Fertilizer effect on the yield and terpene components from the flowerheads of Chrysanthemum boreale M. (Compositae)

Kyung Dong Lee, Min Suk Yang, Non Renseigné, Donald L. Smith

Agron. Sustain. Dev. 25 (2005) 205–211
© INRA, EDP Sciences, 2005
DOI: 10.1051/agro:2005022

Research article

Fertilizer effect on the yield and terpene components from the flowerheads of *Chrysanthemum boreale* M. (Compositae)

Kyung Dong LEE[a], Min Suk YANG[b,c]*, Supanjani[a], Donald L. SMITH[a]

[a] Department of Plant Science, Macdonald Campus, McGill University, 21111 Lakeshore Road, Ste-Anne-de-Bellevue, QC, Canada H9X 3V9
[b] Department of Agricultural Chemistry, Division of Applied Life Science, Gyeongsang National University, 900 Kaswa-dong, Chinju, 660-701, Korea
[c] Institute of Agriculture and Life Sciences, Gyeongsang National University, Chinju, 660-701, Korea

(Accepted 12 January 2005)

Abstract – *Chrysanthemum boreale* M. is an important medicinal plant that has been historically used in natural medicine and in the food industry throughout East Asia. Most flowerheads used for food are taken from the wild. However, natural sources are limited and there is not enough to meet current demand. To fulfill current and future increasing demand, cultivation systems that produce a greater amount of flowerheads with good quality and yield are required. A field experiment was conducted during the growing seasons of 2000 and 2001 to determine the effects of fertilization treatments [without fertilizer (WF), NPK fertilizer (F), NPK plus lime (FL) and NPK plus swine manure (FS)] on flowerhead yield and the content of essential oils. Fertilizer application increased both flowerheads and essential oil yields in both growing seasons. In addition, the contents of terpene, monoterpenoids and sesquiterpenoids were improved only in FL as compared with WF or F treatments. Yield ha^{-1} of cumambrin A, a sesquiterpene compound exhibiting blood-pressure regulating activity, was increased by all fertilization treatments, but its concentration in the flowerheads was only increased by FL treatment. Cumambrin A and calcium contents were correlated in flower parts of *C. boreale* M. This suggests adding calcium could increase the yields and quality of *C. boreale* M. In general, the correct fertility regime could increase both flowerhead production and concentration of health-promoting substances.

***Chrysanthemum boreale* M. / fertilizer / terpene / essential oil**

1. INTRODUCTION

In recent years, the demand of *Chrysanthemum boreale* Makino for natural medicine has increased steadily with the growing desire for health-improving foods in East Asia. However, the amount of commercially available *C. boreale* M. is not sufficient to satisfy demand, because most of the plants are taken from the wild in mountainous areas. Harvesting of *C. boreale* M. from natural resources is becoming more difficult, especially with growing concern about environmental protection. Therefore, the future demands of *C. boreale* M. will have to be filled primarily from harvest of cultivated plants.

C. boreale M., a member of the family Compositae, is a perennial aromatic shrub with yellow flowers, which grows to 1.0–1.5 m (Choi, 1992). Flowerheads of the plant have been used as a natural medicine for sedation of the central nervous system, as a purgative for intestinal bacteria, and for control of headaches and dizziness, since ancient times (Lee, 1985; Nicolaus, 1991; Choi, 1992). Nam and Yang (1995a, b) reported antibacterial activity and cytotoxic activity of the extract from flowerheads of wild *C. boreale* M. In particular, they found that cumambrin A isolated from wild *C. boreale* M. has clear effects on blood-pressure reduction, and is used as an industrial product (Yang et al., 1996; Hong et al., 1999). Most members of the family Compositae contain high levels of terpenes (mainly sesquiterpenes and monoterpenes), known to have bioactivity (Picmam, 1986). These terpenes are synthesized from acetyl CoA via the mevalonic acid pathway and are derived from the combination of 5-carbon elements that have the branched carbon skeleton of isoprene (Chappell, 1995).

