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Role of Calcium in Yield and Medicinal Quality of *Chrysanthemum coronarium* L.

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Abstract: A greenhouse experiment was conducted to investigate the effect of calcium fertilizer on yields and effective components of *Chrysanthemum. coronarium* L. Dried soil fertilized with N-P_2O_5-K_2O was packed into a Wagner pot. Calcium was evaluated by using slaked lime. Predicted maximum of leaf yield was achieved at 2.2 t ha^{-1} lime and of flower yield at 2.2 t ha^{-1}. The dry weights of leaf and flower increased significantly with increasing lime up to 2.0 t ha^{-1} lime. Increasing lime application rate correlated positively with flower sesquiterpene lactone contents and yields, with predicted maximum yields achieved at 2.2 t ha^{-1}. Predicted maximum yield of leaf essential oil was obtained, but correlation between essential oil content and calcium content in the leaves was not found. In conclusion, calcium could increase yields and medicinal quality of *C. coronarium* L. and an integrated calcium management with application rate of 2.0-2.5 ton lime ha^{-1} is suggested.

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

INTRODUCTION

Chrysanthemum coronarium L. contains high iron, potassium, calcium and dietary fiber[1] and has been used to treat cardio vascular diseases[2] and as strong antioxitant[3]. The elucidation of the bioactive substances from this plant has been important to increase medicinal value of cultivated *C. coronarium* L. Cumambrin A and dihydrochrysanolide are commercial products from flower head of *C. coronarium* L. which have high medicinal value for blood-pressure reduction[4,5] and anticancer activity[6]. Most members of Compositae family contain a range of terpenes (mainly sesquiterpenes and monoterpenes), known for their bioactivity[7]. Plant terpenes are synthesized from acetyl CoA via the mevalonic acid pathway and are derived from the union of 5-carbon elements that have the branched carbon skeleton of isoprene[8].

Most studies of *C. coronarium* L. have been focused on the analysis of medicinally effective components, whereas the production aspects have been largely neglected. Best agronomic management practice through the application of fertilizer should get a priority for evaluation to produce the high yield of *C. coronarium* L. with high content of effective compounds. In general, it has been known that soil application of P and N+P fertilizer reduced the concentrations of terpenoid lactones in *Ginkgo biloba* seedling[9]. Phosphate is one of the important ions on phosphorylation pathways to produce essential oils in plant. Magnesium is known as a cofactor in the biosynthesis of isoprenoid in tabacco[10]. *C. coronarium* L. contains high calcium in the leaf tissue as compared with general vegetables. However, information on the role of calcium on terpene biosynthesis is not known. Suh and Park[11] found that calcium content has the higher correlation with essential oils in plant tissue of basil (*Ocimum basilicum* L.) than nitrogen content. We found similar results in *Chrysanthemum boreale* M.[12]. For this reason, calcium (Ca^{2+}) should have a role in terpene biosynthesis in plants. In this study, we determined the optimum levels of calcium application with lime to obtain maximum yield and effective compounds of *C. coronarium* L. plants cultivated in pots. The relationship between the calcium content and the concentration of effective components, like sesquiterpene lactones and essential oils, are also determined.

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MATERIALS AND METHODS

To determine the effects of calcium on yields, seeds of *C. coronarium* L. were sown in trays containing perlite:vermiculite (1:1, v/v) in a greenhouse under natural light conditions, a daytime temperature of 25°C and relative humidity of 65-70%. Seedlings with 10 cm height were transplanted into soil-containing Wagner pots (1/2,000 a size), one plant per pot. The soil is characterized as silt loam (17% clay, 56% silt and 27% sand), containing 4.8 g kg^{-1} OM, 4.0 g kg^{-1} available P_2O_5, 2.0 cmol (+) kg^{-1} exchangeable calcium and pH 5.0. About 16 kg of dried soil was packed into a pot and slaked lime (alkalinity: 60%) was applied at rate of 0, 1, 2, 3, 4 or 5 t ha^{-1}. The application rate of chemical fertilizer was as follows: Basal fertilizer, 105 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, 56 kg K_2O ha^{-1} was applied and 30% of the required nitrogen and potassium were side-dressed at approximately 20 days before the flowering. Pots were arranged in a completely randomized block design with four replications. Seedling of *C. coronarium* L. was transplanted on April 12, 2001 and harvested on July 12, 2001 at the full bloom stage. Flower heads and leaves were separated and air-dried at room temperature over 10 days. Dried materials were sampled for analyzing chemical characteristics. The growth and yield characteristics were measured by using RDA methods[13].

