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Effect of Forms and Levels of Nitrogen Fertilizer on Plant Growth and Essential Oil Content of *Agastache rugosa*

Hyun Choong Ohk*†, Ji Sook Song* and Young Am Chae*

**Department of Agronomy and Research Center for New Biomaterials in Agriculture, Seoul National University, Suwon 441-744, Korea*

ABSTRACT : This study was carried out to investigate the effect of forms and levels of nitrogen fertilizer on plant growth and essential oil production of *Agastache rugosa*. Calcium nitrate had more influenced on length and width of leaves and lateral branch length than did urea. When nitrogen fertilizer level was increased from 12 kgN/10a to 24 kgN/10a, plant growth was stimulated and dry matter of leaf and inflorescence were increased. Top dry matter of plant with calcium nitrate treatment (38.4 g) was heavier than that of urea treatment (32.8 g). Interactions among accession and nitrogen form and nitrogen rate were not significantly different for top dry matter. The forms and rate of nitrogen fertilizer did not affect estragole content. The estragole contents was higher in leaf (91.8%) than that of inflorescence (81.3%). While the essential oil content was not affected by different nitrogen forms, nitrogen level affected the essential oil contents positively by increasing dry matter. Essential oil yield was not affected by accession or nitrogen form, but by nitrogen rate. With increasing N application from 12 kgN/10a to 24 kgN/10a, essential oil yield was increased by 95.8%.

Keywords : *Agastache rugosa*, nitrogen fertilizer, morphological traits, estragole, essential oil.

Agastache rugosa O. Kuntze is a perennial herb that belongs to the *Labiatae* family, and is widely in Korea, China, India, Japan and other East Asian countries (Lee, 1980). Anti-fungus, anti-spirillum, antitumor and cytotoxic activity of *A. rugosa* were reported (Weverstahl *et al.*, 1992). The whole plant with sweet-smell has been used as an agent for the treatment of cholera, vomiting, diarrhea, foul breath, stomach trouble and miasma. The leaves of this plant are used as a spice for fish-based foods and used as a edible herb at its early vegetative stage. The flowers of *A. rugosa* are good source of honey (Chung *et al.*, 1990).

Svoboda *et al.* (1995) and Wilson *et al.* (1992) reported that the major components of essential oil from *A. rugosa* were variable (estragole, pulogone, isomenthone, etc.) depending on the origin of plants. Based on our previous research and others, more than 80% of the essential oil was composed of estragole in *A. rugosa* naturally grown in Korea (Ahn & Yang, 1991; Chae *et al.*, 1999). Estragole, which is also known as methyl chavicol, p-allylanisole, and 1-methoxyl-4-(2-propenyl) benzene, is a colorless liquid possessing an aromatic odor and sweet taste reminiscent of anise. It is an important ingredient in perfumes. It is also used for flavoring of foods and liquors and reducing unpleasant meat flavor. It forms an important constituent in root beer and can be used to prepare other well-known aromatic compounds such as anethole and anisaldehyde (Mazza and Kiehn, 1992). Besides estragole, some minor or even trace constituents are also important factors of odor in *A. rugosa*. These include anisaldehyde, chavicol, p-methoxycinnamyl alcohol, jasmone, limonene, beta-caryophyllene and oct-1-en-3-yl acetate (Weverstahl *et al.*, 1992). Therefore, much attention was paid to *A. rugosa* for commercial utilization of its essential oil.

It was reported that the essential oil contents on dry basis of *A. rugosa* from Korea ranged between 0.3 to 1.4% in leaves, 0.4 to 2.1% in flowers, 0 to 0.5% in stem. The oil content was significantly higher at full bloom stage than other growth stages (Ahn *et al.*, 1991; Chae *et al.*, 1999; Lee *et al.*, 1994), although no significant change of essential oil constituent between vegetative and productive stage or organs (leaf, flower, stem) were reported (Chae *et al.*, 1999). In contrast to the increasing economical importance of *A. rugosa,* most of studies were focused on analysis of essential oil component. Study of agronomic factors affecting herbage yields and essential oil contents of *A. rugosa* was not investigated thoroughly. Among various agronomic practices, plant population and nitrogen fertilization are important factors for securing maximum herbage yield. We investigated the effect of levels and forms of nitrogen fertilizer on plant growth and essential oil content in *A. rugosa* to develop efficient crop system for *A. rugosa.*

MATERIALS AND METHODS

Plant materials

Three accessions of *A. rugosa* were locally collected from Jindo, Mokpo and Soonchun of Chunlanam-do. After field-

†Corresponding author: (Phone) +82-31-290-2310 (E-mail) hycok@chollian.net <Received April 7, 2000>

grown in 1998 at the College Experimental Farm, Seoul National University, Suwon, mature seeds from each accession were harvested on November when the plants were in their ripening stage.

