal. In the Indian system of medicine, turmeric is used to some extent as a stomachic, tonic and blood purifier. It is also prescribed as an anti-periodic (Anonymous, 1950; Shrivashankara et al., 2003). The juice of the fresh rhizomes is used for treating skin infections, indolent ulcers, inflamed joints and in purulent ophthalmia. (Anonymous, 1950) The oil of turmeric in small doses acts as a carminative, stomachic appetizer and tonic. In large doses, however, it appears to act as an anti-spasmodic (Chopra et al., 1941). The essential oil of *C. longa* is reported to possess antibacterial, anti-inflammatory, anti-arthritic**,** anti-fungal, hepatoprotective, anti-tumour, hypolipidaemic and antithrombotic activities (Chandra et al., 1972; Srimal et al., 1993).

There are considerable variations in the reported *C. longa* oil composition, which could be due to differences in genetics, ontogeny and analytical methods (Dung et al., 1995; Richmond et al., 1997). Consequently, the amounts and ratios of oil components can be altered by both biotic and abiotic stress, such as salinity, which is considered one of the major factors affecting plant growth and the biosynthesis of natural plant products (Dow et al., 1981). Salinity is one of the most important environmental stresses which affected nearly half of the irrigated surface (Flagella et al., 2002). It limits crop productivity by decreasing the water potential of the root medium, the ion toxicity due to excessive sodium and chloride uptake, and the nutrient ion imbalance by the disturbance of essential intracellular ion concentrations (Greenway et al., 1980). It may induce different morphological, physiological and biochemical and anatomical changes (Teester et al., 2003). Salinity is known to affects several aspects of plant metabolism, including lipid metabolism in many species (Erdei et al., 1980; Pal et al., 2012). Salt stress may affect the biosynthesis of secondary metabolites in plants such as essential oil compounds (Dow et al., 1981). In India about 6.9 Mha, lands are classified as unsuitable for agriculture due to the salt-stress problem. Among the salt affected soil, sodic soils are characterized by high pH (10.1-11.3), high exchangeable sodium percentage (45.0-85.5%), adverse physical and biological properties of soil and reduced availability of some essential nutrients. Crops grown on these soils are always affected by nutritional deficiencies, leading to low crop yield.

Not much attention has been previously paid to the salinity-induces changes in the essential oil content and composition (Neffati et al., 2008; Pal et al., 2012). To the best of our knowledge no information is available on the influence of the soil salinity to the production of the leaf biomass and volatile profile of C. *longa* leaves. Thus, in the present work, we examined changes in the C. *longa* leaf biomass production and essential-oil yield/composition induced by different salinities of the growing medium.

2. MATERIALS AND METHODS

2.1. Plant material

Field experiments were carried out in the 2009-2010 growing seasons at the Banthra Research Experiment Station located at 25 km east of Lucknow. The *C. longa* was grown

in mid-June 2009 under uniform agronomic management. After 10 days, selected plant specimens were transferred in quarter-strength Hoagland's solution with 0.0 (control), 25.0, 50.0 or 75.0 mM of NaCl. The nutritive solutions were continuously aerated and were replaced every 4 days. The experiments were performed in a greenhouse under control conditions (18-25°C; artificial light of 141 μmolm-2s-1 and 6,000 lux). Leaves were harvested in the end of January 2010.

2.2. Essential-oil isolation

Dry *C. longa* leaves (four different samples (each in triplicate) grown under different salinity conditions; 50 g each) were subjected to hydrodistillation during 90 min. Hydrodistillation conditions (duration) were chosen via preliminary kinetic survey which included measuring of the changes in the volume of the isolated essential oil after 30, 60, 90 and 120 min (Msaada *et al*, 2007). The distillates were extracted with diethyl-ether and dried over anhydrous sodium sulfate. The solvent was then removed on rotary evaporator. The essential oil was stored at 20°C prior to analysis.

2.3. Gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS)

GC analysis of essential oil samples (dissolved in diethyl ether, 1:100, v/v) was performed using a Thermo Scientific gas chromatograph equipped with a flame ionization detector (FID). A polar TR-50MS column (length: 30 m; diameter: 0.25 mm; film thickness: 0.25 μm) was used. The carrier gas was He with a flow rate of 1.6 ml min^{-1}. The split ratio was 60:1. The analysis was performed using the following temperature program: oven was initially held isothermal at 35°C for 10 min, then, temperature was raised from 35 to 250°C at the heating rate of 3°C min^{-1} and finally held isothermal at 205°C during 10 min. Injector temperature was 250°C. Quantitative data were obtained from the electronic integration of the FID peak areas.

