韓藥作誌(Korean J. Medicinal Crop Sci.) 17(5) : 363 – 368 (2009)

질소시비가 감국의 생육 및 유효성분에 미치는 영향

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Effects of Nitrogen Application on Growth and Bioactive Compounds of *Chrysanthemum indicum* L. (Gamgug)

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ABSTRACT : To fulfill the increasing demand for a high quality of flower, we investigated the effects of nitrogen application on plant growth, yield and bioactive compounds of *Chrysanthemum indicum* L.. *C. indicum* L. was cultivated in a pot scale, and nitrogen applied with the level of 0 (N0), 50 (N50), 100 (N100), 150 (N150), 200 (N200) and 300 (N300) kg ha^{-1} to suggest optimum rate of nitrogen fertilization. Phosphate and potassium applied the same amount of 80-80 kg ha^{-1} (P_2O_5-K_2O) in all treatments. Growth characteristics and yields of *C. indicum* L. were significantly affected by nitrogen application. Maximum yield achieved in 265 and 295 kg ha^{-1} N treatment on the whole plant and the flower parts, respectively. The nitrogen content and uptake of whole plant significantly increased by the increase of nitrogen application. Five major components of essential oil, α-pinene, 1,8-cineol, chrysanthenone, germacrene-D, and α–curcumene in flowerheads of *C. indicum* L. occupied approximately 40% of peak area, germacrene-D decreased by the increase of nitrogen application among them. Howerer, cumambrin A contents in the flower parts of *C. indicum* L. were affected negatively by the increase of nitrogen application, but total yields of cumambrin A in flower part significantly increased. Conclusively, nitrogen fertilization could increase the yield of flowerheads. The optimum application level of nitrogen fertilizer might be on the range of 265-295 kg ha^{-1} in a mountainous soil.

***Key Words* : *Chrysanthemum indicum* L., Nitrogen, Terpene, Essential oil, Cumambrin A contents, Yield**

INTROCUCTION

Chrysanthemum indicum L. (Gamgug), widely distributed throughout the fields of Korea, is regarded as an important source materials for traditional herbal medicine. *C. indicum* L. known as a perennial plants of the family Compositae, which is included to *C. boreale* M. (Sangug), *C. zawadskii, C. monifolium* etc. (Byun and Shin, 2008), has yellow blossoms that bloom in the middle of October. In fact, morphological distinction of *C. boreale* M. and *C. indicum* L. in the field is difficult, but generally may different hereditarily. The flowerheads have been indicated for headache and dizziness (Cheng *et al*., 2005; Shunying *et al*., 2005). As the important active components of *C. indicum* L. and *C. zawadskii* var. latilobum, essential oils, flavonoids and other phenolic derivatives are studied (Lee and Lee, 2007). Plant essential oils may important as complimentary and alternative medicine and household life goods, and their effects have been reported to have antimicrobial agents(Shin and Lim, 2004), lowering blood pressure (Derwick, 2002; Kil *et al*., 1996) and antioxidant activities (Kil *et al*., 1996). In recent years, with increasing concerns for health-improving foods, the demand for *C. indicum* L. has become higher than ever. However, the amount of wild *C. indicum* L. collected from mountainous areas is not enough to cover all demands. The cultivation system and fertilization strategy are required to meet increasing demand on *C. indicum* L. with a good quality. The content of essential oil and its composition in a harvested herbal are affected by different of factors, primarily by its genetics (Merk *et al.,* 1988;

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Received 2009 September 4 / Revised 2009 October 20 / Accepted 2009 October 22

Muzika *et al.,* 1989; Lee and Yang, 2003; Lee *et al*., 2004) and cultivation condition, such harvesting time, climate and the use of fertilizer and plant growth regulators (PGRs). Terpene content decreases with increased nitrogen fertilizer application in *Juniperus horizontalis* (Fretz, 1976). In addition, Lee *et al*., (2002) reported that nitrogen fertilization of the range of 225-250 kg ha^{-1} in *C. boreale* M. increase total yields and enhance quality. The contents of cumambrin A, which has the effect on blood-pressure reduction, anticancer, antibacterial and inflammation (Hong *et al*., 1999), decreased with increasing nitrogen application. However, the amount of cumambrin A in flower increased as nitrogen level increased because of increasing yield of flowerheads. Mihaliak and Linclon (1985), and Waring *et al*., (1985) found that nitrogen limiting conditions increase the production of volatile terpenes in an annual plant. Although the efforts to improve terpene production by fertilization have been reported in other species, there is no report on the cultivation methods of *C. indicum* L. In this paper, we report the effects of nitrogen fertilizer on growth, yields and bioactive components, such as sesquiterpene lactones and essential oils from flowerheads of *C. indicum* L. to suggest optimum rate of nitrogen fertilization for producing the flowerhead of high quality.