Soil samples were collected before and after the experiment and air-dried for chemical analysis. Soil samples were sieved (<2 mm) and analyzed for the following: pH and EC (1:5 water extraction), organic matter content[14], available P content[15] and contents of exchangeable Ca^{2+}, Mg^{2+} and K^+ (1 M NH_4-acetate pH 7, AA, Shimazu 660). Flower and leaf tissues were separated after harvesting and air-dried at 70°C for 6 days. Dried materials were grounded 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.

Essential oil contents of *C. coronarium* L. were determined with Simultaneous Distillation Extraction (SDE) apparatus, using the methods by Schultz *et al.*[16]. Cumambrin A, a major active component of *C. coronarium* L. Flower heads, was analyzed by using HPLC (Waters 201, Waters, USA) after $CHCl_3$ extraction at room temperature for 2 days [6,17]. 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 25É; sample size of 5 µL: maximum absorption at 254 nm. The retention time of cumambrin A and dihydrochrysanolide was 6.59 and 13.57 min, respectively. The individual peak areas were calculated using concentration curves of purified cumambrin A and dihydrochrysanolide as standards.

Yields and effective component contents were analyzed statistically by analysis of variance using the Statistical Analysis System (SAS) computer package[18]. When analysis of variance showed a significant treatment effect ($p<0.05$) on plant growth, productivity or effective component variables, the Least Significant Difference (LSD), at a 0.05 level of significance[19] was used to compare treatment means

RESULTS AND DISCUSSION

Dry matter yield of leaf and flower increased with increasing lime level up to 2 t ha^{-1} (Table 1), further lime increase decreased the yields, although at highest rate (5.0 t ha^{-1} lime) the plants did not show any symptom of calcium toxicity. In Table 1 plant dry weight increased from 40.5 g $plant^{-1}$ in non-treated control to 64.3 g $plant^{-1}$ in 2.0 t ha^{-1} lime treatment. Predicted maximum yield was achieved in 2.2 ton lime ha^{-1} for leaf weight ($Y = -3.474X^2+15.410X+34.645$, $r = 0.954$, $p<0.001$) and in 2.2 ton ha^{-1} for flower weight ($Y = -0.404X^2+1.836X+5.589$, $r = 0.982$, $p<0.001$). Plant height showed similar tendency to yield response; it increased from 101 cm in non treated control to 113 cm in 2.0 t ha^{-1} lime treatment. Stem diameter and the number of branch showed similar pattern with plant height.

Calcium levels in soil affected the nutrient content in leaves and flowers. Calcium content of flowers and leaves of *C. coronarium* L. increased with increasing lime application rate and so did the total amount of calcium per plant (Table 2). Calcium content increased from 130 mg $plant^{-1}$ in control to 1,659 mg $plant^{-1}$ in 3 t ha^{-1} lime treatment. Similar results were reported in many other studies. In tomato and in radish, it was reported that the growth of leaf, stem and root parts increased with increasing calcium concentration in nutrient solution and the calcium uptake of plants also showed the same tendency[20,21].

The effect of the lime application rate on sesquiterpene lactone production, in this case cumambrin A and dihydrochrysanolide, is presented in Table 3. Their yields in the flower of *C. coronarium* L. were affected positively by increasing lime application rate ($Y = -0.9726X^2+4.3482X+7.9414$, $r = 0.986$, $p<0.001$) and the predicted maximum yields were achieved at 2.2 ton lime ha^{-1}. Total content of these two compounds in flower increased from 1.36 g kg^{-1} in control to 1.69 g kg^{-1} in 2 t ha^{-1} lime treatment. A positive correlation existed between sesquiterpene lactone

