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Effects of Ammonium Concentration on the Yield, Mineral Content and Active Terpene Components of *Chrysanthemum Coronarium* L. in a Hydroponic System

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Abstract: *Chrysanthemum coronarium* L. is one of the most important medicinal plants cultivated in East Asia. The objective of this study was to determine the effect of ammonium ion on the yield and effective components of the plant grown in a deep floating technique (DFT) system under greenhouse conditions. Our results indicated that growth characteristics and the dry weight of leaves of *C. coronarium* L. decreased significantly with increasing ammonium concentration in nutrient solution. The maximum flowerhead yield was achieved in 1.8 mM of ammonium. Contents of sesquiterpene lactones were increased by increasing ammonium concentration, but yields were decreased by ammonium treatment. Maximum yields of sesquiterpene lactones were achieved in 3.1 mM ammonium concentration (r=0.976***). A highly negative correlation (r=-0.804***) was observed between sesquiterpene lactone contents and nitrogen content in the flower part. Essential oil yields decreased with increasing ammonium treatment and a high correlation was observed (r=0.865, p<0.001) between essential oil content and nitrogen content in leaves.

Key words: *Chrysanthemum coronarium* L., ammonium, nitrogen, essential oil, terpene

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INTRODUCTION

Most plants in the Compositae family produce significant amounts of terpenes (mainly sesquiterpenes and monoterpenes), known to have bioactivity[1]. These terpenes are synthesized from acetyl CoA via the mevalonic aicd pathway and are derived from the union of 5-carbon elements that have the branched carbon skeleton of isoprene[2]. The consumption of herbal medicine is continuously increasing throughout the world and many active components have been isolated from herbs. The leaves of *Chrysanthemum coronarium* L. are rich in minerals, and the flowerheads have a strong aromatic odor due to the presence of essential oils, primarily monoterpenoid and sequiterpenoid compounds[3]. Bioactive terpenes, such as dihydrochrysanolide and cumambrin A isolated from *C. coronarium* L., have been proven to posses beneficial medicinal effects in cancer prevention[4,5] and blood pressure reduction[6]. The elucidation of the bioactive substances from this plant is important to increase its herbal value.

The content of essential oil and its composition in a harvested herbal plant are affected by different factors, including genetic makeup [7,8] and cultivation conditions, such harvesting time, climate and the use of fertilizer. Magnesium is the main inorganic component of chlorophyll. It also reacts as a cofactor for the chloroplast ribosome (A 70S), which is responsible for the biosynthesis of large subunits of ribulose-1, 5-bisphosphate (RuBP) carboxylase and enzymes that participate in CO_2 fixation[9]. The calcium content in the plant tissue of basil has a higher correlation with the essential oil terpene concentration than with nitrogen content[10]. Soil application of only phosphate and nitrogen plus phosphate fertilizer has reduced the concentrations of terpenoid lactones in *Ginkgo* seedling[11]. Nitrogen limiting conditions increase volatile terpene production in annual herbal plants[12,13]. Nitrogen fertilization has been reported to reduce terpene content in *Juniperus horizontalis*[14]; although it has been reported to increase total oil yield in Thyme[15]. However, studies on agronomic factors such as the ammonium amount in nitrogen fertilizers on terpene content of *C.*

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coronarium L. have not been investigated thoroughly until now.

The objective of this study was designed to determine the effect of ammonium concentration on the yield, mineral content and active terpene components of *C. coronarium* L. cultivated in hydroponic solutions.

MATERIALS AND METHODS

Treatment and composition of nutrient solution: Plants of *Chrysanthemum coronarium* L. 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[16]. The composition of the nutrient solution is indicated in Table 1. Four grades of ammonium ion concentrations were selected as NH_4^+ treatments; 0, 2.5, 5.0 and 7.5 mM. Here, electrical conductivities (EC) of each solution were 1.3±0.03, 1.9±0.05, 1.6±0.04 and 2.1±0.07 mS cm^{-1}, respectively, and the pHs of each solution were 6.9±0.12, 5.5±0.18, 5.2±0.14 and 4.9±0.11, respectively.

