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## Calcium Effects on Yield, Mineral Uptake and Terpene Components of Hydroponic *Chrysanthemum coronarium* L.

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**Abstract:** An experiment was conducted to study the relationship between calcium level in the hydroponic solution on *Chrysanthemum coronarium* L. yield and essential oil contents. Regression analysis showed that the maximum yield was achieved in 30.9 mM of calcium in nutrient solution for leaves ( $r = 0.970^{***}$ ) and 38.0 mM for flowers ( $r = 0.950^{***}$ ). A highly positive correlation ( $r = 0.961^{***}$ ) was found between sesquiterpene lactone and calcium in the flower part. Maximum yield of essential oil in leaf part was achieved in 39.7 mM  $\text{Ca}^{2+}$  treatment ( $r = 0.933^{***}$ ) and the correlation between essential oil and calcium contents of leaves was high ( $r = 0.967^{***}$ ). Therefore, the increase of calcium concentration in nutrient solution had positive effects on the contents and yields of effective component, terpene, from *C. coronarium* L. The content of sesquiterpene lactone increased with increase of calcium concentration in nutrient solution.

**Key words:** *Chrysanthemum coronarium* L., terpene, essential oil, calcium, mineral content

### INTRODUCTION

Bioactive terpenes, such as dihydrochrysanolide and cumambrin A isolated from *Chrysanthemum coronarium* L., were proven to have the medicinal effects on cancer prevention<sup>[1,2]</sup> and blood-pressure reduction<sup>[3]</sup>. These terpenes (mainly sesquiterpenes and monoterpenes) are synthesized from acetyl CoA via the mevalonic acid pathway<sup>[4]</sup>. The elucidation of the bioactive substances from this plant is important to increase its vegetable value. The contents of essential oil including terpenes and its composition in plant are affected by different factors, primarily by its genetics<sup>[5]</sup> and cultivation condition, such as harvesting time, climate and the use of fertilizer. Nitrogen fertilization was reported to reduce terpene content in *Juniperus horizontalis*<sup>[6]</sup>, although it increases total oil yield in Thyme<sup>[7]</sup>. Nitrogen limiting conditions increase volatile terpenes production in annual herbal plants<sup>[8]</sup>. Soil application of P and N plus P fertilizer reduced the concentrations of terpenoid lactones in *Ginkgo biloba* seedling<sup>[9]</sup>.  $\text{Mg}^{2+}$  is known as a cofactor in the biosynthesis of isoprenoid in tobacco<sup>[10]</sup>. Calcium is an essential plant nutrient which constitutes between 0.2 and 5.0% on a leaf dry weight basis<sup>[11]</sup>. In its ionic ( $\text{Ca}^{2+}$ ) form calcium plays important roles as a structural component of the cell wall and cell membranes, as a cytoplasmic

second messenger, mediating various hormonal and environmental cues that provoke appropriate physiological responses and as a counter cation for inorganic and organic anions in the vacuole<sup>[11]</sup>. Calcium deficiency disorder is usually alleviated by helping plants increase their  $\text{Ca}^{2+}$  uptake from the surrounding media and transport the mineral to the  $\text{Ca}^{2+}$ -deficient organs<sup>[11]</sup>. Increasing  $\text{Ca}^{2+}$  content in soil has shown to increase its contents in the plant<sup>[12]</sup>. It was reported that essential oils terpene contents in plant tissue of basil (*Ocimum basilicum* L.) showed a high coefficient correlation with calcium contents, being higher than with nitrogen contents<sup>[13]</sup>. However, study on agronomic factors concerning calcium fertilizations on terpene contents of *C. coronarium* L. was not investigated thoroughly till now. In this study we determined the relationship between calcium nutrient in hydroponics solution and the formation of effective components like sesquiterpene lactones and essential oils of flowerhead of *C. coronarium* L.

### MATERIALS AND METHODS

**Treatment and composition of nutrient solution:** *Chrysanthemum coronarium* L. plants were grown in a hydroponic system with a nutrient solution based on the

formulation suggested by the Applied Plant Research, Division Glasshouse Horticulture, the Netherlands<sup>[14]</sup>. Nutrient solution comprised of 2.0 mM  $\text{KH}_2\text{PO}_4$ , 19.0 mM  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 7.1 mM  $\text{KNO}_3$ , 1.3 mM  $\text{NH}_4\text{NO}_3$ , 4.0 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 2.0 mM  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  and micronutrient. Five grades of calcium ion concentrations were selected as calcium treatments; 10, 20, 30, 40, 50 and 70 mM, adjusted by using  $\text{CaCl}_2$ . The Electrical Conductivities (EC) of each solution were  $2.3 \pm 0.10$ ,  $2.2 \pm 0.08$ ,  $2.6 \pm 0.09$ ,  $2.8 \pm 0.09$ ,  $3.3 \pm 0.11$  and  $3.6 \pm 0.13$   $\text{mS cm}^{-1}$ , respectively and pHs of each solution were  $5.5 \pm 0.19$ ,  $5.5 \pm 0.20$ ,  $5.5 \pm 0.21$ ,  $5.5 \pm 0.21$ ,  $5.4 \pm 0.25$  and  $5.4 \pm 0.29$ , respectively.