GC–MS analyses were carried out on a Thermo Fisher TRACE GC ULTRA, with a fused silica TR-50MS capillary column (length: 30 m; diameter: 0.25 mm; film thickness: 0.25 μm). The analysis was performed using the following temperature program: oven was initially held isothermal at 50°C for 5 min, then, temperature was raised first from 50 to 120°C at the heating rate of 2°C min^{-1} and then from 120 to 250°C at the rate of 3°C min^{-1}; finally column was held isothermal at 250°C for 5 min. Injector temperature were held at 250°C. Injector temperature was 220°C, and carrier gas was helium, with a constant flow 1ml/min; slit ratio was 60:1.

2.4. Identification of essential oil constituents

The identification of essential oil components was achieved by comparing the peak retention times with those of authentic standards. Peak identification was performed by comparing the relative retention times with those of commercial standards. Essential oil components were identified by comparison of their retention indices (RI) relative to (C8–C22) n-alkanes with those of authentic compounds under the same conditions (Davies et al., 1990). Further identification was made by matching their recorded mass spectra with those stored in the (NIST/Wiley) mass spectral library and other published mass spectra

(Adams et al., 2001). The corresponding pure standards were obtained from Fluka and Sigma Aldrich. The results of the GC and GC–MS analyses are summarized in Table 1.

2.5. Statistical analysis

The data were examined to statistical analysis using statistical program package STATISTICA (Statsoft, 1998).The one-way analysis of variance (ANOVA) followed by Duncan multiple range test were employed and differences between individual means were deemed to be significant at $p<0.005$.

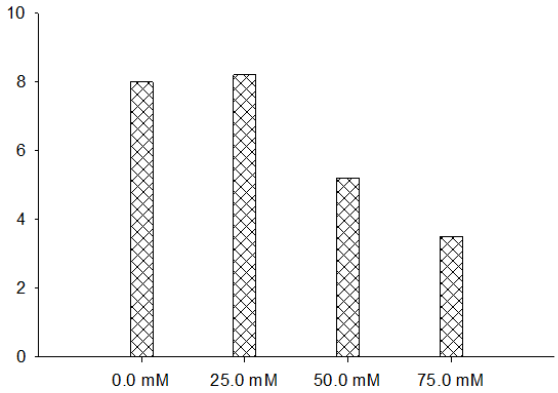

Fig. 1 The influence of NaCl-induced stress on the leaf biomass production in *C. longa*; x-axis: the concentration of NaCl in the growth medium (mM); y-axis: biomass production (leaves' dry weight (g) per plant specimen)

3. RESULTS AND DISCUSSION

3.1. The effect of salinity on growth of *C. longa*

The results regarding the influence of the NaCl-induced stress on the production of leaves' biomass in *C. longa* are summarized in Figure 1. No significant differences were observed between the control and the sample grown on a medium with 25 mM NaCl. However, leaves' biomass production significantly decreased in the case of mediums of higher salinities (up to 34% at 50 mM NaCl, i.e. up to 56% at 75 mM, Figure 1). This is in accordance with the results of Ashraf and Orooj (2006), who reported marked reduction in ajwain (*Trachyspermum ammi*) vegetative growth upon increasing NaCl concentration in the growth medium. Mentioned decrease was thought to be related to the low osmotic potential of the medium (Osawa et al., 1963), which could cause a reduction in chlorophyll content and consequently photosynthesis efficiency (Viera et al., 1991; Castonguay et al., 1991; Nunez-Barrious et al., 1991; Abdul-Hamid et al., 1990).

3.2. The effect of salinity on the yield of *C. longa* leaves essential oil

The results regarding the influence of the NaCl-induced stress on the production/ accumulation of volatile secondary metabolites (essential oil yield) in the leaves of *C. longa* are

summarized in Figure 2. The essential oil content in control *C. longa* sample (0 mM NaCl in the growth medium) was 2.0 % (w/w). In the case of samples grown on the mediums of higher salinities (25 and 50 mM NaCl), 25% and 40% increase in the oil yield was observed (2.5 and 2.8%, respectively). However, further increase of medium's salinity resulted in decrease of oil yield to 1.6% (it was 20% lower in comparison to the control sample), Figure 2. Salinity-induced increase in the oil yield has previously been reported in a large number of plant species, such as sage (Hendawy et al., 2005) and peppermint (Abou El-Fadl et al., 1990). The stimulation of oil production could be due to a higher oil gland density. Alternatively, stress may increase the absolute number of glands produced prior to leaf emergence (Charles et al., 1990). In contrast to these studies, (Ansari et al., 1998) suggested that the oil content and yield decreased with an increase in water salinity in three *Cymbopogon* species. In addition, Dow and associates (1991) were of the view that salinity reduced the oil yield in plants of Lamiaceae, presumably by inhibiting the supply of cytokinin from the roots to the shoots and thus altering the ratio between leaf cytokinin and abscisic acid (Greenway et al., 1980). Salt stress may also indirectly affect the oil yield by a partitioning of assimilates among the growth and differentiation processes. For the leaves, the authors' results partly agree with the growth differentiation balance and carbon-nutrient balance hypothesis (Lorio et al., 1986).When a specific resource like water limits the growth, plants tend to accumulate other resources (e.g., carbon) and use them to increase biosynthesis of secondary metabolites such as oil compounds (Hermes et al., 1992). At low levels of resource availability, the rates of relative growth and stimulation of oil biosynthesis are positively correlated (75 mM), although at moderate one, these two parameters are negatively correlated (50 mM) (Hermes et al, 1992).