Until recently, most studies of *C. boreale* M. were focused on analysis of components effective as natural medicines. Studies of agronomic factors affecting flowerhead yields and effective components of *C. boreale* M. are needed in order to make cultivation profitable. Among various agronomic practices, plant population and fertilization are important factors for securing maximum yield and quality. The terpene response to fertilization has been attributed to terpene production being under strong genetic control (Merk et al., 1998; Muzika et al., 1989). Terpene content decreases with increased nitrogen fertilizer application in *Juniperus horizontalis* (Fretz, 1976). In addition, Mihaliak and Linclon (1985) and Waring et al. (1985) found that nitrogen-limiting conditions increase production of volatile terpenes in an annual plant. Hasler and Meier (1993), as well as Son et al. (1998), reported that P and N plus P fertilizer applications reduced the concentrations of terpenoid lactones in

* Corresponding author: Min Suk Yang

Ginkgo biloba seedlings. Magnesium is known as a cofactor in the biosynthesis of isoprenoids, which are related to terpene synthesis, in tabacco (Facchini and Chappell, 1992). Calcium content in plant tissues of basil (*O. basilicum* L.) is more highly correlated with essential oil contents than nitrogen content (Suh and Park, 2000), but the role of calcium in this biochemical pathway is not clear. Therefore, the terpene concentration and composition responses to changes in fertility are likely to vary among plant species, growth stages and environmental conditions. Although efforts to improve terpene production by fertilization have been reported in other species, there is no report in *Chrysanthemum* spp. such as *C. boreale* M. In this paper, we report the effects of fertilizer on yields and effective components, such as sesquiterpene lactones and essential oils, in view of developing efficient cultivation methods of flowerheads of *C. boreale* M.

2. MATERIALS AND METHODS

2.1. Field experiments

A field experiment was conducted during each of the two growing seasons of 2000 and 2001 at the Gyeongsang National University, Kaswa-dong, Chinju, South Korea (35° 12' N, 128° 07' E; Elevation 120 m). The soil texture was a silt loam with 3.6 $g \cdot kg^{-1}$ organic matter (OM), 2.0 $cmol(+) \cdot kg^{-1}$ exchangeable calcium, and a pH of 5.2. Combinations of fertilizer treatments included a control without any fertilizer addition (WF), a control with NPK fertilizers (F), NPK plus lime (FL), and NPK plus swine manure (FS). NPK fertilizers were applied at the same level ($N\text{-}P_2O_5\text{-}K_2O$ = 150-80-80 kg ha^{-1}, in the form of urea, superphosphate and muriate of potash) in all treatments except WF treatments. Phosphate was applied as basal fertilizer; nitrogen and potassium were split, 70% as basal and 30% applied approximately 20 days prior to flowering. Lime was applied at 2 $ton \cdot ha^{-1}$, whereas swine manure was applied at 120 $ton \cdot ha^{-1}$ (Kowald et al., 1982). Analyses showed that the swine manure contained 424 g kg^{-1} OM, 13.5 g kg^{-1} N, 21.5 g kg^{-1} P and pH 6.0. The fertilizers were spread evenly on the field one week before seedlings were transplanted into the field. For each year, the soil content of organic matter, nitrogen and exchangeable calcium were determined before planting, the average being 4.2 g kg^{-1}, 0.4 g kg^{-1} and 2.7 cmol(+) kg^{-1} in 2001 and 6.5 g kg^{-1}, 1.0 g kg^{-1} and 3.6 cmol(+) kg^{-1} in 2002, respectively. Plots were arranged in a randomized complete block design with three blocks. Each plot was 6.0 by 5.4 m consisting of six rows of plants, with a spacing of 90 cm between rows and 30 cm within the rows. The space between adjacent blocks was 2.0 m and the space between plots was 1.0 m. Seedlings of *C. boreale* M. were transplanted on May 20, 2000 and on May 18, 2001. Plots were hand-weeded as needed. The flowerhead yields were determined at the full bloom stage (on October 24, 2000 and on October 20, 2001) and the flowerheads were air-dried at room temperature over 10 days for cumambrin A analysis. The fresh flowerheads were picked before the full bloom stage (October 10–20) and put immediately into a deep-freeze (–70 °C) for essential oil determination. The yield characteristics were investigated by RDA methods (RDA, 1995).

2.2. Analysis of inorganic chemicals

Soil samples were collected before and after the experiment and air-dried for chemical analysis. Soil samples were sieved (2-mm screen) and analyzed for the following: pH and EC (1:5 water extraction), organic matter content (Wakley and Black method; Allison, 1965), available P content (Lancast; RDA, 1988) and contents of exchangeable Ca^{2+}, Mg^{2+}, and K^+ (1 M NH_4-acetate pH 7, Atomic Absorption, Shimazu, 660). Flower, stem and leaf tissues were separated after harvesting and air-dried at 60 °C for 72 h. 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 and Ca.