These accessions were planted on nursery on 24 April 1999 after soaking in GA_3 of 100 ppm solution for 24 hrs. Fifty-day-old seedlings were transplanted with 40×30 cm density at the College Experimental Farm. The experiment was laid out in a split-split-plot design with 3 replication, by allotting accession to the main plot, N forms of urea and calcium nitrate to the subplot, and N levels of 12, 18 and 24 kgN/10a to the sub-subplot. Each plot size was 3 m×4 m. Half of fertilizer was applied at 7 days after planting and the rest on 27 July 1999.

Plants were harvested at 2 cm above the ground level at the full bloom stage, when the first lateral branch was in bloom in early September. The growth traits such as stem length, length of the longest branch per plant, number of lateral branches per plant, leaf length and width, inflorescence length were examined. After harvest, plants were divided to leaves, stem, and inflorescence. They were dried under the shade before dry matter was measured and used for analysis.

Essential oil extraction

The extraction of essential oil was performed with Liceus and Nickerson's simultaneous steam distillation and extraction (SDE) apparatus modified by Schultz *et al.* (1977). 10~15 g of dried stem, leaves and inflorescence were extracted in 80 ml n-pentane : diethyl ether mixed liquid (1:1 v/v) for 2 hrs. After extraction, the solvent layer was fractionated and dehydrated with sodium sulfate anhydrous and, then, condensed at 40 in ambient pressure.

Gas chromatography

Gas chromatography (GC; Hewlett-Packard 6890) conected to Chemstation software was used for analysis of essential oil. The operating conditions were as follows : HP-5 capillary column (32 m×0.32 m×0.25 μm); helium carrier gas was adjusted to a linear flow at 1 ml/min; injector and detector temperatures at 250°C; oven temperature was programmed to increase from 100 to 230°C at 5°C/min and then held isothermal for 10 min; sample size of 0.5 μl and 250; split as 40:1. The compounds were identified by their GC retention times. Reference oil components for comparison were obtained from Fluorochem Ltd, Roth, Sigma.

GC-MS Analysis

GC-MS analysis was carried out with a HP58799GC/HP5971MS fitted with a HP-5 capillary column (32 m×0.32 m×0.32 μm) with the following conditions: Oven temperature was programmed to increase from 50°C to 240°C at 3°C/min, held at 50°C for 5 min and at 240°C for 10 min; injector temperature at 250°C; helium carrier gas was adjusted to a linear flow of 1 ml/min; splitting ratio at 40:1; Mass spectrometer ionization energy at 70 eV and; ion source temperature at 230°C. The identification of the GC peak mass spectra was made with using the mass spectrum data from Wiley 275 Library and Standard reagent.

Table 1. Morphological traits at full blooming stage among different origins of accessions cultivated with different forms and rates of nitrogen in *A. rugosa.*

