Influence of organic fertilizer on rhizome yield and α-tocopherol content of *Codonopsis lanceolata*

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Summary

Codonopsis lanceolata (Sieb. et Zucc.) Trautv. is a valuable wild vegetable in East Asian countries, especially Korea. We investigated effects of organic fertilizer application on rhizome yield and quality under Andosol soil conditions in the AFC field of Shinshu University. Commercial barnyard manure, which was fermented with crushed bark and beef cattle dung, and Japanese oak leaf mold were applied at the rates of 0, 5, 10, 15, and 20 g m^{-2}. Barnyard manure increased the fresh weight yield of rhizomes but decreased the DL-α-tocopherol (vitamin E) content. The rhizome yield was drastically influenced by the C/N ratio of the soil from the surface to 30 cm below ground level that was controlled by the application ratio of barnyard manure to leaf mold. Total N, soluble P, exchangeable K, Ca, and Mg did not significantly affect the rhizome yield. There was a significant negative correlation between the rhizome fresh weight and vitamin E content at harvest time. The highest vitamin E content of 9-10 mg 100 g^{-1} dry matter was obtained at a leaf mold application rate of 10 g m^{-2}. Nitrogen supply from the soil to plants primarily influenced the yield and quality of rhizomes as vegetables or drugs. The vitamin E content under a higher soil C/N ratio was the highest among commercial vegetables available in common Japanese food markets.

Key words : barnyard manure, C/N ratio, *Codonopsis lanceolata*, leaf mold, rhizome, vitamine E.

1. Introduction

Food safety and nutrition are essential for the consumption of food. Traditional vegetables grown by organic farming are popular in the East Asian market. Organic cultivation of vegetables has increased because organic farming is effective in conserving agricultural environment in addition to maintaining human health.

Codonopsis lanceolata (Sieb. et Zucc.) Trautv. is a perennial climbing herb used as a popular and exotic vegetable that is in large demand as organic food in Korea. However, it has been recognized as a rare and unusual wild plant in mountainous areas of Japan (Figure 1). *C. lanceolata* is chemotaxonomically similar to *C. pilosula* (Wang et al., 1995)[18)], a major source of the traditional Chinese drug, "Dangshen" (Iwai et al., 1992 ; Namba et al., 1992a ; 1992b)[8,11,12)]. *C. lanceolata* is naturally distributed in shady, damp sites in Korea, China, and Japan. Many farmers have domesticated this species for sale as rhizome vegetables, distilled spirits, traditional drugs, or fodder in Korea (Figure 2). The Korean common name of this species is "Deo-Deog." Korean and Chinese populations have been carefully protected by the use of this plant as a useful vegetable and drug for centuries (Park, 1991 ; Pemberton and Lee, 1996 ; Sakamoto, 1998)[13,14,15)].