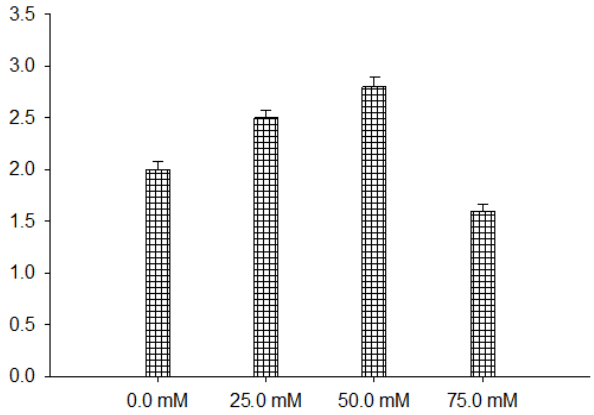

Fig. 2 The influence of NaCl-induced stress on the yield of the essential oil of *C. longa* leaves; x-axis: the concentration of NaCl in the growth medium (mM); y-axis: oil yield (%, w/w (dry leaves))

Herein presented results are in general agreement with the previous studies, but suggest that the observed differences in the oil yield for plant samples grown on media of different salinities are the outcome of several different processes with opposing effects (e.g. the increase of the yield at lower medium salinities and the decrease at the higher medium salinities).

3.3. The effect of salinity on the chemical composition of *C. longa* leaves essential oil

The results of the GC and GC–MS analyses of the essential-oil samples obtained from the leaves of *C. longa* samples grown in the media of different salinities are summarized in Table 1. The analyses revealed the presence of 35 different constituents which accounted for c.a. 99% of the total essential oils (Table 1). α-Phellandrene was the major

Table 1 The chemical composition (%; mean ± standard deviation) of the essential-oil samples obtained from the leaves of *C. longa* grown in the media of different salinities (0 – 75 mM NaCl)

Compound[a]	0 mM (control)	25 mM	50 mM	75 mM
α-Phellandrene	38.3±1.5	42.4±1.6	39.8±1.7	38.7±1.4
p-Cymene	8.8±0.5	9.6±0.6	6.3±0.4	5.2±0.4
Terpinen-4-ol	8.4±0.4	10.5±0.7	7.4±0.1	5.6±0.4
Geraniol	7.7±0.5	7.9±0.5	7.0±0.6	5.6±0.4
α-Thujene	7.3±0.3	6.8±0.6	5.9±0.4	4.5±0.3
β-Sesquiphellandrene	6.8±0.4	6.5±0.4	6.0±0.2	4.8±0.2
β-Myrcene	3.0±0.2	3.2±0.2	3.8±0.2	2.6±0.2
α-Bisabolol	2.7±0.1	1.5±0.1	1.6±0.1	1.7±0.1
Octane	1.9±0.1	2.8±0.1	2.2±0.1	2.4±0.1
β-Pinene	1.2±0.1	0.6±0.1	1.6±0.1	1.0±0.1
β-Linalool	1.0±0.1	1.4±0.1	1.8±0.1	0.9±0.1
Nonanal	0.7±0.1	0.8±0.1	1.2±0.1	1.0±0.0
Sabinene	0.7±0.1	0.7±0.0	0.6±0.0	0.4±0.0
α-Pinene	0.7±0.1	0.6±0.1	0.8±0.1	1.0±0.1
β-Ocimene	0.7±0.1	0.3±0.0	0.2±0.0	tr[b]
α-Terpinene	0.6±0.0	0.2±0.0	1.3±0.1	1.1±0.1
α-Caryophyllene	0.4±0.0	1.1±0.2	0.7±0.1	0.3±0.0
trans-*p*-Menth-2-en-1-ol	0.4±0.0	0.3±0.0	0.4±0.0	0.3±0.0
α-Terpineol	0.4±0.0	tr	0.2±0.0	0.2±0.0
Eugenol	0.3±0.0	0.7±0.0	0.6±0.0	0.3±0.0
Undecanal	0.3±0.0	0.6±0.1	0.4±0.0	0.1±0.0
Carvacrol	0.3±0.0	0.5±0.0	0.3±0.0	2.6±0.1
Terpinolene	0.3±0.0	0.4±0.0	0.6±0.0	0.7±0.0
Camphor	0.2±0.0	0.8±0.0	0.8±0.1	1.0±0.1
γ-Elemene	0.2±0.0	0.2±0.0	0.3±0.0	0.8±0.0
β-Turmerone	0.2±0.0	0.1±0.0	tr	tr
Linalyl acetate	0.2±0.0	0.1±0.0	0.3±0.0	0.4±0.0
β-Farnesene	0.2±0.0	tr	0.2±0.0	tr
α-Bergamotene	0.2±0.0	tr	tr	tr
1,8-Cineole	0.1±0.0	0.2±0.0	0.3±0.0	0.1±0.0
α-Turmerone	0.1±0.0	0.2±0.0	0.1±0.0	0.2±0.0
Caryophyllene oxide	0.1±0.0	0.1±0.0	0.2±0.0	0.2±0.0
Borneol	tr	0.2±0.0	0.2±0.0	0.2±0.0
γ-Terpinene	tr	tr	tr	tr
α-Humulene	tr	tr	tr	tr

