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# Yield, essential oil and pigment content of *Calendula officinalis* L. flower heads cultivated under salt stress conditions

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ABSTRACT

Flower heads of *Calendula officinalis* L. are used for medicinal or culinary purposes. Since Egyptian agricultural lands contain salt, this study investigated the effects of saline irrigation water on yield (fresh and dry weights of flower heads), essential oil (EO) yield, chemical constituents of the EO and total flavonoids and carotenoids of flower heads at three flowering stages, i.e. initial (21 days after bud formation (DABF)), full flowering (81 DABF) and final (111 DABF). After plants were treated with different levels of saline irrigation water (0.39, 1.56, 3.13, 4.69, 6.25, 7.81 and 9.38 dS m$^{-1}$) consisting of NaCl, $CaCl_2$ and $MgCl_2$ salts, the flower head yield and pigment (total flavonoids and carotenoids) content were significantly reduced. Irrigation with saline water increased the EO content and its main components (α-cadinol, γ- and Δ-cadinene). Fresh and dry weights of flower heads and EO increased near 81 DABF while the content of pigments increased by 111 DABF.



## 1. Introduction

*Calendula officinalis* L. (English marigold, pot marigold) belongs to the *Asteraceae* (*Compositae*) family; it is an annual with bright or yellow orange daisy-like flowers which are used for medicinal or culinary purposes (Bcerentrup and Robbelen, 1987; Cromack and Smith, 1988). *C. officinalis* can be broadly applied as an antiseptic, anti-inflammatory and cicatrizing (Correa Júnior, 1994) as well as a light antibacterial (Chiej, 1988) and antiviral (Bogdanova and Farmakol, 1970) agent. Many *Calendula* species have a characteristic scent or taste caused by mono- and sesquiterpenes within the essential oil (EO), which in many cases are the reason for their application in folk medicine (Yoshikawa et al., 2001). Recently, many attempts have been made to better characterize their therapeutic properties and to enhance the production of these useful compounds within their EOs. Selected *Calendula* chemotypes growing in soil or *in vitro*, for example, flowers of the cadinol chemotype, are very important in European and western Asian folk medicines and are used to treat inflammatory conditions (Masayuki et al., 2001). Distinct subspecies of *C. officinalis* have been reported from various countries (Chalchat et al., 1991; Nicoletta et al., 2003), i.e. Herbaria, Mecsek, Melius, Golden Dragon and Adamo (Bakó et al., 2002). *C. officinalis* can be used as a colorant because it primarily contains two classes of pigments, the flavonoids and carotenoids, which can be used as yellow and orange natural colors, respectively. Natural colors are gaining considerable attention since several synthetic colorants have given rise to allergic, toxic and carcinogenic effects (Lea, 1988). Flavonoids have antioxidant activities which play an important role in food preservation and human health by combating damage caused by oxidizing agents (Meda et al., 2005). Carotenoids are important to humans and other animals as precursors of vitamin A and retinoids. In addition, they act as antioxidants, immunoenhancers, inhibitors of mutagenesis and transformation, inhibitors of premalignant lesions, screening pigments in primate fovea, and nonphotochemical fluorescence quenchers (Castenmiller and West, 1998).

Saline soil can be defined as soil having an electrical conductivity of the saturated paste extract (ECe) of 4 dS m$^{-1}$ (4 dS m$^{-1}$ ∼40 mM NaCl) or more. Salinity is a major factor reducing plant growth and productivity worldwide; it affects about 7% of the world's total land area (Flowers et al., 1997; Zhu, 2002) and is the major environmental factor limiting plant growth and productivity (Allakhverdiev et al., 2000). The detrimental effects of high salinity on plants can be observed at the whole-plant level such as the death of plants or necrosis of plant organs and/or decreases in productivity. Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within cells (e.g. Kobayashi, 2008). During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, and energy and lipid metabolism are affected (Parida and Das, 2005). The ear-

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liest response is a reduction in the rate of leaf surface expansion, followed by a cessation of expansion as the stress intensifies (Parida and Das, 2005). Growth often resumes when the stress is relieved. Carbohydrates, which among other substrates are needed for cell growth, are supplied mainly through the process of photosynthesis, and photosynthetic rates are usually lower in plants exposed to salinity, especially to NaCl (Parida and Das, 2005).

Many researchers have studied the effect of soil salinity on growth, EO and chemical composition of several plants. For example, Abou El-Fadl et al. (1990) indicated that even though soil salinity more than 2000 ppm decreased peppermint (*Mentha arvensis*) plant growth, the EO yield and that of its components increased. By increasing the levels of soil salinity plant growth of *Ocimum basilicum* (basil) was significantly decreased but EO and its main components increased (El-Shafy et al., 1991). The same trend was found for the EO and its components of damsesea (*Artemisia absinthium*) plant which increased with increasing salinity levels (Abd-El Nabi and Hussein, 1996). Khalid (2001) demonstrated that increasing salinity decreased plant growth of black cumin (*Nigella sativa*). Salinity significantly increased sage (*Salvia officinalis*) EO yield and its main components (Hendawy and Khalid, 2005). The EO content in sweet fennel (*Foeniculum vulgare* var. 'Dulce') fruits decreased progressively with an increase in NaCl concentration (Ashraf and Akhtar, 2004). NaCl salt reduced the fruit weight and oil content but increased the moisture content of olive (*Olea europaea* L.) fruits (Chartzoulakis, 2005). Ashraf and Orooj (2006) reported that seed oil concentration did not change with an increase in external salt level of ajwain (*Trachyspermum ammi* L.). Parida et al. (2004) indicated that salt significantly decreased the carotenoid content of mangrove (*Aegiceras corniculatum*). *Salvinia natans* pigments were significantly reduced by 50 mM NaCl and higher (Jampeetong and Brix, 2009). Increasing soil salinity strongly increased *Rosmarinus offfcinalis* (rosemary) EO biosynthesis (Solinas and Deiana, 1996). Nevertheless, investigations dealing with the effect of this stress on EO production are scarce although in general, it affects the composition and causes a reduction in yield of the EOs of medicinal and aromatic species (Dow et al., 1981; El-Keltawi and Croteau, 1987).