2.3. Essential oils

Essential oil contents of *C. boreale* M. flowerheads were determined with simultaneous distillation extraction (SDE) apparatus, using a modification of the methods by Schultz et al. (1977) and Pino et al. (2001). Briefly, diethyl ether extracts were separated with a Supelcowax 10 fused silica capillary column (length 60 m × inside diameter 0.32 mm × film thickness 0.25 μm) on a Hewlett-Packard 5880 gas chromatograph equipped with a FID detector. The operating conditions of the GC were as follows: oven temperature was held at 50 °C for 5 min, then programmed to increase at 3 °C min^{-1} to 225 °C where it was maintained for 30 min. The injector and ion detector temperatures were 225 °C and 230 °C, respectively. The carrier gas was nitrogen, at a flow rate of 1.86 mL min^{-1}. Peak areas were measured by electronic integration. The relative amounts of the individual components are based on the peak areas. MS spectra were determined by GC-MSD (HP5890 and HP5970, Hewlett-Packard, USA), with the electron impact source operating at 70 eV and 240 °C. The injector and ion source detector temperatures were the same as above. Terpene compounds were identified by computer matching of the mass spectra and confirmed by GC retention times.

2.4. Cumambrin A content

Cumambrin A, a major active component of *C. boreale* M. flowerheads, was analyzed by HPLC (Waters 201, Waters, USA) after $CHCl_3$ extraction at room temperature for 2 days (Robbins et al., 1996; Lee et al., 2002). 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 time of cumambrin A was 7.94 min. The individual peak area was calculated using concentration curves of purified cumambrin A (confirmed by Yang et al., 1996) as standards.

2.5. Statistical analysis

Yields and effective component contents were analyzed statistically by analysis of variance using the Statistical Analysis System (SAS) computer package (SAS Institute Inc, 1990). When analysis of variance showed significant treatment effects ($P < 0.05$), the least significant difference (LSD), at a 0.05 level of significance (Steel and Torrie, 1980), was used to compare treatment means of plant growth, productivity and effective component variables.

Table I. Dry matter and essential oil yields of flowerheads of *C. boreale* M. cultivated in four soil fertilization regimes in Korea during the two growing seasons of 2000 and 2001.

Treatment	Dry matter (kg ha^{-1})			Essential oil (kg ha^{-1})		
	2000	2001	Mean	2000	2001	Mean
Without fertilizer	611.1	659.3	635.2	1.96	2.18	2.07
Fertilizer	755.6	781.5	768.5	2.61	3.05	2.83
Fertilizer + Lime	796.3	825.9	811.1	2.97	3.63	3.30
Fertilizer + SMC	851.9	881.5	866.7	3.60	4.07	3.84
$LSD_{0.05}$	22.9	29.5	21.8	0.20	0.15	0.10

$LSD_{0.05}$ = least significant difference at probability level of 5%; SMC = swine manure compost.

3. RESULTS AND DISCUSSION

3.1. Dry matter and essential oil yield

The dry matter and essential oil yields of flowerheads of *C. boreale* M. in relation to fertilizer treatments during the growing seasons of 2000 and 2001 are shown in Table I. The average from the 2000 and 2001 experiments indicated that NPK fertilizer (F treatment) increased total flowerhead dry weight of *C. boreale* M. by 21%, from 635 kg ha^{-1} in soil without fertilizer (WF). Additions of lime (FL) or swine manure (FS) further increased total flowerhead dry weight, averaged over the two growing seasons ($P < 0.05$) by 12.8 and 5.5%, respectively, as compared with the F treatment. A similar trend was observed for the harvest index and morphological traits at the full bloom stage. There was a variation between the two years; the total flowerhead yield was 4% higher in 2001 than 2000. This is probably due to adequate and regular rainfall in October 2001, the period of the early blooming stage, which provided a more favorable environment for plant growth than in October 2000 (Fig. 1). Most variables were larger with fertilizer (F, FL and FS) treatments than without fertilizer treatment (WF).