Plant preparations and cultivation: This study was carried out in the greenhouse of Kyeongsang National University. The seeds of *C. coronarium* L. were purchased from the Hungnong Seed Company in Seoul, Korea. The seeds were sown on the surface of sterilized sand saturated with deionized water. After 3 weeks, plants that were 10 cm in height were transferred into the modified nutrient solution treatment. On February 12, the plants were transplanted in the container (60x 40x 15 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, at a daytime temperature of 25°C and a 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 harvest the plant characteristics were investigated by the RDA methods[17].

Inorganic element and chlorophyll content: Leaf and flowerhead tissues were separated after harvesting and air-dried at 70°C for 6 days. Dried materials were ground and then digested in H_2SO_4 for total nitrogen or in a ternary solution (HNO_3:H_2SO_4:$HClO_4$= 10:1:4 with volume) for the determination of P, K, Ca and Mg[18]. 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[19].

Sesquiterpene content and essential oil: Cumambrin A, a major active component of *C. coronarium* L. flowerheads, was analyzed by HPLC (Waters 201, Waters, USA) after $CHCl_3$ extraction at room temperature for 2 days[4,20]. The operating conditions were as follows: Adsorbosphere silica 5 µm column and Lamda-max detector; eluent of a dichloromethane: isopropanol (49:1) mixture; column temperature at 25°C; sample size of 5 µl: maximum absorption at 254 nm. The retention times of cumambrin A and dihydrochrysanolide were 6.59 and 13.57 min, respectively. The essential oil content of *C.coronarium* L. was determined with a simultaneous distillation extraction (SDE) apparatus, using the methods by Schultz *et al* [21].

Statistical analysis: All statistical analysis was conducted by using a one-way ANOVA of the Statistical Analysis System (SAS) computer package[22]. The means of the different treatments were compared by using the Duncan's multiple range tests. The effects of treatments were considered as 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 DISCUSSIONS

Growth and yield characteristics: Growth characteristics and dry weight of leaves and flowerheads of *C. coronarium* L. decreased significantly with increasing ammonium concentration in nutrient solution (Table 2). 0 mM ammonium treatment, which treated only nitrate as nitrogen source, showed the best results in plant growth and yield. Yields of the leaf and flowerhead in 5.0 ammonium treatment decreased 54% and 27% compared to the 0 mM treatment, respectively. The statistical analysis showed high correlation coefficients between leaf yield and ammonium concentration ($y=-0.254X^2+0.934X+11.288$, $r=0.995$, $p<0.001$), and flowerheads and ammonium concentration ($y=-0.016X^2-1.092X+12.714$, $r=0.963$, $p<0.001$) in the nutrient solution (Table 2). It appears that the maximum *C. coronarium* L. flowerhead yield achieved in 1.8 mM of ammonium level. The optimum concentration for the leaf yield was not established. The chlorophyll content increased from 36.8 µg ml^{-1} in 0 mM calcium to 48 µg ml^{-1} in 5 mM calcium concentration in nutrient solution. The mineral content in leaves and flowerheads of *C. coronarium* L. was largely affected by ammonium levels

Table 1: Composition of the nutrient solution in the ammonium treatments

Reagent	Ammonium (mM)			
	0	2.5	5.0	7.5
NH_4NO_3	-	2.5	0.25	2.5
$(NH_4)_2SO_4$	-	-	4.75	5.0
$Ca(NO_3)_24H_2O$	9.5	2.0	9.5	-
KNO_3	4.0	4.0	-	-
KH_2PO_4	1.0	1.0	1.0	1.0
$MgSO_47H_2O$	3.0	3.0	3.0	3.0
KCl	-	-	2.0	-
$CaCl_22H_2O$	-	7.5	-	7.5
K_2SO_4	-	-	4.0	8.0
$NaNO_3$	1.15	-	-	-

Micronutrient[a] Fe-EDTA, H_3BO_3, $MnSO_44H_2O$, $Na_2MoO_42H_2O$, $ZnSO_47H_2O$

[a]Micronutrients in this experiment were based on the formulation suggested by the Applied Plant Research, Division Glasshouse Horticulture, the Netherlands[16].