However, not all studies have shown a positive effect of salt stress on EO parameters: NaCl, $CaCl_2$ and $MgCl_2$ significantly reduced the EO yield of lemon balm (*Melissa officinalis* L.) (Ozturk et al., 2004) and sage (*Salvia offficinalis*) (Taarit et al., 2010). The highest flower dry weight and EO content of chamomile (*Matricaria chamomila*) plants were observed under non-salinity stress (Razmuoo et al., 2008). The major volatile compound in *Coriandrum sativum* L. (coriander) leaves and the content of these compounds were affected differently by the saline level (Neffati and Marzouk, 2008). These results from the literature indicate that in fact there is no safe-fast trend and that the effects of salinity on EO yield need to be tested on an individual plant or species basis.

The EO concentration in plant tissue under salt stress increased as compared with untreated controls, suggesting that oil synthesis and/or oil degradation processes were less sensitive to salt stress than similar processes in peppermint (*Mentha* × *piperita* L.), pennyroyal (*Mentha pulegium* L.) and apple mint (*Mentha suaveolens* Ehrh.) (Aziz et al., 2008). Within the EO, the relative level of various constituents increased, decreased, or did not change in mint (*Mentha* sp.) plants under NaCl salt compared with non-stressed control plants (Aziz et al., 2008). The EO content from the aerial part of thyme (*Thymus Maroccanus* Ball.) did not change with an increase in external salt level (Belaqziz et al., 2009). Increasing salinity of irrigation water significantly decreased vegetative growth and green yield of sweet fennel (*F. vulgare* var. 'Dulce') (Zaki et al., 2009). The EO yield of marjoram (*Origanum majorana*) shoots was 0.12% in the control and 0.10% at 50 mM NaCl but an important decrease was observed at 100 mM (0.05%) Baatour et al., 2010: 33 components were identified belonging to different chemical classes in the control, the EO was found to be rich in *trans*-sabinene hydrate (47.67%), terpinen-4-ol (20.82%) and *cis*-sabinene hydrate (7.23%) and the proportions of these main compounds were significantly affected by salt. Ahmed and Jabeen (2009) showed a significant decrease in growth characters of sunflower (*Helianthus annuus* L.) with an increase in salt concentration. Salt stress enhanced *M. pulegium* EO yield by ∼2.75-fold and affected the percentage of menthone, which is the major compound (∼51%), increasing that of menthone, pulegone, and neomenthol, which constitute the monoterpene class and which were the principal components (Karray-Bouraoui et al., 2009). The chemical composition of EO in clary sage (*Salvia sclarea* L.) was strongly affected by NaCl treatments (Taarit and Msaada, 2010).

*C. officinalis* is produced in many countries such as France, Egypt, Algeria, Morocco, South Africa, China and Hungary. In 2009, the cultivated area in Egypt with *C. officinalis* was approximately 3500 ha. *C. officinalis* flower heads produce 75–100 kg of EO/ha. Many countries are an important market for *C. officinalis* flower heads and EO, namely Germany, Russia, Netherlands, the UK and the USA (Haikal and Omar, 2009).

The Egyptian climate is mostly arid and semi-arid, where water availability is a major problem for crop 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 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 government over the last few decades. In this study, we investigate the possible effect of saline irrigation water on the yield, EO content, and pigment content of *C. officinalis* flower heads, an economically important medicinal plant in Egypt.

## 2. Materials and methods

Experiments were carried out at the National Research Centre (NRC), Giza, Egypt, during two seasons, 2007/2008 and 2008/2009. *C. officinalis* 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 (meteorological data at Giza, Egypt during the two growing seasons are presented in Table 1) within the greenhouse of the NRC. Each pot was filled with 10 kg of air-dried clay loam 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 2. Eight weeks after sowing the seedlings were thinned to three plants per pot.

After 45 days from sowing plants were subjected to different levels of saline irrigation water, 0.39 (tap water as control), 1.56, 3.13, 4.69, 6.25, 7.81 and 9.38 dS $m^{-1}$. To prepare irrigation water with different salinity levels, highly soluble NaCl, $CaCl_2$ and $MgCl_2$ salts were used. These salts were used because they are found naturally in the irrigation water in Egypt (El-Sherif et al., 1990). Sodium (Na) adsorption ratio (SAR) was kept at about 1 at all salinity levels so that only the effect of salinity could be evaluated without any negative effect of Na. When the SAR value is >10, evaluation of Na