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**PLANT SCIENCE**

# Effect of potassium uptake on the composition of essential oil content in *Calendula officinalis* L. flowers

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## Abstract

Potassium (K) is an important macronutrient and the most abundant cation in higher plants. K has been the target of some researchers mainly because it is essential for enzyme activation such as enzyme of essential oil synthesis. The highest accumulation of essential oil (0.29% and 0.095 g plant$^{-1}$) was recorded at the treatment of 173.2 kg ha$^{-1}$ compared with control treatment (0.13% and 0.015 g plant$^{-1}$). 28 constituents were identified in the essential oil. The main constituents (α-cadinol, Δ-and γ -cadinene) increased with K level. The highest amount of the main constituents [α-cadinol (33.11%), Δ-cadinene (18.41%), and γ -cadinene (9.99%)] was produced from the F treatment (346.4 kg ha$^{-1}$). Alcohols were the major constituents of the heavy oxygenated compounds (HOC) of *Calendula* essential oil in which α-Cadinol represented the highest concentration.

*Key words*: *Calendula officinalis* L., Essential oil, Potassium (K), α-cadinol, Δ-and γ –cadinene

## Introduction

*Calendula officinalis* L. (English marigold, pot marigold) is an annual plant belonging to the *Asteraceae* (*Compositae*) family with bright yellow or orange daisy-like flowers which are used for medicinal or culinary purposes (Beerentrup 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 and antiviral agent (Chiej, 1988). Many *Calendula* species have a characteristic scent or taste caused by mono- and sesquiterpenes within the essential oil, which in many cases are the reason for their application in folk medicine (Msayuki 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 essential oils. 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 (Msayuki et al., 2001).

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Plant nutrition is one of the most important factors that increase essential oil production. Potassium (K) is an important macronutrient and the most abundant cation in higher plants. K has been the target of some researchers mainly because it is essential for enzyme activation such as enzyme of essential oil synthesis (Machner, 2001; Silva, 2004). High K level decreased the essential oil content of bracts and leaves of *Origanum dictamnus* L. plants (Economakis, 1993).

Hornok (1980) indicated that K fertilization was effective on the essential oil content of dill (*Anethum graveolens* L.) while, Hussien (1995) reported that K fertilization was more affected on the dill essential oil constituents. Also, K fertilization increased the essential oil content of some medicinal *Apiaceae* plants (Khalid, 1996). K uptake in *Tagetes minuta* L. plants increases essential oil content (Somida, 2002). Kahlid et al (2007) indicated that fertilization with potassium affected essential oil and its constituents in rue (*Ruta graveolens* L.). The highest content of peppermint (*Mentha piperita*) essential oil production was obtained with fertilization with K at 218 mg L$^{-1}$ (Mollafilabi et al., 2010). Furthermore, addition of K at 123 kg ha$^{-1}$ year$^{-1}$ enhanced the total essential oil yield of palmarosa (*Cymbopogon* *martinii* [roxb.] wats. var. motia burk) plant by 23.7% in comparison to no K application (Munnu, 2008). Similarly, K fertilization increased essential oil and anethole contents of anise (*pimpinella anisum* L.) plant (Al-Awak, 2010). When treated with different concentrations of K, caraway (*Carum carvi* L.) experienced an increased in essential oil content, linalool, and limonene but no considerable differences was observed in carvone (Ezz El-Din et al., 2010). In this study, we investigate the possible effect of K on the essential oil content of *C. officinalis* flower heads.

## Materials and Methods

Experiments were carried out at the National Research Centre (NRC), Giza, Egypt under natural coditions, for 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) as 10 seeds per pot. The viability of the seeds was approximately 92%. In the third week of December during both seasons, the pots were transferred to natural conditions within the greenhouse of the NRC were each pot was filled with 10 kg of air-dried clay loam soil (meteorological data at Giza, Egypt during the two growing seasons are presented in Table 1). 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. All agriculture practices operations other than experimental treatments were done according to the recommendations of Ministry of Agriculture, Egypt. Pots were divided into 6 main groups adjusted to $K_2SO_4$ (48%, $K_2O$) as a source of potassium with varying amounts of K as [A] 0 (control), [B] 115.5, [C] 173.2, [D] 231.00, [E] 288.7 and [F] 346.4 kg $ha^{-1}$ (as $K_2O$ form) respectively.

## Harvesting

Fresh flower heads were collected from each treatment at flowering stages during both seasons and air dried. The flower yield (g $plant^{-1}$ of fresh and dry weights of flower heads) was recorded.