**Plant preparations and cultivation:** The seeds of *C. coronarium* L. were purchased from Hungnong Seed Company in Seoul, Korea. The seeds were sown on the surface of sterilized sand saturated with deionized water. After 3 weeks, the plants with 10 cm height were transferred into treatment modified nutrient solution. On 12 February, the plant was transplanted in the container (60x40x15 cm, 18 L per pot) in the Deep Floating Technique (DFT) system. Modified nutrient solutions was circulated continuously by air pump and were grown in a greenhouse under natural light conditions, a daytime temperature of 25°C and relative humidity of 65-70%. Containers were arranged in a completely randomized block design with three replications. The nutrient solution was replaced with a fresh solution every 7 days in the early growth stage (12 February - 12 March) and every 3 days in the later growth stage (13 March - 20 April). The leaves and flowers were harvested on March 12, 2001 and April 20, 2001, respectively. At harvesting time, the plant characteristics were investigated by the RDA methods<sup>[15]</sup>.

**Inorganic element and chlorophyll contents:** Flower-heads and leaf tissues were separated after harvesting and air-dried at 70°C for 6 days. Dried materials were grounded and then digested in  $\text{H}_2\text{SO}_4$  for total nitrogen or in a ternary solution ( $\text{HNO}_3$ : $\text{H}_2\text{SO}_4$ : $\text{HClO}_4$  = 10:1:4 with volume) for the determination of P, K, Ca and Mg<sup>[16]</sup>. Chlorophyll was extracted by 80% (v/v) acetone and its contents were determined at 663 nm and 645 nm by a Hitachi U-2000 dual length spectrophotometer<sup>[17]</sup>.

**Essential oil and sesquiterpene contents:** Essential oil contents of *C. coronarium* L. were determined with Simultaneous Distillation Extraction (SDE) apparatus, using the methods described by Schultz *et al.*<sup>[18]</sup>. Cumambrin A, a major active component of *C. coronarium* L. flower heads, was analyzed by using HPLC (Waters 201, Waters, USA) after  $\text{CHCl}_3$  extraction at

room temperature for 2 days<sup>[1,19]</sup>. The operating conditions were as follows: Adsorbosphere silica 5  $\mu\text{m}$  column and Lamda-max detector; eluent of a dichloromethane: isopropanol (49:1) mixture; column temperature at 25°C; sample size of 5  $\mu\text{L}$ ; maximum absorption at 254 nm. The retention time of cumambrin A and dihydrochrysanolide was 6.59 and 13.57 min. The individual peak area was calculated using concentration curves of purified cumambrin A and dihydrochrysanolide as standards.

**Statistical analysis:** All statistical analysis was conducted by using one-way ANOVA of the Statistical Analysis System (SAS) computer package<sup>[20]</sup>. The different between means of treatments were compared by using the Duncan's multiple range tests. The effects of treatments were considered as a significant when the probability for observed *F*-value was equal to or smaller than 5%. Multiple regression analysis was conducted to determine the effect of mineral element on productivity and effective components.

## RESULTS AND DISCUSSION

Plant height was significantly affected by calcium level, increased from 79.3 cm in 10 mM calcium to 86.3 cm in 40 mM calcium level (Table 1). Stem diameter and leaf length showed similar pattern with those of plant height. Yield response showed similar tendency with growth characteristics. Dry weights of leaves and flowers of *C. coronarium* L. increased significantly with increasing calcium concentration in nutrient solution. Correlation coefficients were observed between leaf yields and calcium concentration in nutrient solution ( $y = -0.0043X^2 + 0.266X + 8.242$ ,  $r = 0.970$ ,  $p < 0.001$ ) and flower yields and calcium concentration in nutrient solution ( $y = -0.0041X^2 + 0.312X + 5.488$ ,  $r = 0.950$ ,  $p < 0.001$ ). The maximum yields were achieved in 30.9 mM of calcium in nutrient solution for leaves and 38.0 mM for flowers. Chlorophyll contents increased from 36  $\mu\text{g mL}^{-1}$  in 10 mM calcium to 48  $\mu\text{g mL}^{-1}$  in 40 mM calcium concentration in nutrient solution (Table 1). Similarly, Takano and Yamamoto<sup>[21]</sup> reported that the yield of leaf was increased significantly by increasing calcium level in basil (*Ocimum basilicum* L.).