	Stem length (cm)	Lateral branch length (cm)	Lateral branch number	Leaf length (cm)	Leaf width (cm)	Inflorescence length (cm)
Collection site						
Jindo	75.7 ± 2.33[†]	49.6 ± 2.55	23.6 ± 0.63	8.9 ± 0.27	5.9 ± 0.17	10.2 ± 0.55
Mokpo	81.5 ± 1.35	53.4 ± 1.49	22.1 ± 0.50	8.9 ± 0.14	6.1 ± 0.13	11.5 ± 0.33
Soonchun	76.5 ± 2.02	44.6 ± 2.26	23.6 ± 0.68	8.0 ± 0.26	5.8 ± 0.21	10.0 ± 0.44
$LSD_{0.05}$	5.21	5.78	1.76	0.60	0.49	1.22
Nitrogen form						
$CO(NH_2)_2$	76.5 ± 1.52	45.7 ± 1.65	22.4 ± 0.63	8.3 ± 0.21	5.7 ± 0.15	10.5 ± 0.44
$Ca(NO_3)_2$	80.1 ± 1.36	52.6 ± 1.52	23.1 ± 0.41	8.9 ± 0.14	6.1 ± 0.12	10.9 ± 0.28
$LSD_{0.05}$	4.19	4.63	1.41	0.49	0.39	0.99
Nitrogen rate (kgN/10a)						
12	75.2 ± 1.86	44.5 ± 2.10	22.3 ± 0.68	8.3 ± 0.23	5.6 ± 0.19	10.2 ± 0.45
18	79.2 ± 1.66	51.7 ± 1.83	23.2 ± 0.51	8.7 ± 0.20	6.1 ± 0.16	11.0 ± 0.42
24	81.5 ± 1.80	53.1 ± 1.97	23.0 ± 0.63	8.9 ± 0.20	6.2 ± 0.14	11.1 ± 0.39
$LSD_{0.05}$	4.95	5.48	1.68	0.58	0.45	1.17

[†]Mean ± standard error

RESULTS AND DISCUSSION

Morphological traits

When morphological traits of *A. rugosa* from the different collections at full blooming stage were examined, most of morphological traits of the Mokpo collection except for lateral branch number were larger than the other collections (Table 1). Especially, inflorescence length (11.5 cm) of Mokpo collections was the longest. Though, the collections from Jindo and Soonchun showed similar morphological traits, leaf and lateral branch length of Jindo collection were longer than that of Soonchun. Plant growth of collections responded to nitrogen fertilizer application was enhanced in the order of Mokpo, Jindo and Soonchun collection.

Morphological traits of *A. rugosa* showed different responses to nitrogen forms. The morphological traits with treatment of calcium nitrate were larger than that of urea treatment. The length and width of leaf and the length of lateral branch with treatment of calcium nitrate were significantly longer than those of urea treatment. When the rate of nitrogen fertilizer was increased from 12 kgN/10a to 24 kgN/10a, plant growth was enhanced. Most of morphological traits except lateral branch number and inflorescence length were increased significantly. However, significant differences between 18 kgN/10a and 24 kgN/10a was not observed for all of morphological traits.

There was no significant interactions in morphological traits among accession, nitrogen forms and nitrogen application rate (Table 2).

Top dry matter

It is one of important factor to increase the top dry matter in order to increase essential oil production of *A. rugosa*. It was reported that leaf and inflorescence were the main production organs for the essential oil in *A. rugosa* (Chae *et al.*, 1999; Lee *et al.*, 1994) and their increase of dry matter would be important to increase essential oil yield. According to the analysis of variance results (Table 3), there was no significant interaction among accession, nitrogen forms and nitrogen application rates for top dry matter. Also, accession effect was not detected for top dry matter. However, mean squares with treatment of nitrogen forms and nitrogen application rate were significantly different for dry matter. Nitrogen forms had the effect to increase the stem and inflorescence dry matter, while nitrogen application rate the leaf dry matter. These results suggest that nitrogen forms as well as nitrogen level should be considered as the factors determining the top dry matter.

Top dry matter per plant at full blooming stage showed no differences significantly among the accessions (Table 4). From the results of Table 1 and Table 4, calcium nitrate fertilizer was more effective than the urea form to increase plant growth of *A. rugosa*. The major inorganic form of N absorbed by plants are NO_3^- and NH_4^+. Both form of N can be present naturally in the soil solution, NH_4^+ from decay of organic matter and NO_3^- from nitrification of NH_4^+, while both forms of N can be applied, NH_4^+-N is less costly and most often applied. Ammonium is rapidly nitrified to NO_3^-

Table 3. Analysis of variance for top dry matter in different form and application rate of nitrogen fertilizer in three *A. rugosa* collection.