## Essential oil isolation

Fresh flower heads were collected from each treatment and from the flowering stage in both seasons, air dried and weighted to extract the essential oil. Dry flower heads (500 g) from each of these treatments was hydro-distilled for 3 h using a Clevenger-type apparatus (Clevenger, 1928). The essential oil content was calculated as a percentage. Also g essential oil $plant^{-1}$ was calculated according to the dry weights of flower heads per plant.

Table 2. Physical and chemical properties of the soil.

| Sand (%) | 26 |
|---|---|
| Silt (%) | 36 |
| Clay (%) | 38 |
| Texture class | Clay loam |
| CaCo3 (%) | 4.5 |
| O.M. | 1.3 |
| pH | 1.7 |
| Soluble cations and anions (mequiv./l) | |
| EC | 0.57 |
| $Na^+$ | 2.23 |
| $Mg^{++}$ | 0.88 |
| $Ca^{++}$ | 1.11 |
| $K^+$ | 1.48 |
| $HCO3^{-1}$ | 1.12 |
| $Co^{+}3$ | 0.73 |
| $Cl^{-1}$ | 2.10 |
| $SO4^{-2}$ | 1.62 |

*GC/MS:* The essential oil was analyzed on a VG analytical 70- 250S sector field mass spectrometer, 70 eV, using a SPsil5, 25 m x 0.30 mm, 0.25 µm coating thickness, fused silica capillary column, injector 222°C, detector 240°C, linear temperature 80°–270°C at 10°C/min. One microliter of diluted essential oil (1/100, v/v, in *n*-pentane) was injected manually in the GC at 250 °C, with the splitless mode flame ionization detection (FID) using the HP Chemstation software on a HP 5980 GC with the same type column as used for GC/MS and same coditions program.

## Qualitative and quantitative analyses essential oil

Identifications of essential oilcomponents were made by library searches (Adams, 2007) combining MS and retention data of authentic compounds and comparison of their GC retention indices (RI) with those of the literature (Adams, 2007). The retention indices were determined in relation to a homologous series of *n*-alkanes (C8–C22) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley 5 Libraries or with mass spectra from literature. Component relative concentrations were calculated based on GC peak areas without using correction factors.

Table 1. Meteorological data at Giza, Egypt during the two growing seasons.

| Months | 1st season (2007/2008) | | | | | 2nd season (2008/2009) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | T (°C) | | Rs ($MJm^{-2} d^{-1}$) | RH (%) | ETp ($mmd^{-1}$) | T (°C) | | Rs ($MJm^{-2} d^{-1}$) | RH (%) | ETp ($mmd^{-1}$) |
| | Max. | Min. | | | | Max. | Min. | | | |
| November | 22.6 | 10.5 | 13.0 | 48.2 | 23.1 | 22.8 | 12.0 | 12.2 | 38.9 | 24.2 |
| December | 19.4 | 11.6 | 14.4 | 48.9 | 27.4 | 21.3 | 11.5 | 13.5 | 40.2 | 26.8 |
| January | 18.0 | 10.9 | 24.9 | 48.3 | 28.3 | 19.6 | 10.2 | 22.6 | 49.3 | 32.5 |
| February | 23.8 | 12.6 | 25.9 | 55.6 | 29.6 | 22.1 | 16.2 | 26.9 | 50.2 | 33.5 |
| March | 27.5 | 14.3 | 26.7 | 70.6 | 30.4 | 28.7 | 17.6 | 27.2 | 52.4 | 34.0 |
| April | 28.9 | 14.4 | 27.5 | 80.4 | 36.5 | 29.1 | 18.1 | 30.5 | 56.2 | 37.5 |
| May | 31.2 | 16.3 | 31.6 | 88.9 | 42.5 | 30.4 | 20.1 | 32.5 | 74.2 | 44.2 |
| June | 35.4 | 20.5 | 35.8 | 90.3 | 49.6 | 33.8 | 21.3 | 33.8 | 84.2 | 51.1 |

Monthly average. T: temperature; Rs: solar radiation; RH: relative humidity; ETp: potential evapotranspiration.

## Statistical analysis

In this experiment, 1 factor was considered: potassium amount (control, 115.5, 173.2, 231.00, 288.7 and 346.4 kg $ha^{-1}$). For each treatment there were 4 replicates, each of which had 10 pots; in each pot 3 individual plants were planted. The experimental design followed a complete random block design according to Snedecor Cochran (1990). The flower samples were collected from each replicate (30 plants). The averages of data were statistically analyzed using analysis of variance one - way (ANOVA-1). Significant values determined according P values ($P<0.05$ = significant, $P<0.01$ = more significant and $P<0.001$ = highly significant). The applications of that technique were according to the STAT-ITCF program (Foucart, 1982).