Addition of lime or swine manure to NPK fertilization further increased flower yield, suggesting that the nutrient status of the soil before transplanting was poor. Essential oil yields, relative to F, were increased ($P < 0.05$) by 13.8 and 37.9% in 2000 and by 19 and 33.4% in 2001 due to FL and FS treatments, respectively, but, relative to F, WF treatment decreased essential oil yield by 24.9 and 28.5% each year, respectively (Tab. I). The lower essential oil content (%) obtained in 2000 could be attributed to moisture stress, which also affected plant dry weight. In contrast, the higher essential oil content in 2001 was due to the favorable moisture conditions maintained throughout the plant growth period (Fig. 1). FS treatment resulted in higher plant nitrogen contents than the control F, and increased total essential oil concentrations and contents of *C. boreale* M. Increases in essential oil following NPK additions at optimum levels have been reported in other herbs, such as in basil, bergamot mint and Japanese mint (Rao et al., 1983; Haelvae, 1987; Kothari et al., 1987). Haelvae (1987) also reported that optimum fertilizer application increased herbal plant and essential oil yields, but the relationship between fertilizer application and essential oil concentration was poorly explained. For this reason we carried out more detailed research to understand better the relationship between essential oil components and soil fertility.

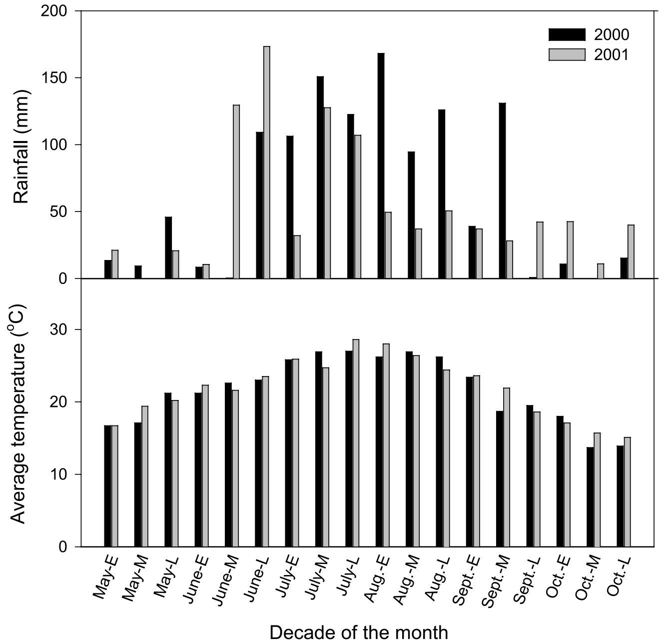

Figure 1. Rainfall and temperature during the 2000 and 2001 growing seasons.

3.2. Terpene composition of essential oils

Changes in essential oil concentrations of medically valuable flowerheads of *C. boreale* M., along with their relative percentage change over two years, as affected by soil amendment and by WF, relative to F, are given in Table II. Major essential oils isolated from the flowerheads of *C. boreale* M. were camphor (20–23%), followed by β-caryophyllene, cis-chrysanthenol, camphene, germacrene-D and caryophyllene epoxide. In contrast to yield of essential oil, which was increased by FL and FS fertilizer application treatments, flowerheads of the without fertilizer control treatment (WF) contained higher terpene oils (5% monoterpenoid and 14.6% sesquiterpenoid) than control F flowerheads (Fig. 2). These results are in agreement with those reported by Bernath (1986) and Tuomi et al. (1994), who found that plants grown in less fertilizer had increased terpene

Table II. Major component contents (%) of monoterpenoid and sesquiterpenoid groups of essential oil of *C. boreale* M. flowerheads as affected by different soil fertilizations. Changes of major component (%) in essential oil of *C. boreale* M. flowerheads (average of two years).