Source	df	Mean squares			
		Stem	Leaf	Inflorescence	Total
Collection (C)	2	10.52	23.11	0.31	60.30
Nitrogen form (F)	1	413.88*	81.40	9.17*	1049.41*
Nitrogen rate (R)	2	86.82	382.22**	13.01	1026.99*
C × F	2	21.42	33.98	3.88	69.71
C × R	4	50.56	20.46	3.27	69.54
F × R	2	26.97	14.07	7.26	41.69
C × F × R	4	9.16	3.90	6.78	44.87

*,**Significant at the 0.05 and 0.01 levels of probability, respectively.

Table 2. Analysis of variance for morphological traits in different form and application rate of nitrogen fertilizer in three *A. rugosa* collection.

Source	df	Mean squares					
		Stem length	Lateral branch length	Lateral branch number	Leaf length	Leaf width	Inflorescence length
Collection (C)	2	745.59	682.57	14.98	18.03	2.57	19.04
Nitrogen form (F)	1	164.07	253.03	1.92	0.24	0.65	0.01
Nitrogen rate (R)	2	423.76	244.63	9.62	4.40	4.66	25.17
CF	2	1317.23	135.72	79.44	4.56	0.31	4.57
CR	4	94.06	154.70	10.11	2.51	1.58	9.16
FR	2	94.61	176.69	8.07	0.87	0.44	3.38
CFR	4	78.24	63.15	10.77	0.64	1.02	4.10

Table 4. Comparison of top dry matter among different origins of accessions at different form and rate of nitrogen in *A. rugosa* at full blooming stage.

	Dry matter (g/plant)			
	Stem	Leaf	Inflorescence	Total
Collection site				
Jindo	19.8 ± 1.70†	13.2 ± 0.95	4.0 ± 0.39	36.8 ± 2.81
Mokpo	19.5 ± 1.47	13.8 ± 1.06	3.8 ± 0.50	37.3 ± 2.31
Soonchun	17.7 ± 1.10	12.4 ± 0.67	3.7 ± 0.29	33.9 ± 1.55
$LSD_{0.05}$	3.96	2.46	1.09	6.17
Nitrogen form				
$CO(NH_2)_2$	16.6 ± 0.99	12.5 ± 0.66	3.7 ± 0.26	32.8 ± 1.63
$Ca(NO_3)_2$	20.9 ± 1.18	13.6 ± 0.73	4.0 ± 0.34	38.4 ± 1.80
$LSD_{0.05}$	3.08	1.98	0.87	4.86
Nitrogen rate (kgN/10a)				
12	17.5 ± 1.28	9.7 ± 0.55	3.2 ± 0.31	30.4 ± 1.72
18	19.2 ± 1.36	14.7 ± 0.68	4.0 ± 0.30	38.0 ± 1.92
24	20.3 ± 1.57	15.9 ± 0.99	4.5 ± 0.53	40.7 ± 2.66
$LSD_{0.05}$	3.93	2.04	1.05	5.80

†Mean ± standard error

under most soil conditions (Haynes, 1986). However, there are intervals of time in which the NH_4^+-N can constitute the major portion of N available for the plant. Because NH_4^+ can increase water stress due to decrease in root hydraulic conductivity, the NH_4^+-N application to plants may have undergone mild water stress that could decrease cell elongation (Adler *et al.*, 1987).

As expected, top dry matter was increased with increase of the nitrogen fertilizer level from 12 kgN/10a to 24 kgN/10a. In 24 kgN/10a plot, dry matters of leaf and inflorescence were increased by 63.9% and 40.6%, respectively, compared to the 12 kgN/10a plot. When dry matter between 18 kgN/10a and 24 kgN/10a was compared, there was no significant difference even though dry matter in 24 kgN/10a plot was slightly heavier than that of 18 kgN/10a plot. Therefore, it is necessary to consider nitrogen fertilization rate in the aspect of economical efficiency.

Table 5. The composition and contents of essential oil from leaves of *A. rugosa* at vegetative growth stage from different origins.