## Results

### Effect of fertilizing potassium amount on the essential oil content

As shown in Table 3, essential oil yield increased with the potassium level compared with control treatment. The highest accumulation of essential oil (0.29% and 0.095 g $plant^{-1}$) was recorded at potassium treatment of 173.2 kg $ha^{-1}$, compared with that of the control treatment (0.13% and 0.015 g $plant^{-1}$). The yield of essential oil yield was low at potassium treatments 115.5, 231.00, 288.7 and 346.4 kg $ha^{-1}$ compared with that of treatment 173.2 kg $ha^{-1}$. The changes in essential oil yield were highly significant ($P<0.001$) for K treatments (Table 3).

Table 3. Yield (% and g $plant^{-1}$) of *Calendula officinalis* L. flower heads essential oil after fertilization with different concentrations of potassium.

| $K_2O_5$ (kg $ha^{-1}$) treatments | Essential oil (%) | Essential oil (g $plant^{-1}$) |
|---|---|---|
| Control | 0.13 | 0.015 |
| 115.5 | 0.18 | 0.035 |
| 173.2 | 0.29 | 0.095 |
| 231.0 | 0.24 | 0.054 |
| 288.7 | 0.25 | 0.051 |
| 346.4 | 0.26 | 0.052 |
| F ratio* | 22.31*** | 840.84*** |

* $P \leq 0.05$ (significant) according to F-values of the 2-way analysis of variance (ANOVA-2).

** $P < 0.01$ (more significant) according to F-values of the 2-way analysis of variance (ANOVA-2).

*** $P < 0.001$ (highly significant) according to F-values of the 2-way analysis of variance (ANOVA-2).

F ratio* = Calculated F

### Effect of K on the chemical composition of essential oil

Table 4 shows that 28 constituents, amounting 99.20 - 99.64% of the essential oil were identified. The main constituents of *Calendula* essential oil as detected by GC-MS were α-cadinol, Δ-and γ -cadinene. The highest values of the main components (33.11, 18.41, both at 9.99%) were obtained from potassium treatment of 346.4 kg $ha^{-1}$. The relative level for other various constituents were changed (increased or decreased) in *C. officinalis* flower heads under different K treatments compared with untreated control treatment.

Table 4 included the compounds obtained from *C. officinalis* essential oil under K treatments grouped into classes (monoterpenes, sesquiterpenes, light oxygenated compounds, heavy oxygenated compounds) and their percentages compared with the control treatment. From the same table, it is noticeable that the concentration of heavy oxygenated compounds was the highest in essential oil with all in plants treated with K compared to the control and other chemical classes (light oxygenated compounds, sesquiterpene and monoterpene). Alcohols were the major constituents of heavy oxygenated compounds in *Calendula* essential oil with α-Cadinol as the highest concentration among these alcohols. This indicates that most of *Calendula* essential oils, the experimental conditions belong to the α-cadinol chemotype. The highest amount of light oxygenated compounds (11.31%) resulted from the control treatment; the highest amount of sesquiterpenes (46.53) resulted from the 173.2 kg $ha^{-1}$ treatment; the highest amount of monoterpene (1.78) resulted from the 231.0 kg $ha^{-1}$ treatment; while the highest amount of heavy oxygenated compounds (46.50) resulted from the 346.4 kg $ha^{-1}$ treatment. The main components of essential oil (α-cadinol, γ-and Δ-cadinene) increased with the potassium level compared with control treatment. ANOVA indicated that the changes in all essential oil constituents were highly significant for K treatments except for aromadendrene (allo) [more significant ($P<0.01$)] and α-cadinol (insignificant). On the other hand, changes in monoterpene and sesquiterpenes were highly significant ($P<0.001$) while it was more significant in light oxygenated compounds but it was insignificant heavy oxygenated compounds for K treatments (Table 4).

Table 4. Effect of potassium on the essential oil constituents extracted from *Calendula officinalis* L. flower heads.