MATERIALS AND METHODS

1. Nitrogen application and growth management

This experiment was simultaneously conducted with *C. boreale* M. (Sangug) (Lee *et al*., 2002) on May 20-30, 2000 at Geongsang National University. The used soil was Ap soil in an alpine area (Elevation 160 m). Texture and chemical properties of soils are summarized in Table 5. Nitrogen levels in a Wagner pot (1/2000a), which packed 18 kg of dried soil, were applied at the level of 0, 50, 100, 150, 200 and 300 kg ha^{-1} in N0, N50, N100, N150, N200 and N300 treatments, respectively. Phosphate and potassium were applied at at the level of P_2O_5-K_2O (80-80 kg ha^{-1}) for all treatments. Fertilizers were separately applied as follows: basal fertilizer, 70% of nitrogen levels, 80 kg P_2O_5 ha^{-1} and 56 kg K_2O ha^{-1} was applied together with soil packing in pots on May 30, 2000, and the remain of nitrogen and potassium were side-dressed at approximately 35 days before the flowering. Pots were arranged in a complete randomized plot design and each treatment was carried out in triplicate. The flowerhead yields were determined at the full bloom stage on October 20, 2000 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) and put immediately into a deep-freeze (−70℃) for essential oil determination. The yield characteristics were investigated by RDA methods (R.D.A., 1995).

2. Analysis of inorganic elements

Soil sample collected before and after the experiment and were dried by air for chemical analysis. Soil samples were sieved (2 ㎜ 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 (R.D.A., 1988) and the contents of exchangeable Ca^{2+}, Mg^{2+}, and K^+ (1 M NH_4-acetate pH 7, Atomic Absorption, Shimazu, 660). Plant samples were carried out by RDA methods (R.D.A., 1988).

3. Analysis of bioactive compounds

Essential oil contents of *C. indicum* L. flowerheads were determined with simultaneous distillation extraction (SDE) apparatus, using a modificated 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 ㎜ × film thickness 0.25 ㎛) on a Hewlett-Packard 5880 gas chromatograph equipped with a FID detector. The operating conditions of the GC were similar with Lee *et al*., (2005) for *C. boreale* M. flowerheads. Terpene compounds were identified by computer matching of the mass spectra and confirmed by GC retention times. Cumambrin A, as bioactive component of *C. indicum* L. flowerheads, was analyzed by HPLC (Waters 201, Waters, USA) after $CHCl_3$ extraction at room temperature for 2 days (Lee *et al*., 2005). The operating conditions were as follows: Adsorbosphere silica 5 ㎛ column and Lamda-max detector; eluent of a dichloromethane: isopropanol (49 : 1) mixture; column temperature at 25℃; sample size of 5 ㎕: maximum absorption at 254 ㎚. 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.

4. Statistical analysis

All data were analyzed statistically by an analysis of variance using CoStat software (CoHort Software, Monterey, USA). Nitrogen treatments were tested in an experiment using a randomized complete block model with three replications. Mean comparisons were conducted using an ANOVA protected least significant difference (LSD) test ($P < 0.05$).

RESULTS AND DISCUSSION

1. Plant growth and yield

The dry matter and growth characteristics of *C. indicum* L. as affected by nitrogen application levels are shown in Table 1. Nitrogen application increased yields of whole plants, and significant difference was found among treatments with nitrogen application. Maximum yield was achieved in 265 kg ha^{-1} N treatment on the total plant weight ($Y = -0.0021X^2 + 1.1136X + 29.348$, $R^2 = 0.98$, $p < 0.001$) but in 295 kg ha^{-1} N treatment on the flower part ($Y = -0.0004X^2 + 0.2361X - 0.2724$, $R^2 = 0.98$, $p < 0.001$) (Fig. 1), which is a valuable part in *C. indicum* L. as an herbal medicine. This means that the high correlation coefficient in the yield of whole plant responded the most positively with nitrogen application level. A similar result reported that maximum yield of *C. boreale* M. was achieved in 246 kg ha^{-1} N treatment for total dry weight and in 226 kg ha^{-1} N treatment for flowerheads (Lee *et al*., 2002). Growth characteristics, such as leaf length, stem diameter, plant height and the number of branch showed similar pattern to yield responses.