Compounds	Without fertilizer		Fertilizer		Fertilizer +Lime		Fertilizer +SMC	
	Mean	SD[a]	Mean	SD	Mean	SD	Mean	SD
Monoterpenoids								
α-pinene	2.28	0.125	2.23	0.169	2.84	0.118	2.49	0.101
Camphene	3.65	0.107	3.60	0.117	3.77	0.146	3.68	0.098
β-pinene	2.94	0.103	2.98	0.114	3.14	0.097	2.91	0.108
Sabinene	0.53	0.038	0.59	0.037	0.62	0.041	0.54	0.039
Myrcene	1.37	0.067	1.33	0.066	1.50	0.057	1.45	0.057
p-cymene	1.38	0.073	1.26	0.065	1.38	0.054	1.29	0.066
1,8-cineol	1.99	0.161	1.68	0.155	2.07	0.171	1.96	0.162
α-thujone	1.45	0.072	1.34	0.078	1.94	0.087	1.08	0.070
Camphor	21.23	0.771	20.41	0.889	21.72	0.690	22.06	0.748
cis-chrysanthenol	5.27	0.192	4.65	0.160	5.52	0.189	3.81	0.167
Total monoterpenoids	42.09		40.07		44.50		41.27	
Sesquiterpenoids								
α-copaene	0.61	0.043	0.45	0.056	0.60	0.045	0.57	0.035
β-elemene	1.62	0.086	1.68	0.080	1.85	0.089	1.77	0.081
β-caryophyllene	5.88	0.215	5.30	0.200	6.41	0.228	5.86	0.219
β-farnesene	1.35	0.075	1.37	0.078	1.82	0.075	1.62	0.074
epi-sesquiphellandrene	2.06	0.067	1.41	0.061	2.08	0.079	1.32	0.042
Germacrene D	4.59	0.171	3.55	0.168	5.82	0.183	3.61	0.118
β-selinene	0.88	0.050	0.70	0.062	0.80	0.058	0.84	0.054
ar-cucumene	0.63	0.061	0.65	0.051	0.83	0.066	0.67	0.054
Caryophyllene oxide	4.16	0.182	3.83	0.156	4.50	0.168	4.17	0.173
Nerolidol	0.78	0.054	0.75	0.062	0.95	0.057	0.79	0.063
Total sesquiterpenoids	22.56		19.69		25.66		21.22	
Total monoterpenoid + sesquiterpenoid	64.65		59.76		70.16		62.49	
Yield of essential oil components (kg ha^{-1})[b]	1.34c		1.69b		2.32a		2.40a	

[a] SD: standard deviation (n = 3); [b] the values followed by the same letter were not statistically different in an $LSD_{0.05}$ protected ANOVA.

concentrations, while plants grown with increased nitrogen fertilizer show a decrease. Other reports demonstrate that environmentally harmful growth conditions and limited nutrients in the soil cause an increase in terpene compounds, while increasing nitrogen and mineral nutrients decrease terpene concentrations (Bryant et al., 1983; Kainulainen et al., 1992; Doran and Bell, 1994; Nerg et al., 1994). However, it is difficult to find similar results, regarding compound change of monoterpenoids and sesquiterpenoids; the bulk of the differences in these seem to relate to interspecies variability.

Most of the essential oil components for plants grown in amended soils (FL and FS) were increased over the control F treatment. The degree of these effects varied between terpene groups. FL and FS treatments increased sesquiterpenes, 30.3 and 7.8%, respectively, more than monoterpenoids (11.1 and 3.0%, respectively). The most remarkable increase was for germacrene-D and epi-sesquiphellandrene content: 63.9 and 47.5%, respectively, due to amendment with FL and 29.3 and 46.1% due to the without fertilizer (WF) treatment, respectively. FS, which resulted in plants that contained high levels of nitrogen, also increased germacrene-D, but less than for plants treated with high levels of calcium. In addition, β-caryophyllene content in FS-treated plants increased compared with the control F treatment. Similar results were reported in patchouli (Singh et al., 2002). These results show that changes in essential oil concentration are dependent on soil fertility. Our results regarding the effect of liming treatment (FL) is in agreement with those reported in basil by Suh and Park (2000) who found that increased calcium concentrations in nutrient solutions increased sesquiterpene contents more than monoterpene

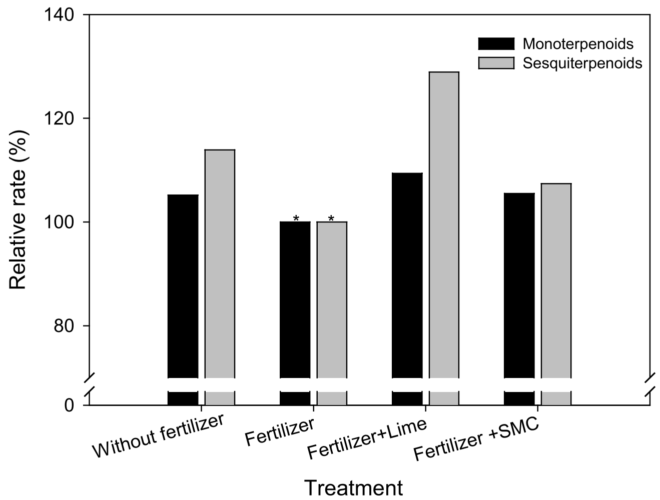

Figure 2. Relative comparison of total monoterpenoids and total sesquiterpenoids concentrations from flowerheads of *C. boreale* M. grown in different fertilizers. *: Standard (100%).