Table 2: Yield and growth characteristics of *C. coronarium* L. in the different ammonium treatments

NH_4^+(mM)	Growth characteristics (cm)			Dry weight (g plant^{-1})^b		Chlorophyll (ug ml^{-1})		
	Plant height	Stem diameter	Leaf length	Leaf	Flower	Chl.a	Chl.b	Total
0.0	74.0a^a	9.6a	14.7a	12.3a	11.1b	23.5b	13.3c	36.8b
2.5	73.6a	9.1a	14.0a	11.1b	12.3a	26.4b	14.3b	40.7ab
5.0	69.7b	8.0b	12.7b	5.6c	8.1c	32.3a	15.3a	47.6a
7.5	45.3c	6.6c	12.2b	4.0d	2.0d	24.0b	13.8c	37.8b

[a]Mean values within ammonium concentration followed by the same letter are not significantly different according to the Duncan's multiple range test at the 5% level

[b]The plants were transplanted on February 12, 2001 and harvested March 12, 2001 for leaf yield and April 20, 2001 for flower yield

Table 3: Mineral composition and N uptake of *C. coronarium* L. cultivated hydroponically at different ammonium levels

NH_4^+(mM)		Mineral content (g kg^{-1})					N uptake (mg plant^{-1})
		T-N	P	K	Ca	Mg	
Leaf	0.0	84c^a	4.3c	95.2bc	24.9b	3.2a	1037b
	2.5	120b	4.7c	87.9c	36.3a	2.7b	1333a
	5.0	141ab	6.3b	99.2b	24.5b	2.6b	797c
	7.5	149a	8.7a	117.4a	23.0b	2.3c	593d
Flower	0.0	137a	7.2a	131.2c	7.4b	3.7a	1520a
	2.5	116c	6.8b	134.3b	14.8a	2.8b	1428b
	5.0	123b	6.6b	136.0ab	13.8a	2.8b	997c
	7.5	125bc	6.5b	139.5a	8.8b	2.3c	246d

[a]Mean values within ammonium concentration followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% level

in the nutrient solution (Table 3). Several cations in the nutrient solution were inhibited by the increasing ammonium concentration. The Mg content was decreased significantly in the leaf and the flowerhead. The nitrogen uptake in the leaf and the flowerhead decreased significantly with increasing ammonium concentration in the nutrient solution.

Table 4: Sesquiterpene lactone content and yields of the flowerhead of *C. coronarium* L. cultivated hydroponically at different ammonium levels

NH_4^+(mM)	Sesquiterpene lactone content (g kg^{-1}, D.W.)			Sesquiterpene lactone yield (mg $plant^{-1}$, D.W.)		
	Dihydro-chrysanolide	Cumambrin A	Total	Dihydro-chrysanolide	Cumambrin A	Total
0.0	0.440c[a]	0.878c	1.318c	4.88b	9.75ab	14.63b
2.5	0.625b	0.903b	1.528b	7.69a	11.11a	18.79a
5.0	0.725ab	1.069a	1.792ab	5.86ab	8.66b	14.52b
7.5	0.798a	1.086a	1.884a	1.60c	2.17c	3.77c

[a]Mean values within ammonium concentration followed by the same letter are not significantly different according to Duncan's multiple range tests at the 5% level

Table 5: Essential oil content and yields of the leaf part of *C. coronarium* L. cultivated hydroponically at different ammonium levels

NH_4^+(mM)	Essential oil content (ml kg^{-1})	Essential oil yield (ml $plant^{-1}$)
0.0	14.8b[a]	0.182a
2.5	15.1b	0.168b
5.0	16.1a	0.090c
7.5	15.8a	0.064d

[a]Mean values within ammonium concentration followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% level