**Mineral uptake:** The nutrient content in leaves and flowers of *C. coronarium* L. was significantly affected by calcium concentration levels in nutrient solution (Table 2). The calcium contents of leaves and flowers were increased significantly by increasing calcium concentration. However, P and Mg mineral contents of leaves and flowers in  $\text{Ca}^{2+}$  treatment of hydroponics

Table 1: Yield and growth characteristics of *C. coronarium* L. cultivated in the different calcium treatment

Ca <sup>2+</sup> (mM)	Growth characteristics (cm)			Dry weight (g plant <sup>-1</sup> ) <sup>b</sup>		Chlorophyll content (µg mL <sup>-1</sup> )		
	Plant height	Stem diameter	Leaf length	Leaf	Flower	Chl.a	Chl.b	Total
10	79.3b <sup>a</sup>	8.9bc	12.2c	10.6c	8.4d	32b	14a	46b
20	80.2b	9.1bc	12.7bc	11.7c	9.4d	34a	13a	47a
30	85.2ab	9.6b	13.9ab	12.1bc	11.5c	35a	13a	48a
40	86.3a	9.9a	14.5a	12.7a	11.8b	35a	13a	48a
50	72.3bc	9.2bc	13.7a	10.4a	10.2a	34a	12a	46b
70	64.5cd	8.7c	12.3c	5.9b	7.1b	31b	10b	41c

<sup>a</sup>Mean values in a column followed the same letter (s) did not significantly different at Duncan's Multiple Range Tests at the 5% level

<sup>b</sup>The plants were transplanted on February 12, 2001 and harvested March 12, 2001 for leaf and April 20, 2001 for flower

Table 2: Mineral composition and calcium uptake of *C. coronarium* L. grown in the different levels of calcium treatment

Ca <sup>2+</sup> (mM)	Mineral contents (g kg <sup>-1</sup> )					Ca <sup>2+</sup> uptake (mg plant <sup>-1</sup> )
	T-N	P	K	Ca	Mg	
<b>Leaf</b>						
10	88c <sup>a</sup>	5.6a	99bc	18.0e	3.5b	190e
20	89c	5.7a	105b	32.6d	3.8ab	380c
30	110a	4.8b	109a	40.3c	4.1a	489b
40	104b	4.7b	100bc	42.5b	2.5c	539a
50	103b	4.5b	92c	45.4a	2.4c	471b
70	84c	4.9b	105b	44.8ab	2.1d	263d
<b>Flower</b>						
10	126c	7.4ab	167b	7.9d	4.1a	67e
20	140ab	7.8a	174a	11.7c	3.1b	110d
30	145a	7.0b	172a	18.1ab	2.7bc	201ab
40	148a	6.8bc	170a	19.7a	2.4cd	233a
50	137b	6.6c	169ab	18.4ab	2.4cd	190b
70	127c	6.5c	168ab	17.7b	2.2d	126c

<sup>a</sup>Mean values in a column followed the same letter (s) did not significantly different at Duncan's Multiple Range Tests at the 5% level

decreased significantly with increasing calcium concentration in nutrient solution. Many other studies reported similar results that the growth of leaf, stem and root part of crops increased with increasing calcium concentration in nutrient solution and the calcium uptake of plants also showed the same tendency<sup>[22,23]</sup>. Antagonism relationships of calcium and magnesium absorption of plants in the nutrient solution have also been reported in several studies<sup>[22,24]</sup>.

**Sesquiterpene lactone:** Calcium in the hydroponic medium affected sesquiterpene lactone contents in the flower of *C. coronarium* L. and their yield (Table 3). Both cumambrin A and dihydrochrysanolide, as representatives of sesquiterpene lactone, showed a similar response to increasing calcium concentration in the nutrient solution and a 71% increase in sesquiterpene lactone was achieved when calcium in the hydroponic medium was increased from 10 to 40 mM. Regression analysis showed that maximum yields of cumambrin A and dihydrochrysanolide were achieved in 40.9 mM Ca<sup>2+</sup> treatment ( $Y = -0.0115X^2 + 0.941X + 0.439$ ,  $r = 0.935$ ,  $p < 0.001$ ). Total yields of the two compounds from the flower part increased to about 2.4 times in 40 mM Ca<sup>2+</sup> treatment, from 9.13 mg plant<sup>-1</sup> in 10 mM Ca<sup>2+</sup> treatment. Calcium

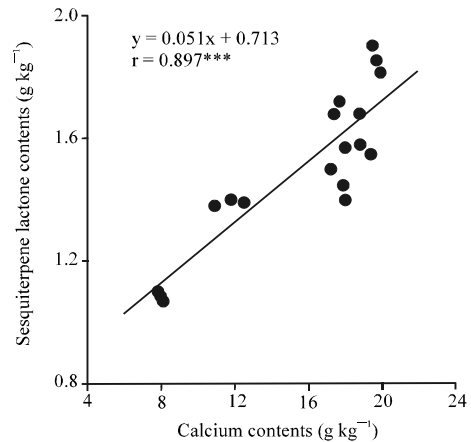


Fig. 1: Relationship between sesquiterpene lactone contents and calcium contents in the flower of *C. coronarium* L.