Table III. Effect of soil fertilization on cumambrin A contents and yields of flowerheads of *C. boreale* M.

Treatment	Cumambrin A: Content ($g\ kg^{-1}$, dry weight) 2000	2001	Mean	Yield ($kg\ ha^{-1}$) 2000	2001	Mean
Without fertilizer	2.82	2.73	2.77	1.72	1.80	1.76
Fertilizer (NPK)	2.73	2.70	2.71	2.06	2.11	2.09
Fertilizer + Lime	3.08	2.98	3.03	2.45	2.50	2.47
Fertilizer + SMC	2.65	2.58	2.61	2.26	2.28	2.27
$LSD_{0.05}$	0.23	0.21	0.18	0.22	0.22	0.18

HPLC retention time (min): 7.94; $LSD_{0.05}$ = least significant difference at probability level of 5%.

contents. This means that calcium treatment increased essential oil levels more by activating sesquiterpene than monoterpene biosynthesis and cyclase. McAinsh et al. (1990) reported that increased levels of calcium in the stroma cytoplasm induced the synthesis of abscisic acid (ABA), a sesquiterpene compound. It has been established that intracellular levels of calcium play a central role in transducing environmental and hormonal signals in plant cells (Cote and Crain, 1993; Sanders et al., 2002).

3.3. Cumambrin A

C. boreale M. contains high concentrations of the sesquiterpene lactone, cumambrin A, which has blood-pressure reduction activity (Hong et al., 1999). This is found primarily in flowerheads as compared with other plant parts (Lee et al., 2003). Cumambrin A concentration in flowerheads of plants cultivated during the 2000 and 2001 growing seasons was increased by WF, and by FL and FS soil fertility/amendment treatments (Tab. III). The greatest increases ($P < 0.05$) in both years, averaged about 11.8%, were obtained in FL, which contained high concentrations of calcium. A similar pattern occurred for the contents of sesquiterpene essential oils (see above). However, FS, which contained considerably less calcium but contained increased levels of nitrogen, decreased cumambrin A concentration, although the cumambrin A yield, on a per plant basis, was higher than in the control ($P < 0.05$) due to higher flowerhead yields.

The yields of FL and FS treatments were increased by 18.2 and 8.6% for 2000 and 2001, respectively, while WF decreased yield (15.8%). There was no difference in cumambrin A yields between 2000 and 2001, suggesting that cumambrin A content is not affected by variations in soil water availability. In contrast, liming (FL), which supplied calcium, increased cumambrin A yield, while FS treatment, which increased nitrogen availability, did not affect yields. In practice, our experiment demonstrated a high correlation between cumambrin A and calcium concentrations in flowerheads of *C. boreale* M. (Tab. IV). Similar

Table IV. Mineral content of flowerhead as affected by fertilizer application in the soil and their correlation coefficients with cumambrin A in *C. boreale* M. at two year harvests.

Treatment	N ($g\ kg^{-1}$, dry weight) 2000	N 2001	P 2000	P 2001	K 2000	K 2001	Ca 2000	Ca 2001
Without fertilizer	17.7	19.5	3.2	3.1	22.2	23.5	8.5	9.5
NPK	20.2	20.7	3.6	3.9	26.3	29.0	9.3	9.9
NPK + Lime	20.5	21.7	3.7	3.9	27.2	27.6	13.2	14.8
NPK + SM	23.9	22.6	4.1	4.3	30.6	31.3	9.3	10.3
$LSD_{0.05}$	1.20	2.51	0.79	0.60	2.47	2.38	1.82	1.78
Correlation coefficients								
Cumambrin A	0.040	0.032	0.021	0.001	0.030	0.030	0.711**	0.726**

** denote significance at 1% levels, respectively.

results have been reported by Suh and Park (2000) who found that the concentration of essential oils in basil is closely related to calcium content of plant tissue, rather than to nitrogen content. Since cumambrin A is a sesquiterpene, calcium supplied by FL is expected to increase cumambrin A concentration.

4. CONCLUSIONS

Application of suitable fertilizers could improve effective component concentration, and should be recommended in the cultivation of *C. boreale* M. Our experiment demonstrated that NPK fertilization with additional lime is one important management tool allowing increased yield of medically valuable flowerheads of *C. boreale* M. and increased production of effective components such as cumambrin A and essential oils. Further research should elucidate the pathway of terpene biosynthesis, which is currently unclear. It has been difficult to determine the biosynthetic mechanism of sesquiterpene and sesquiterpene lactone in plants. It is difficult to identify the cofactors of enzymes involved, and their possible relationships to biosynthesis functions, since the biological reactions are very divergent. Further, these pathways are dependent on many factors, such as plant species, growth stage and environmental conditions. Understanding terpene biosynthesis is a prerequisite for more detailed research into the role of calcium in this process.