[a]Compounds are listed in order of decreasing abundance (ratio of the peak area to total peak area);
[b]tr-less than 0.05%

constituent of all four essential-oil samples (38.3-42.4%), and was followed by terpinene-4-ol (5.6-10.5%), geraniol (5.6-7.9%), *p*-cymene (5.2-9.6%), α-thujene (4.5-7.3%), β-sesquiphellandrene (4.8-6.8%), β-myrcene (2.6-3.8%) and α-bisabolol (1.5-2.7%). The herein obtained results regarding the volatile profile of *C. longa* leaves are in agreement with previous ones (Oguntimcin et al., 1990). As evident from the data given in Table 1, the distribution of the relative abundances of the *C. longa* volatiles was somewhat different in four studied oil samples, suggesting that salinity-induced stress differently affects biosynthesis of different secondary metabolites. However, these differences, that are in general agreement with the previous studies regarding susceptibility of plant volatile profile to environmental conditions (Gil et al., 2002), are not as pronounced as one might expect.

4. CONCLUSION

Production of the volatile metabolites by *C. longa* leaves was stimulated at low and moderate salinity of growth medium. However, high salinity levels suppress biosynthesis/accumulation of volatiles in *C. longa* leaves. The same is true for the production of leave biomass. The dominant constituents of all of the studied essential oils (different salinities of growth medium) were α-phellandrene, *p*-cymene, terpinene-4-ol, geraniol, α-thujene and β-sesquiphellandrene. It seems that the relative amounts of these constituents were not susceptible to the changes of the salinity of growth medium.

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UTICAJ STRESA IZAZVANOG POVEĆANIM SALINITETOM HRANLJIVOG MEDIJUMA NA PRODUKCIJU I ISPARLJIVI PROFIL LISTOVA BILJNE VRSTE *CURCUMA LONGA* L.

U ovom radu je po prvi put ispitivan uticaj stresa izazvanog povećanim salinitetom hranljivog medijuma na rast i isparljivi profil listova biljne vrste Curcuma longa *L.* C. longa *je gajena na četvorostruko razblaženom Holandovom rastvoru u koji su dodate različite količine natrijum-hlorida, takve da njegova konačna koncentracija bude 0 (kontrola), 25, 50 ili 75 mM. Produkcija biomase listova u slučaju biljaka gajenih u hranljivom medijumu u kom je koncentracija NaCl bila 25 mM se nije razlikovala od one kod kontrolnog uzorka. Međutim, rast listova je bio značajno smanjen u slučaju podloga sa višim vrednostima saliniteta (50 i 75 mM NaCl). Isparljivi sastojci listova su izolovani hidrodestilacijom i analizirani pomoću GC i GC/MS metoda. Prinos etarskog ulja je u slučaju kontrolnog uzorka bio 2,0%, a povećan je bio kod biljaka gajenih u 25 i 50 mM NaCl medijumima (2,5 i 2,8%). Nasuprot tome, prinos etarskog ulja se smanjio (1,6%) u slučaju biljaka gajenih u 75 mM NaCl medijumu. Glavni isparljivi sastojci listova vrste* C. longa *bili su α-felandren (38,3-42,4%; više od jedne trećine ukupnog ulja), terpinen-4-ol (5,6-10,5%), geraniol (5,6-7,9%), p-cimen (5,2-9,6%), α-tujen (4,5-7,3%), β-seskvifelandren (4,8-6,8%), β-mircen (2,6-3,8%) i α-bisabolol (1,5-2,7%).*

Ključne reči: *Curcuma longa* L.*, etarsko ulje, NaCl, α-felandren*