and sesquiterpene lactone contents in the flower part of *C. coronarium* L. have a highly positive correlation ( $r = 0.961$ ,  $p < 0.001$ ) (Fig. 1). We found similar results in *Chrysanthemum boreale* M.<sup>[25]</sup>. In *C. boreale*, cumambrin A increased to 34 and 19% by lime and fly ash application in soil and positive correlation between calcium contents and cumambrin A in the flower part of *C. boreale* M. was also found<sup>[25]</sup>. This means that relationship increases of sesquiterpene lactone cyclase and synthase by calcium were reported directly in sesquiterpene lactone productivity. The physiological role between sesquiterpene lactone and calcium is unclear till now and more study is required.

**Essential oil:** Calcium in nutrient solution increased essential oil yields in the leaves of *C. coronarium* L. to maximum 0.163 mL plant<sup>-1</sup> in 40 mM calcium, which was 2.3 times higher than in 10 mM Ca<sup>2+</sup>. However, further increase over 40 mM Ca<sup>2+</sup> in nutrient solution reduced the essential oil yield in leaves (Table 4). Regression analysis demonstrated that maximum yields of essential oil in leaf were achieved in 39.7 mM Ca<sup>2+</sup> treatment ( $Y = -0.00001X^2 + 0.0069X - 0.014$ ,  $r = 0.933$ ,

Table 3: Sesquiterpene lactone contents and yields of the flower part of *C. coronarium* L. grown in the different levels of calcium treatment

Ca <sup>2+</sup> (mM)	Sesquiterpene lactone content (g kg <sup>-1</sup> , D.W.)			Sesquiterpene lactone yield (mg plant <sup>-1</sup> , D.W.)		
	Dihydro-chrysanolide	Cumambrin A	Total	Dihydro-chrysanolide	Cumambrin A	Total
10	0.316e <sup>a</sup>	0.772c	1.088c	2.65d	6.48d	9.13d
20	0.523cd	0.869bc	1.392b	4.92bc	8.17c	13.09c
30	0.517d	1.140a	1.657ab	5.95b	13.11ab	19.06ab
40	0.667a	1.190a	1.857a	7.87a	14.04a	21.91a
50	0.605b	1.000ab	1.605ab	6.17b	10.20b	16.37b
70	0.535c	0.911b	1.446b	3.80c	6.47d	10.27cd

<sup>a</sup>Mean values in a column followed the same letter (s) did not significantly different at Duncan's Multiple Range Tests at the 5% level

Table 4: Essential oil contents and yields of the leaf part of *C. coronarium* L. grown in the different levels of calcium treatment

Ca <sup>2+</sup> (mM)	Essential oil contents (mL kg <sup>-1</sup> )	Essential oil yield (mL plant <sup>-1</sup> )
10	8.65c <sup>a</sup>	0.072d
20	11.71ab	0.111c
30	13.40a	0.154b
40	13.77a	0.163a
50	11.66ab	0.119c
70	10.73b	0.077d

<sup>a</sup>Mean values in a column followed the same letter (s) did not significantly different at Duncan's Multiple Range Tests at the 5% level

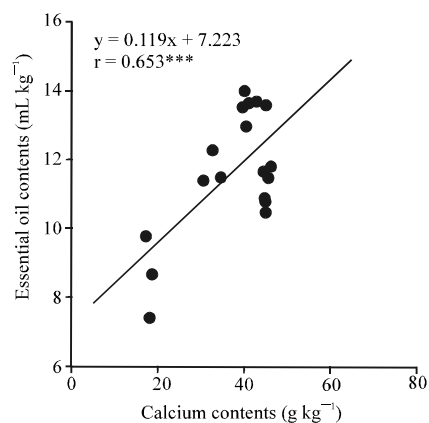


Fig. 2: Relationship between essential oil contents and calcium contents in the leaf parts of *C. coronarium* L.

$p < 0.001$ ) and a high correlation was observed between essential oil and calcium contents of leaves ( $r = 0.967$ ,  $p < 0.001$ ) (Fig. 2). Optimum fertilization could increase the yield of essential oils in the herbal plant like lemon-grass, bergamot mint and Japanese mint<sup>[26]</sup>. These results were contradictory to the results reported in basil (*Ocimum basilicum* L.) by Suh and Park<sup>[13]</sup> that increasing calcium concentration in nutrient solution remarkably decreased the essential oil yield.

This study demonstrates the importance of calcium application to improve plant growth and essential oil yield in *C. coronarium* L.

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