REFERENCES

Allison L.E. (1965) Organic carbon, in: Black C.A. (Ed.), Methods of soil analysis, Am. Soc. Agron. Inc. Publ., Madison, USA, pp. 1367–1376.

Bernath J. (1986) Production ecology of secondary plant products herbs, spices and medical plants: Recent advances in botany, horticulture and pharmacology, Oryx press, Phoenix, USA, pp. 185–234.

Bryant J.P., Chapin F.S., Klein D.R. (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory, Oikos 40, 357–368.

Chappell J. (1995) The biochemistry and molecular biology of isoprenoid metabolism, Plant Physiol. 107, 1–6.

Choi Y.J. (1992) Korean traditional herbal plants, Academic Press, Seoul, Korea, 53 p.

Cote G.G., Crain R.C. (1993) Biochemistry of phosphoinositides, Ann. Rev. Plant Physiol. Mol. Biol. 44, 333–356.

Doran J.C., Bell R.E. (1994) Influence of non-genetic factor on yield of monoterpenes in leaf oils of *Eucalyptus camaldulensis*, New Forest 8, 363–376.

Facchini P.J., Chappell J. (1992) Gene family for an elicitor-induced sesquiterpene cyclase in tobacco, Proc. Natl. Acad. Sci. (USA) 89, 11088–11092.

Fretz T.A. (1976) Effect of photoperiod and nitrogen on the composition of foliar monoterpenes of *Juniperus horizontalis* Moench. cv Plumosa, J. Am. Soc. Hortic. Sci. 101, 611–613.

Haelvae S. (1987) Studies on fertilization of dill (*Anethum graveolens* S.) and basil (*Ocimum basilicum*). III. Oil yield of basil affected by fertilization, J. Agr. Sci. Finland 59, 2529–2535.

Hasler A., Meier B. (1993) *Ginkgo biolba* content of flavonoids and terpenes from leaves during the harvest time and from full extracts determined by chromatographic and biological methods, Planta Med. 59, 632–635.

Hong Y.G., Yang M.S., Park Y.B. (1999) Effect of cumambrin A treatment on blood pressure in spontaneously hypertensive rats, Korean J. Pharmacogn 30, 226–230.

Kainulainen P., Oksanen J., Palomaki V., Holpainen J.K., Holopainen T. (1992) Effect of drought and waterlogging stress on needle monoterpenes of *Picea abies*, Can. J. Bot. 70, 1613–1616.

Kothari S.K., Singh V., Singh K. (1987) Effect of rates and methods of phosphorus application on herb and oil yields and nutrient concentrations in Japanese mint (*Mentha arevensis* L.), J. Agr. Sci. 108, 691–693.

Kowald R., Bahtiyar M., Ditter P. (1982) Effect of refuse compost on the properties and the crop yield of an arable field, Z. Kulturtech. Flurbereinig. 23, 178–189.

Lee C.H. (1985) Illustrated encyclopedia of flora in Korea, Hyagmoonsa, Seoul, Korea, 648 p.

Lee K.D., Yang M.S., Ha T.J., Park K.M., Park K.H. (2002) Isolation and identification of dihydrochrysanolide and its 1-epimer from *Chrysanthemum coronarium* L., Biosci. Biotech. Biochem. 66, 862–865.

Lee K.D., Yang M.S., Jung Y., Sohn B., Cho J., Lee S.T., Kim P.J. (2003) Effect of NPK fertilization on the yields and the effective components of *Chrysanthemum boreale* M., J. Korean Soc. Agr. Chem. Biotechnol. 46, 134–139.

McAinsh M.R., Brownlee C., Hetherington A.M. (1990) Abscisic acid-induced elevation of guard cell cytosolic Ca^{2+} precedes stomatal closure, Nature 343, 186–188.

Merk L., Kloos M., Schonwitz R., Ziegler H. (1998) Influence of various factors on quantitiative composition of leaf monoterpenes of *Picea abies* (L.) Karst., Trees 2, 45–51.