R. T.†	Component	Mean of GC area %		
		Soonchun	Mokpo	Jindo
5.45	cis-3-hexenal	0.16	0.05	0.11
7.68	1-octen-3-ol	0.48	0.68	0.30
7.77	3-octanone	0.43	0.35	0.17
7.92	alpha-pinene	0.06	0.05	0.04
8.09	beta-myrcene	0.12	0.11	0.07
8.89	limonene	8.11	9.04	5.89
10.20	oct-1-en-3yl acetate	0.46	0.89	0.49
11.90	estragole	88.13	87.42	90.71
15.34	beta-bourbonene	0.02	-	-
15.84	beta-caryophllene	0.79	0.73	1.10
16.62	germacrene-D	0.12	0.20	0.07
16.81	germacrene-B	0.05	0.06	0.06
	unknown compounds	1.07		

†R.T.; Retention time

Essential oil composition

Estragole has been reported as a major component of essential oil of *A. rugosa* grown naturally in Korea including the accessions used in this study (Ahn *et al.*, 1989; Chae *et al.*, 1999; Lee *et al.*, 1994). While field-grown in 1998, it has been revealed that the composition and contents of essential oil almost similar regardless of accessions (Table 5). Limonene (5.9~9.0%) was the second major component after estragole (87.4~90.7%). The minor components included 1-octen-3-ol, oct-1-en-3yl acetate, beta-caryophllene and germacrene-D.

It has been reported that the composition of essential oil can be partially affected by nitrogen form and level. For example, in sweet basil, while N form did not alter the percentage of monoterpenes and aromatic polypropanoides, NH_4^+ increased the total sesquiterpene percentage (Adler *et al.*, 1989). In dragonhead plant, nitrogen fertilization stimulates the formation of the acyclic, monoterpenes. In mint, with increasing the level of fertilizer, the content of menthylacetate, caryophylene, neom-

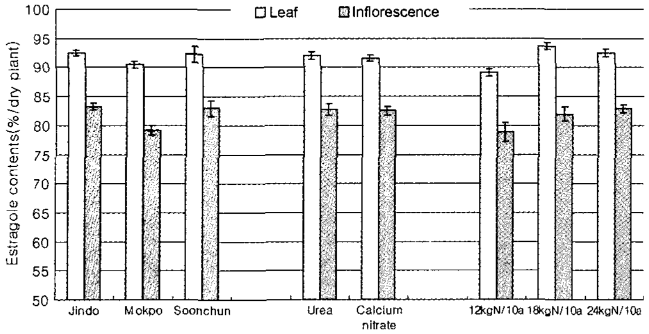

Fig. 1. Estragole contents at full blooming stage among differdent origins of accessions cultivated with different forms and rates of nitrogen in *A. rugosa*. Each bar represents the means ± standard error.

enthol and neoisomenthol was also increased, however, they did not record changes in the menthol content (Singh *et al.*, 1989). However, in these studies, the total amount of major constituents has not been significantly affected by nitrogen form and levels. In this study, estragole contents was not affected by form and rate of nitrogen fertilizer, either. Regardless of accessions, form or rate of nitrogen fertilizer, more than 80% of estragole were contained in both leaf and inflorescence (Fig. 1). While estragole contents was not affected by accessions, form or rate of nitrogen fertilizer, the contents between leaf and inflorescence were significantly different. Estragole content in leaf (91.8%) was 10.5% more than that of inflorescence (81.3%). Therefore, it is necessary to include minor as well as major component when comparing the difference of composition and content of essential oil among the plant organ.

Essential oil content

In order to maximize the essential oil production, essential oil contents per dry weight is also important as much as herbage yields because the essential oil yield is calculated based on herbage yields and its essential oil content. Table 6 shows essential oil contents per dry plant in different origin of accessions and the effect of nitrogen form and rate in *A. rugosa* at full blooming stage. Though essential oil contents in leaves from Jindo (1.53%) and Soonchun (1.59%) collection were more than that of Mokpo (1.24%) collection, oil contents was not significantly affected by accessions. Adler *et al.* (1989), reported that essential oil contents could be changed by nitrogen form. In this study, the significant difference in essential oil contents between urea and calcium nitrate treatment was not observed.