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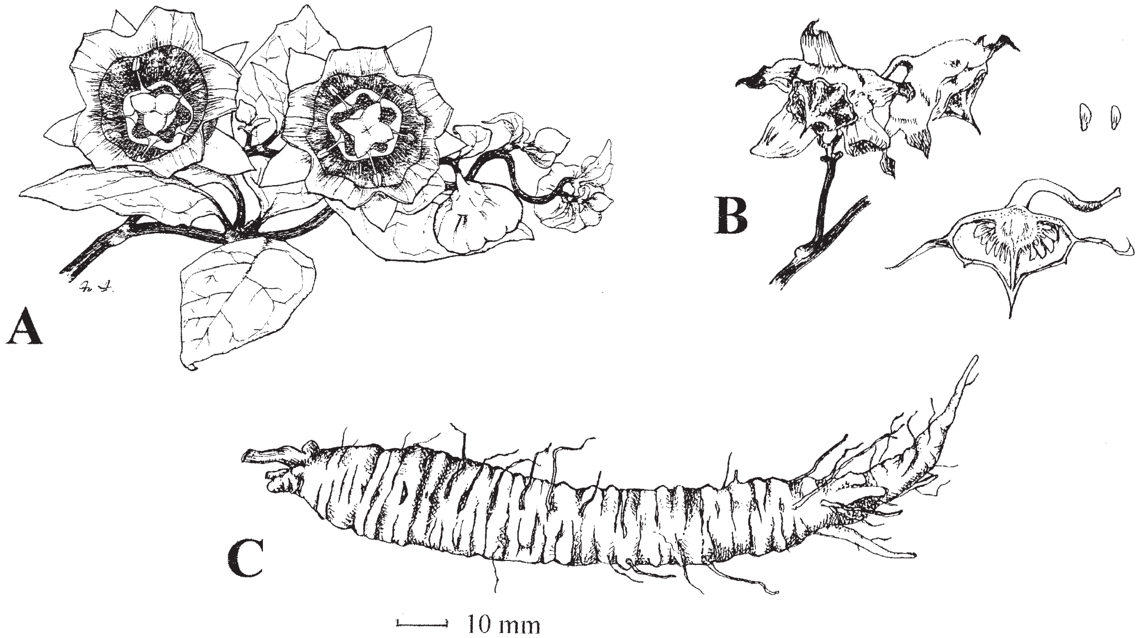
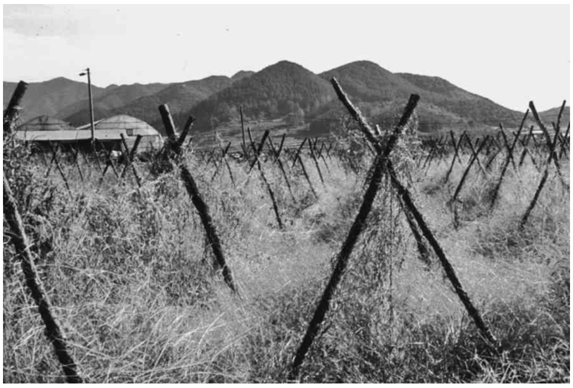


Figure 1 *Codonopsis lanceolata* (Sieb. et Zucc.) Trautv. (family *Campanulaceae*).
A : inflorescence and vine, B : capsule and single seed, C : rhizome.
The plant sample was collected at the mountainous area of Young Weoul in Kangweon-do, Korea, cultivated on the AFC fields and drawn by Inoue.

A



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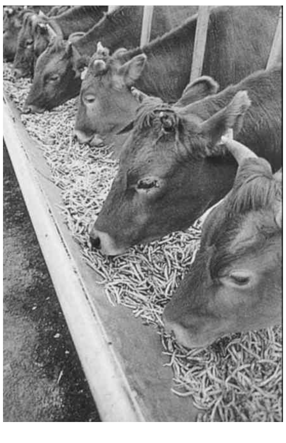
A : Cultivation site in Chinan, Chollabuk-Do, Korea (left).
B : Fresh rhizome as vegetable at Kyungdong market, Seoul, Korea (right).

C

D



E

C : Bulgogi with *Codonopsis lanceolata* in Hoengseong, Kangweon-do, Korea (left).
D : Fodder for Korean native cattle in Hoengseong, Kangweon-do, Korea (center).
E : Distilled spirit in the local food market “Green farm” in Ina, Nagano, Japan (right).

Figure 2 Cultivation and use of *Codonopsis lanceolata* in Korea and Japan.

In addition, *C. lanceolata* has been used as a minor vegetable and for the production of distilled spirits in Central Japan (Figure 2E) (Inoue, 1998a ; 1998b)[5,6]. The Ainu aboriginal people have used this species as a common vegetable, drug, and "magical medicine plant" (Shiraoi Ainu Museum, 1989 ; Annetai et al., 1996 ; Fukuoka and Sato, 1995 ; Hayashi, 1968)[2,4,16]. The Japanese common name of *C. lanceolata* is "Turu-ninjin" ("turu" means vine and "ninjin" means rhizome), and the Ainu names are "Chir-muk" and "Tope-muk" (Inoue, 2003)[7]. In Central Japan, its local names are "Tou-Do," "To-Dog," and "Jii-Sob" (Inoue, 2003 ; Shirado, 1993)[7,17].

The cultivation area of *C. lanceolata* has increased to >1000 ha in South Korea because the edible rhizome has a good aromatic flavor (Lee *et al.*, 1996)[9], delicious texture when chewed, and high anti-oxidant activity (Maeng and Park, 1991)[10]. However, the food chemical composition usually fluctuates with cultivation conditions, and the best soil conditions for producing high-quality rhizomes are still unknown. For producing high-quality vegetables for healthy functional foods, productivity testing is necessary to clarify the effect of the application rate of organic fertilizer on the yield and DL-α-tocopherol (vitamin E) content, which has anti-oxidant activity.