| No | Components (%) | KI* | $K_2O_5$ (kg ha $^{-1}$) treatments Control | 115.5 | 173.2 | 231.0 | 288.7 | 346.4 | *F -ratio* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | α-Pinene | 938 | 1.75 | 1.32 | 1.33 | 1.78 | 0.63 | 1.39 | 77.65*** |
| 2 | β-Farnesene | 1450 | 0.19 | 0.22 | 0.35 | 1.56 | 1.43 | 1.32 | 1753.95*** |
| 3 | α-Humulene | 1455 | 0.22 | 1.12 | 0.89 | 1.67 | 0.88 | 0.98 | 2613.60*** |
| 4 | α-Patchoulene | 1457 | 0.22 | 1.12 | 0.78 | 1.66 | 1.98 | 2.11 | 3314.47*** |
| 5 | Aromadendrene | 1463 | 1.20 | 1.23 | 1.23 | 1.12 | 1.13 | 1.34 | 8.85** |
| 6 | γ-Gurjunene | 1474 | 2.41 | 2.34 | 1.34 | 1.11 | 1.89 | 1.63 | 1135.10*** |
| 7 | γ-Muurolene | 1478 | 0.43 | 1.56 | 0.78 | 1.14 | 1.96 | 1.37 | 4526.20*** |
| 8 | α-Muurolene | 1500 | 0.55 | 0.34 | 0.67 | 0.45 | 0.87 | 0.55 | 80.68*** |
| 9 | γ-Cadinene | 1514 | 9.74 | 10.34 | 11.89 | 9.88 | 9.96 | 9.99 | 3231.40*** |
| 10 | Δ-Cadinene | 1523 | 17.80 | 18.33 | 20.67 | 17.97 | 18.38 | 18.41 | 1731.88*** |
| 11 | β-Patchouli alcohol | 1539 | 3.41 | 3.44 | 2.16 | 2.67 | 1.98 | 1.67 | 3729.80*** |
| 12 | α-Calcorene | 1541 | 1.72 | 1.13 | 1.34 | 1.89 | 1.32 | 1.38 | 413.21*** |
| 13 | Muurol-5-enen-4-B-oL | 1546 | 1.32 | 1.34 | 1.22 | 1.65 | 0.57 | 0.45 | 643.36*** |
| 14 | β-Calacorene | 1564 | 3.24 | 2.34 | 1.45 | 1.76 | 0.98 | 1.23 | 1842.32*** |
| 15 | Nerolidol (*E*) | 1565 | 5.70 | 3.45 | 1.67 | 1.12 | 1.15 | 1.11 | 4011.6*** |
| 16 | Guaiol | 1596 | 1.14 | 1.13 | 1.32 | 1.67 | 1.98 | 1.76 | 1501.92*** |
| 17 | β-Acorenol | 1635 | 3.61 | 2.24 | 2.67 | 2.22 | 2.65 | 2.13 | 1521.33*** |
| 18 | Cadinol (epi-α) | 1639 | 0.86 | 1.45 | 1.12 | 2.24 | 2.87 | 2.65 | 1681.99*** |
| 19 | Muurolol | 1640 | 0.13 | 0.17 | 0.16 | 1.34 | 1.87 | 1.33 | 4093.32*** |
| 20 | α-Eudesmol | 1653 | 2.26 | 1.29 | 1.34 | 0.98 | 1.22 | 1.24 | 844.92*** |
| 21 | ά-Patchouli alcohol | 1654 | 1.62 | 1.54 | 1.34 | 1.96 | 2.23 | 2.39 | 812.02*** |
| 22 | Bulnesol | 1658 | 1.28 | 1.39 | 1.82 | 1.44 | 1.78 | 1.95 | 408.87*** |
| 23 | α-Cadinol | 1663 | 32.00 | 32.37 | 34.76 | 32.78 | 32.56 | 33.11 | 0.71 |
| 24 | Eudesmol | 1669 | 0.96 | 1.11 | 1.23 | 1.76 | 1.56 | 1.99 | 2063.70*** |
| 25 | Isocedranol | 1670 | 0.75 | 0.77 | 0.69 | 1.55 | 1.35 | 1.37 | 315.00*** |
| 26 | α-Bisabolol | 1684 | 1.72 | 1.69 | 1.11 | 1.23 | 1.26 | 1.38 | 33.77*** |
| 27 | Bisabolol | 1687 | 0.27 | 1.45 | 1.23 | 1.13 | 1.45 | 1.29 | 518.68*** |
| 28 | Pentacosane | 2501 | 2.80 | 2.98 | 2.98 | 1.87 | 1.42 | 2.12 | 682.94*** |
| | Total identified | | 99.30 | 99.20 | 99.54 | 99.60 | 99.31 | 99.64 | |
| | Monoterpenes | | 1.75 | 1.32 | 1.33 | 1.78 | 0.63 | 1.39 | 648.46*** |
| | Sesquiterpenes | | 43.93 | 46.49 | 46.53 | 44.75 | 44.18 | 44.10 | 215.77*** |
| | Light oxygenated compounds | | 11.31 | 8.27 | 6.78 | 7.25 | 8.65 | 7.65 | 5.86** |
| | Heavy oxygenated compounds | | 42.31 | 43.12 | 44.90 | 45.82 | 45.85 | 46.50 | 1.00 |

* $P \leq 0.05$ (significant) according to F-values of the 2-way analysis of variance (ANOVA-2).