Effect of nitrogen fertilization rate on essential oil contents was previously reported. El-Gengaihi and Wahba (1995) reported that the percentage of volatile oil in dragonhead plant did not change by nitrogen fertilization. On the other hands, Singh *et al.* (1989) reported that oil concentration of mint was the most without nitrogen fertilizer and decreased with increasing nitrogen application, explaining that plants growing with nitrogen fertilization were less mature and at an earlier stage of development than those with no nitrogen. The results from this study indicated that the contents of essential oil of *A. rugosa* had increased by nitrogen application. Particularly, the essential oil contents of inflorescence and the whole above-ground plant were significantly increased by 32.6%, 40.7% respectively, in

Table 6. Essential oil contents at full blooming stage among different origins of accessions cultivated with different forms and rates of nitrogen in *A. rugosa.*

	Stem	Leaf	Inflorescence	Total
		(%)		
Collection site				
Jindo	0.56 ± 0.18[†]	1.53 ± 0.28	2.24 ± 0.18	1.08 ± 0.12
Mokpo	0.30 ± 0.04	1.24 ± 0.11	2.22 ± 0.26	0.87 ± 0.09
Soonchun	0.35 ± 0.05	1.59 ± 0.21	2.14 ± 0.11	1.00 ± 0.10
$LSD_{0.05}$	0.328	0.636	0.580	0.310
Nitrogen form				
$CO(NH_2)_2$	0.46 ± 0.12	1.45 ± 0.15	2.12 ± 0.15	1.02 ± 0.09
$Ca(NO_3)_2$	0.34 ± 0.05	1.46 ± 0.20	2.29 ± 0.15	0.95 ± 0.08
$LSD_{0.05}$	0.276	0.526	0.450	0.259
Nitrogen rate (kgN/10a)				
12	0.33 ± 0.06	1.29 ± 0.13	1.90 ± 0.11	0.81 ± 0.05
18	0.43 ± 0.11	1.44 ± 0.20	2.19 ± 0.11	1.01 ± 0.08
24	0.45 ± 0.16	1.64 ± 0.28	2.52 ± 0.22	1.14 ± 0.13
$LSD_{0.05}$	0.355	0.642	0.474	0.278

[†]Mean ± standard error

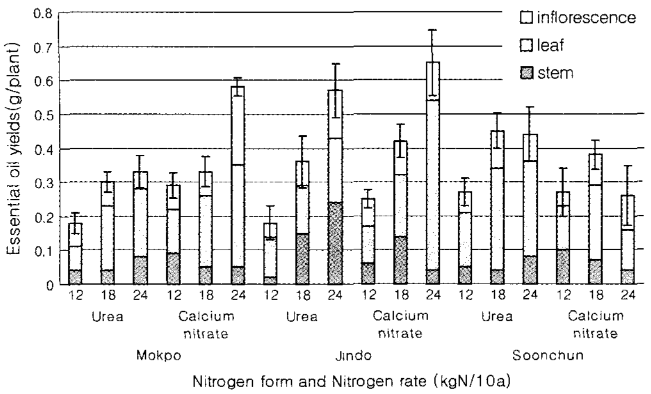

Fig. 2. Essential oil yields at full blooming stage among different origins of accessions cultivated with different forms and rates of nitrogen in *A. rugosa*. Each bar represent the means ± standard error.

24 kgN/10a plot than in 12 kgN/10a plot. *A. rugosa* was well supplied with nitrogen nutrient at full bloom stage because half of fertilization was applied at early reprodcutive stage. It is necessary to study the details in fertilizer application affecting plant growth and essential oil for habituation of *A. rugosa*.

Essential oil yield

The average essential oil yield from a whole dry plant at full blooming stage was 0.4 g per plant; 0.1g from stem, 0.2 g from leaf, 0.1g from inflorescence. As shown in Fig. 2, the essential oil yield was the highest in Jindo collection with calcium nitrate 24 kgN/10a application followed by Mokpo collection with calcium nitrate 24 kgN/10a and Jindo collection with urea 24 kgN/10a. As seen in the essential oil contents, essential oil yield was not affected by accession or nitrogen form but by nitrogen rate. With increasing from 12 kgN/10a to 24 kgN/10a, essential oil yield was increased by 95.8% from 0.24 g to 0.47 g. Probably, the increased level of nitrogen application were resulted in increasing the plant growth and essential oil content simultaneously. However, excessive nitrogen fertilization may result in over-grown plant causing lodging and leaf-drop. Therefore, it is necessary to study optimal level of nitrogen fertilization for the highest essential oil yield.

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