Mihaliak C.A., Lincoln D.E. (1985) Growth pattern and carbon allocation to volatile leaf terpenes under nitrogen-limiting conditions, Oecologia 66, 897–902.

Muzika R.M., Pregitzer K.S., Hanover J.W. (1989) Changes in terpene production following nitrogen fertilization of grand fir (*Abies grandis* (Dougl.) Lindl.) seedlings, Oecologia 80, 485–489.

Nam S.H., Yang M.S. (1995a) Antibacterial activities of extracts from *Chrysanthemum boreale* M., Agr. Chem. Biotechnol. 38, 269–272.

Nam S.H., Yang M.S. (1995b) Isolation of cytotoxic substances from *Chrysanthemum boreale* M., Agr. Chem. Biotechnol. 38, 273–277.

Nerg A., Kainulainen P., Vuorinen M., Hanso M., Holopainen J.K., Kurkela T. (1994) Seasonal and geographical variation of terpenes, resin acids and total phenolics in nursery grown seedlings of Scots pine (*Pinus sylvestris* L.), New Phytol. 128, 703–713.

Nicolaus H.F. (1991) Sesquiterpenoid lactones, Methods Plant Biochem. 7, 187–211.

Picman A.K. (1986) Biological activities of sesquiterpene lactone, Biochem. Syst. Ecol. 14, 255–281.

Pino P.A., Chris D.B., Maria J.P. (2001) Antifungal activity of the essential oil of flowerheads of garland chrysanthemum against agricultural pathogens, Phytochemistry 57, 99–102.

Rao B.R.R., Rao E.V.S.P., Singh S.P. (1983) Influence of NPK fertilization on the herbage yield essential oil content and essential oil yield bergamot mint (*Menta citrata*), Ind. Perfum. 27, 77–79.

RDA (Rural Development Administration) (1988) Methods of soil chemical analysis, National Institute of Agricultural Science and Technology, RDA, Suwon, Korea.

RDA (Rural Development Administration) (1995) Standard investigation methods for agricultural experiment, RDA, Suwon, Korea, 601 p.

Robbins M.P., Evans T.E., Morris P. (1996) The effect of plant growth regulators of growth, morphology and condensed tannin accumulation in transformed root cultures of *Lotus corniculatus*, Plant Cell, Tissue, Organ Cult. 44, 219–227.

Sanders D., Pelloux J., Brownlee C., Harper J.F. (2002) Calcium at the crossroads of signaling, Plant Cell S401, 17.

SAS Institute Inc. (1990) SAS user's guide statistics, 5th ed., SAS Inst., Cary, NC.

Schultz T.H., Flath R.A., Mon T.R., Eggling S.B., Tweauishi R. (1977) Isolation of volatile components from a model system, J. Agr. Food Chem. 25, 446–451.

Singh M., Sharma S., Ramesh S. (2002) Herbage, oil yield and oil quality of patchouli (*Pogostemon cablin* B.) influenced by irrigation, organic mulch and nitrogen application in semi-arid tropical climate, Ind. Crops Prod. 16, 101–107.

Steel R.G.D., Torrie J.H. (1980) Principles and procedures of statistics: a biometric approach, McGraw-Hill, New York, USA, 633 p.

Son Y., Kim Z., Hwang J.H., Park J.S. (1998) Fertilization effects on growth, foliar nutrients and extract concentrations in Ginkgo Seedlings, J. Korean For. Soc. 87, 98–105.

Suh E.J., Park K.W. (2000) Effect of calcium ion in nutrient solution on the content and composition of essential oil of sweet basil in hydroponics, J. Korean Soc. Hort. Sci. 41, 598–601.

Tuomi J., Niemela P., Haukioja E., Siren S., Neuvonen S. (1994) Nutrient stress: An explanation for plant anti-herbivore responses to defoliation, Oecologia 61, 208–210.

Waring R.H., MeDoanld A.J.S., Larsson S., Ericsson T., Wiren A., Arwidsson E., Ericsson A., Lohammar T. (1985) Differences in chemical composition of plants grown at constant relative growth rate with stable mineral nutrition, Oecologia 65, 157–160.

Yang M.S., Park K.H., Jang D.S., Choi S.U., Nam S.H., Shiro M. (1996) Cumambrin A in *Chrysanthemum boreale* Makino preparation X-ray crystal structure and ^{13}C- and ^{1}H-NMR study of cumambrin A, Korean J. Pharmacogn 27, 207–211.

To access this journal online:
www.edpsciences.org