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

	Growth characteristics			Dried weight (g plant^{-1})		
Lime (t ha^{-1})	Plant height (cm)	Stem diameter (cm)	Branch (No. plant^{-1})	Leaf	Flower	Total
0	101.20	0.96	16.50	34.80	5.65	40.45
1	106.20	1.11	20.80	43.20	6.99	50.19
2	113.50	1.09	24.40	56.90	7.44	64.34
3	103.80	0.98	21.60	45.80	7.68	53.48
5	85.50	0.72	9.20	25.40	4.61	30.01
$LSD_{0.05}$	6.91	0.13	1.85	3.20	1.86	3.78

Table 2: Mineral content and calcium uptake of *Chrysanthemum coronarium* L. cultivated in the different levels of calcium

	Mineral content (g kg^{-1})					
Lime (t ha^{-1})	T-N	P	K	Ca	Mg	Ca uptake (mg plant^{-1})
Leaf						
0	28.00	1.30	40.60	14.60	9.80	406.0
1	32.30	1.50	41.40	21.40	8.70	924.0
2	35.80	1.50	45.10	24.60	7.60	1402.0
3	36.50	1.40	51.00	36.20	6.80	1659.0
5	36.90	1.30	43.50	37.10	4.10	944.0
$LSD_{0.05}$	0.18	0.07	0.36	0.85	0.06	20.3
Flower						
0	42.00	2.40	34.70	11.10	4.50	63.0
1	48.60	2.30	34.90	16.70	4.30	117.0
2	49.90	2.50	35.10	17.50	3.20	130.0
3	49.80	2.30	39.00	22.40	3.20	172.0
5	49.70	2.20	35.50	35.50	2.00	164.0
$LSD_{0.05}$	0.36	0.17	0.61	0.25	0.23	8.8

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

	Sesquiterpene lactone content (g kg^{-1}, DW)			Sesquiterpene lactone yield (mg plant^{-1}, DW)		
Lime (t ha^{-1})	Dihydro-chrysanolide	Cumambrin A	Total	Dihydro-chrysanolide	Cumambrin A	Total
0	0.500	0.862	1.362	2.83	4.87	7.70
1	0.715	0.981	1.696	5.00	6.85	11.85
2	0.728	0.966	1.694	5.42	7.19	12.61
3	0.673	0.893	1.566	5.17	6.86	12.03
5	0.543	0.635	1.178	2.50	2.93	5.43
$LSD_{0.05}$	0.04	0.03	0.10	0.08	0.10	0.49

Retention time: cumambrin A (6.59 min) and dihydrochrysanolide (13.57 min)

Table 4: Essential oil content and yields of the leaf part of *Chrysanthemum coronarium* L. cultivated in the different levels of calcium

Lime (t ha^{-1})	Essential oil content (mL kg^{-1})	Essential oil yield (mL plant^{-1})
0	16.54	0.577
1	18.13	0.802
2	18.45	1.051
3	16.98	0.778
5	14.69	0.374
$LSD_{0.05}$	0.87	0.040

contents and calcium contents in the flower (r = 0.604, $p<0.05$) (Fig. 1). We found similar results in *C. boreale* M.[12], in which cumambrin A increased to 34 and 19% by lime and fly ash applications, respectively, in soil and positive correlation between calcium content and cumambrin A in the flower part of *C. boreale* M. was also found[12]. The biosynthetic pathway of sesquiterpene and sesquiterpene lactone biosynthesis in plant has not been determined. Although this study demonstrates clear effect of calcium on the production of these compounds, its role in their pathways is not known, especially because the biological reactions vary depending on plant species, growth stages and environmental conditions.

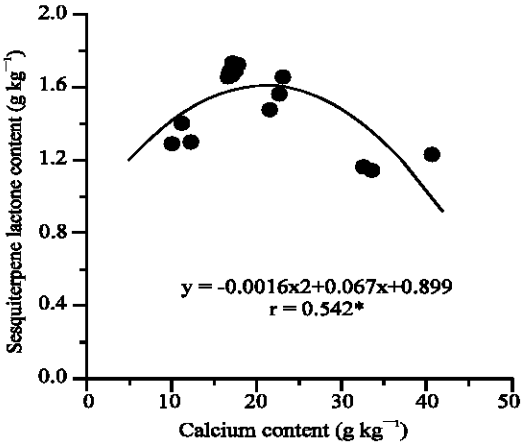

Fig. 1: Relationship between sesquiterpene lactone and calcium content in flowers