2. Nitrogen contents and uptake of plant

The nitrogen content of whole plant significantly increased with increasing nitrogen application (Table 3). Dry matter and nitrogen content had positively increased with increasing nitrogen application. Absorbed nitrogen of plant was increased by 415.2 mg plant^{-1} for flowers and 1,131.4 mg plant^{-1} for whole parts in N300 treatment, and increased by 16 times for whole parts in N300 treatment compared to 68.9 mg plant^{-1} in control (N0 treatment). Similar results

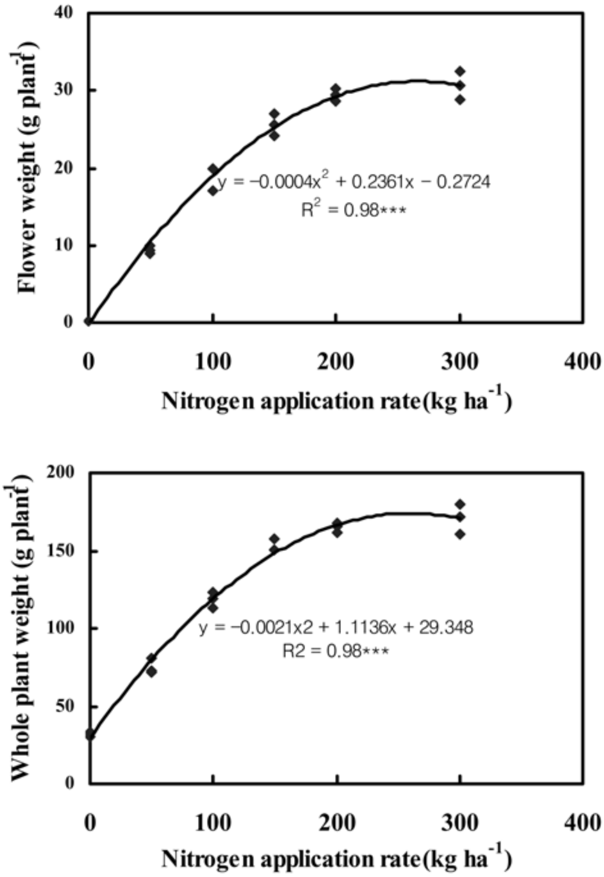

Fig. 1. Relationship between the dry weight of plant and nitrogen application level in *C. indicum* L.

Table 1. Growth characteristics and yield of *C. indicum* L.

Treatment	Dried weight (g plant^{-1})				Plant height (cm)	Stem diameter (cm)	Branch no. (No. plant^{-1})	Leaf length (cm)
	Leaf	Stem	Flower	Total				
N0	5.28	26.4	0.28	32.0	48.7	0.38	1.0	2.3*
N50	13.3	52.2	9.45	75.0	82.3	0.79	16.3	3.2
N100	20.3	79.1	18.9	118.4	94.6	0.96	26.9	3.6
N150	29.1	98.1	25.6	152.9	121.0	1.11	35.3	3.9
N200	30.3	104.6	29.4	164.9	127.1	1.18	36.7	4.0
N300	30.8	109.4	30.6	170.3	129.4	1.26	38.3	4.0
$LSD_{0.05}$	2.24	9.65	2.17	9.65	12.4	0.14	3.73	0.25

*Means separation within columns by LSD at 5% level ($p < 0.05$, $n = 3$)

Table 2. Nitrogen content and uptake of *C. indicum* L.

Treatment	Nitrogen content (g kg^{-1})			Nitrogen uptake (㎎ $plant^{-1}$)			
	Leaf	Stem	Flower	Leaf	Stem	Flower	Total
N0	5.54	1.38	8.8	29.5	36.9	2.5	68.9*
N50	7.72	2.43	10.9	103.3	127.6	102.7	333.6
N100	8.34	2.94	11.5	169.6	230.5	219.2	619.2
N150	9.43	3.15	12.6	275.3	308.5	322.4	906.2
N200	9.89	3.26	12.0	305.7	340.0	381.3	1027.1
N300	10.36	3.69	13.6	314.2	402.1	415.2	1131.4
$LSD_{0.05}$	1.22	0.82	1.67	43.9	55.4	47.2	107.5

*Means separation within columns by LSD at 5% level (p < 0.05, n = 3)

Table 3. Changes of major component (%) in the essential oil of flowerhead of *C. indicum* L.

Compounds	N0	N50	N100	N150	N200	N300
	Mean	Mean	Mean	Mean	Mean	Mean
α-pinene	9.17±0.089	8.56±0.086	9.23±0.077	9.48±0.068	8.22±0.069	8.33±0.075*
1,8-cineol	8.31±0.088	8.36±0.074	8.75±0.075	8.77±0.072	8.96±0.085	8.82±0.081
Chrysanthenone	12.8±0.096	11.66±0.087	11.89±0.082	12.67±0.075	12.43±0.093	12.34±0.075
Germacrene D	6.55±0.065	7.02±0.058	6.99±0.069	6.78±0.053	6.68±0.063	6.36±0.061
α-curcumene	3.85±0.032	3.56±0.036	3.78±0.022	4.31±0.036	4.86±0.039	3.49±0.041
Total (%)	40.68	39.16	40.64	42.01	41.15	39.34

SD*: standard deviation

reported that nitrogen uptake in *C. boreale* M. increased from 130 ㎎ $plant^{-1}$ in control to 1,685 ㎎ $plant^{-1}$ in N250 treatment (Lee *et al*., 2002).