** $P < 0.01$ (more significant) according to F-values of the 2-way analysis of variance (ANOVA-2).

*** $P < 0.001$ (highly significant) according to F-values of the 2-way analysis of variance (ANOVA-2).

KI*= Confirmed by comparison with Kovats indices on DB5 column (Adams, 2001).

## Discussion

In this study, potassium fertilization increased the essential oil content. The results were confirmed by previous literature (Hornok, 1980; Hussien, 1995; Khalid, 1996) in which it was reported that K fertilization increased the essential oil content of plants. All potassium treatment concentrations used in this study induced low essential oil content except for the treatment of 173.2 kg ha$^{-1}$, in agreement with Economakis (1993) who indicted that high K level decreased the essential oil content of bracts and leaves of *Origanum dictamnus* L. plants.

The quality of the essential oil used in this investigation was somewhat similar to that of a French *Calendula* chemotype whose essential oil composition was rich in α-cadinol (Chalchat et al., 1991). We noticed that the changes in the chemical constituents *of C. officinalis* essential oil were confirmed by previous literature (Munnu, 2008; Al-Awak, 2010; Ezz El-Din et al., 2010). Claimed that within the essential oil , the relative level of various constituents increased, decreased, or did not change at all in mint (*Mentha* sp.), palmarosa (*Cymbopogon martinii* [roxb.] wats. var. motia burk) and caraway plants under K treatments, compared with control plants (Munnu, 2008; Mollafilabi et al., 2010). The different essential oil yield and constituents as a result of potassium treatment may be due to their effects of potassium on enzyme activity and metabolism during essential oil production by the plants (Burbott and Loomis, 1969; Machner, 2001; Silva, 2004). α-Cadinol was reported to be up to 26.38% in this French ecotype (Chalchat et al., 1991). Previously, the essential oil of French *C. officinalis* was obtained in low yield (0.3%) by steam distillation of flowers heads, and 66 components were identified by GC-MS, mainly sesquiterpene alcohols; α-cadinol was the main constituent (Chalchat et al., 1991). The essential oil concentrates of *C. officinalis* flower heads extracted by supercritical $CO_2$ was found to consist of methylhexanoate, methyllinoleate, methyl 9, 12, 15-octadecatrienoate, methyloctadecanoate, methyl-tetradecanoate, γ-cadinene and cubenol, δ-cadinene, α-cadinol and oplopanonec (Nicoletta et al., 2003). The main constituents of *C. officinalis* essential oil as detected by GC-MS and cultivated under presowing low temperature were α-cadinol, Δ-cadinene, δ-cadenene and nerolidol (Khalid and El-Ghorab, 2006). The main constituents (α-cadinol, Δ-cadinene) of essential oil extracted from whole *C. officinalis* plants and detected by GC-MS increased when organic fertilizers were applied under soil solarization conditions (Khalid et al., 2006). The main constituents of *C. officinalis* essential oil as detected by GC-MS and cultivated under saline conditions were α-cadinol, Δ-cadinene, δ-cadenene (Khalid and Teixeira da Silva, 2010).

Hornok (1980) indicated that K fertilization was effective on the essential oil content of dill (*Anethum graveolens* L.) while, Hussien (1995) reported that K fertilization was more affected on the dill essential oil constituents. Also, K fertilization increased the essential oil content of some medicinal *Apiaceae* plants (Khalid, 1996). K uptake in *Tagetes minuta* L. plants increases essential oil content (Somida, 2002). Kahlid et al (2007) indicated that fertilization with potassium affected essential oil and its constituents in rue (*Ruta graveolens* L.). The highest content of peppermint (*Mentha piperita*) essential oil production was obtained with fertilization with K at 218 mg $L^{-1}$ (Mollafilabi et al., 2010).

## Conclusion

Essential oil yield increased with the potassium level compared with control treatment. The highest accumulation of essential oil was recorded at potassium treatment of 173.2 kg $ha^{-1}$. Alcohols are the major constituents of the heavy oxygenated compounds (HOC) of *Calendula* EO with α-Cadinol as the main component representing the highest concentration among the alcohols. This indicates that *Calendula* essential oil obtained under growth under potassium belongs to the α-cadinol chemotype. The main constituents (α-cadinol, Δ-and γ -cadinene) increased with K level. Heavy oxygenated compounds were the highest in essential oil with all in plants treated with K compared to the control and other chemical classes (light oxygenated compounds, sesquiterpene and monoterpene). Alcohols were the major constituents of heavy oxygenated compounds.

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