Table 5: Chemical properties of soil with the different levels of calcium after harvesting of *Chrysanthemum coronarium* L

						Ex.Cations^c(cmol(+)kg^{-1})		
Lime (t ha^{-1})	pH (1:5, H_2O)	EC^b (dS m^{-1})	OM^a (g kg^{-1})	T-N (g kg^{-1})	Avail. P_2O_5(mg kg^{-1})	K	Ca	Mg
0	4.8	0.53	5.3	0.7	5.0	0.32	0.9	0.52
1	4.9	0.88	5.4	0.7	5.0	0.32	2.1	0.59
2	5.1	1.15	5.6	0.6	4.9	0.26	3.1	0.60
3	6.3	1.36	6.1	0.6	4.9	0.26	5.1	0.61
5	7.8	1.57	6.4	0.5	5.0	0.32	7.5	0.64
LSD$_{0.05}$	0.5	0.15	0.4	0.12	0.7	0.05	1.3	0.07

^{a}OM: Organic Matter, ^{b}EC: Electrical Conductivity, ^{c}Ex. Cations: Exchangeable Cations

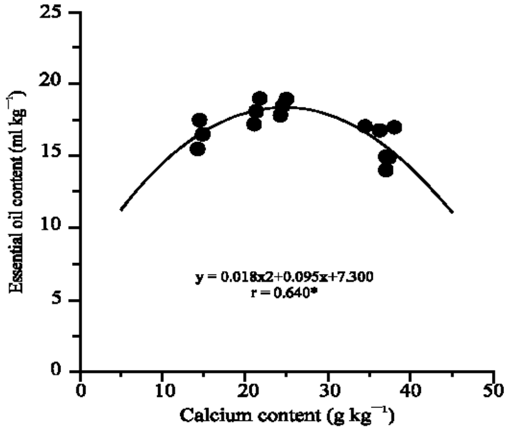

Fig. 2: Relationship between essential oil content and calcium content in leaves

Essential oil content and yield, which included sesquiterpene compounds, in the leaves of *C. coronarium* L. increased with increasing lime up o 2 t ha^{-1} and further increase in liming reduced both essential oil content and yield (Table 4). Predicted maximum yield of essential oil in the leaf was achieved in 2.2 t ha^{-1} lime treatment ($Y = -0.72X^2+0.316X+0.586$, $r = 0.937$, $p<0.001$). Essential oil content and calcium content in leaves of *C. coronarium* L showed quadratic relation ($Y = -0.0016x^2 + 0.0671x + 0.899$, $r = 0.542$, $p<0.05$) (Fig. 2). Suh and Park[11] reported in basil that although increasing calcium concentration in nutrient solution remarkably decreased the essential oil yield in basil but increased the content of terpenes. Present result demonstrated that both essential oil yield and sesquiterpene lactone content have quadratic relation with lime concentration. Similarly, quadratic relation also occurs between calcium concentration in flowerhead and sesquiterpene lactone content and between calcium concentration in leaf and essential oil content. These confirm that *C. conorarium* L. is a non calcicole plant, in which high calcium content has deleterious effect in essential oil production.

Lime application is the most widely used material to correct soil acidity. In our experiment, lime application up to 5.0 t ha^{-1} of slaked lime increased soil pH from 4.8 in non-limed control to 7.8 (Table 5). Exchangeable calcium also increased up to 7.5 cmol (+) kg^{-1} in lime treatments from 0.9 cmol (+) kg^{-1} in the control. Apparent reduction in exchangeable calcium in soil at harvest occurred in control as compared to those before transplanting. Since soil was collected from sterile mountainous area, nutrient contents of soil before transplanting were very low and scarcely changed after harvesting.

In conclusion, greenhouse experiment in this study demonstrated that to produce the high yields and high medicinal quality of *C. coronarium* L., even where soil pH is pH 4.8, liming is needed. This is to improve, in the leaves and Flower heads, the calcium concentration which positively correlated to sesquiterpene lactone content. The optimum calcium application rate might be on the range of 2.0-2.5 ton lime ha^{-1} and higher rate can detrimental to the quality of harvested materials. We suggest that integrated calcium management can improve the yield and medicinal quality of *C. coronarium* L. in field conditions.

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