3. Essential oil content and major components

Changes of essential oil concentrations, along with their relative percentage as affected by nitrogen fertilization, are given in Table 3. Nitrogen application increased the yield of essential oil in the flower of *C. indicum* L. up to a maximum of 0.226 ㎖ $plant^{-1}$ at N300 compared to 0.005 ㎖ $plant^{-1}$ in control. Five major components of essential oil, α-pinene, 1,8-cineol, chrysanthenone, germacrene D, and α-curcumene in flowrheads of *C. indicum* L. occupied approximately 40% of peak area by GC-MS analyzer. Germacrene-D decreased by the increase of nitrogen application, but other compounds were slightly increased. These results were generally similar with the report for *C. boreale* M. by Lee *et al*., (2005) and for *Juniperus horizontalis* by Fretz (1976).

4. Yield of cumambrin A

Cumambrin A, belong to susquiterpene lactone group, has the effect of blood-pressure reduction, anticancer, antibacterial and inflammation (Hong *et al*., 1999), and was contained highly in the flower part among whole plant in *C. indicum* L. (Table 4). However, cumambrin A content in flowerheads of *C. indicum* L. could be less than *C. boreale* M. Moreover, it is hard to recognize morphological characteristics in the field, but the flowerheads of *C. indicum* L. usually were bigger than *C. boreale* M.. Cumambrin A contents in flower parts of *C. indicum* L. were affected negatively by the increase of nitrogen level. In particular, yield of cumambrin A in flower part increased from 0.24 g kg^{-1} in control to 23.1 g kg^{-1} in N300 treatment. These mean that the increase of flowerheads yield caused yield of cumambrin A, and the similar results reported in *C. boreale* M. (Lee *et al*., 2005). The metabolism pathway between cumambrin A and nitrogen content is unclear and needs more study on relationship of them.

5. Changes of soil chemicals

Soil chemicals contents were very low before transplanting and showed a little changes after harvesting (Table 5). For example, organic matter and total nitrogen in soil was increased to from 4.3 to 5.9 g kg^{-1} by nitrogen application.

Table 4. Yields and contents of cumambrin A from the flowerheds of *C. indicum* L.

Treatment	Contents of cumambrin A (g kg^{-1}, dry weight)			Yield of cumambrin A (g kg^{-1}, dry weight)
	Leaf	Stem	Flower	Flower
N0	0.33	0.079	0.87	0.246**[e]
N50	0.34	0.056	0.80	7.56[d]
N100	0.38	0.057	0.78	14.64[c]
N150	0.35	0.068	0.76	19.58[b]
N200	0.36	0.056	0.76	22.27[a]
N300	0.311	0.052	0.75	23.16[a]
$LSD_{0.05}$	ns*	ns	0.054	1.37

ns*: not significant different
**Means separation within columns by LSD at 5% level ($p < 0.05$, $n = 3$)

Table 5. Physical and chemical properties of soils investigated pot tests before and after experiments.

	Treatment	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})	CEC[d] (cmol(+) kg^{-1})	Ex. Cations[c] (cmol(+) kg^{-1})		
								K	Ca	Mg
Before		5.2	0.05	4.0	0.4	6.0	9.7	0.15	2.0	0.46*
after	N0	5.1	0.03	4.3	0.3	9.6	10.0	0.18	2.3	0.51
	N50	5.2	0.04	4.5	0.3	9.8	10.6	0.19	2.3	0.50
	N100	5.1	0.05	5.0	0.5	10.1	`10.8	0.15	2.3	0.54
	N150	5.3	0.05	5.2	0.8	10.6	10.9	0.13	2.1	0.50
	N200	5.2	0.06	5.6	0.9	11.1	11.2	0.14	2.1	0.52
	N300	5.2	0.08	5.6	1.1	11.0	11.8	0.12	2.2	0.54
	$LSD_{0.05}$	0.2	0.03	0.4	0.1	0.9	0.5	0.03	0.12	0.05

*Means separation within columns by LSD at 5% level ($p = 0.05$, $n = 3$)
[a] OM: organic matter
[b] EC: Electrical conductivity
[c] Ex. Cations: Exchangeable cations
[d] Cation exchange capacity

Nitrogen content significantly increased by the increase of nitrogen application and reached the peak of 1.1 g kg^{-1} in N300 level. While the increase of nitrogen application in soils had available phosphorus levels and cation exchange capacity (CEC) higher than the controls, but other physical and chemical properties did not increase exchangeable cation contents and electrical conductivity (EC) in soil at harvest.

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