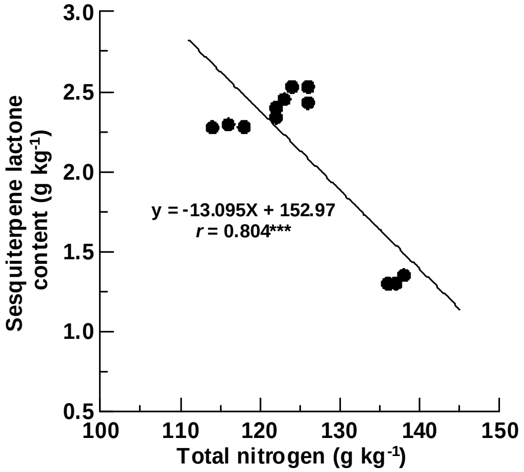

Fig. 1: Relationship between yield of sesquiterpene lactone and total nitrogen in the flowerhead of *C. coronarium* L.

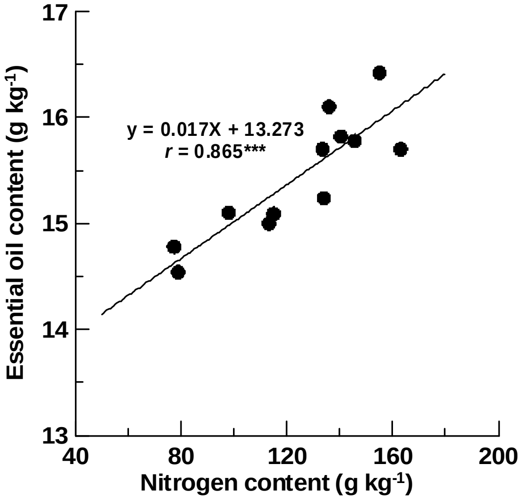

Fig. 2: Relationship between essential oil content and nitrogen content in the leaf parts of *C. coronarium* L.

Sesquiterpene lactone: The effect of ammonium on sesquiterpene lactone production in *C. coronarium* L. is presented in Table 4. The concentration of cumambrin A and dihydrochrysanolide in the flowerhead of *C. coronarium* L. were affected significantly by the ammonium concentration in the nutrient solution. There was a highly negative correlation (r=-0.804, p<0.001) between the sesquiterpene lactone concentration and nitrogen content in the flowerhead of *C. coronarium* L. (Fig. 1). The concentrations of the two compounds were increased significantly with increasing ammonium concentration in the nutrient solution, but the dry weight yield was decreased with increasing ammonium in the nutrient solution. Maximum yields of the two compounds were achieved in the 3.1 mM ammonium treatment($Y=-1.120X^2+6.886X+15.451, r=0.976, p<0.001$). Yields of the two compounds in the flowerhead increased to about 89% from 1.32 g kg^{-1} in 0.0 mM ammonium treatment to 2.50 g kg^{-1} in 7.5 mM ammonium treatment.

Essential oil: Essential oil yields were decreased significantly in the 0.0 mM ammonium treatment (Table 5).

Regression analysis showed that the maximum yield of essential oil was not established in the ammonium treatment on the leaf, but a high correlation was observed (r=0.865, p<0.001) between essential oil concentration and nitrogen content in the leaves (Fig. 2). This means that sesquiterpene lactone cyclase and synthase was increased by ammonium. Alder et al.[23] reported that the ammonium ion in sweet basil (*Ocimum basilicum* L.) decreased the formation of essential oils by 28% compared to the nitrate ion in the hydroponic system and that it could contribute to increased sesquiterpene content.

This study demonstrates the importance of ammonium application to improve plant growth and essential oil yield in *C. coronarium* L. However, to maximize sesquiterpene lactone yield, especially cumambrin A and dihydrochrysanolide, 3.1 mM rates of ammonium should be applied. We recommend that integrated ammonium management can improve the yield and medicinal quality of *C. coronarium* L. in a hydroponic system.

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