In the present study, we conducted field experiments to investigate the effects of commercial barnyard manure, which was fermented with crushed bark and beef cattle dung, and Japanese oak leaf mold on the rhizome yield and vitamin E content at the end of the first year of cultivation.

2. Materials and Methods

The experimental site was located in the AFC experimental fields of Shinshu University in Minamiminowa Village, Nagano Prefecture (altitude 740 m, 35°N, 138°E). The soil taxonomy was Andosol and texture was clay loam as per the FAO/UNESCO system and by the international system, respectively. Commercial barnyard manure, which was fermented with crushed bark (80%) and cattle dung (20%), and Japanese oak leaf mold were prepared before seeding. Table 1 presents the chemical compositions of the soil, barnyard manure, and leaf mold. The two organic fertilizers (barnyard manure and leaf mold) were applied and plowed in to a depth of 35 cm below the soil surface just before transplantion of seedlings. The application rates were 0, 5, 10, 15, and 20 g m^{-2}. The experiment had a random block design with 4 replications. For all treatments, the plot size was 100 × 100 cm with 15 cm spacing between individuals. The nylon nets were set for climbing of the vines.

A local race was selected from Young Weoul, Kang Weon Province, South Korea, and seedlings with 4 leaves were transplanted into each plot in early May. Rhizomes were harvested in early November.

Table 1 Chemical properties of soil and organic fertilizer.

Constituent	Soil	Organic fertilizer	
		Barnyard manure	Leaf mold
pH (H_2O)	6.3	7.1	6.8
EC (ms)	0.06	2.2	0.5
CEC (me)	22.5	36.7	44.6
Total N	0.5	2.2	0.9
Total C	9.3	23.8	23.8
C/N ratio	17.3	10.9	25.9
Inorganic N (NO_3)	2.4	11.6	0.2
Inorganic N (NH_4)	0.7	5.8	1.8
Soluble P	17.7	787.8	58.9
Exchangeable K	41.6	56.6	25.1
Exchangeable Ca	398.4	302.3	239.1
Exchangeable Mg	50	29.2	18.7

Soil samples were collected to a depth of 30 cm before and after fertilization and then air dried indoor. Total carbon and nitrogen contents of the soil were determined with a CN corder (Yanaco, MT700). Ammonium-N and NO_3-N in the soil were extracted with a 2N KCl solution and measured by Bremner's and Devarda's methods as standard methods of soil science, respectively. Soluble P in the soil was measured by traditional Truog's method. Exchangeable K, Ca, and Mg were extracted with a 1 N acetic ammonium solution and measured by atomic absorption spectrochemical analysis.

The DL-α-tocopherol content of rhizomes was determined by High Performance Liquid Chromatografy (HPLC). Samples were treated with a methanol solution and the extracts were solubilized with methanol and injected in HPLC (silica gel column, normal phase). The quantitative determination of DL-α-tocopherol was carried out by HPLC with fluorescence detector. Column was silica gel HPLC column (Shimazu, Shim-pak, HRC-SIL, length 250 mm, diameter 4.6 mm and particle diameter 5μm). Mobile phase was the solvent with normal hexane 99.5% and isopropanol 0.5%. Flow rate was 1.5ml/min and column temperature was controlled at 30°C. Injection volume was 10μl. The excitation and emission wavelengths of fluorescence detector were settled at 298 nm and 325nm. Stock solution (1000 mg/l) as a standard was obtained dissolving 100mg of DL-α-tocopherol (Sigma-Aldrich) in 100ml methanol. Work solutions (0,3ppm, 0,5ppm, 0,75ppm, 1ppm and 2ppm) were obtained progressively diluting stock solution in methanol.

3. Results

Precipitation at the site was 600 mm, the mean air temperature was approximately10-24°C, and mean solar radiation was approximately15000 J cm^{-2} d^{-1} during the growth period.

The application ratio of barnyard manure to leaf mold drastically influenced the rhizome yield at harvest time. Barnyard manure increased the rhizome yield, whereas fermented leaf mold decreased it. Correlations between soil chemical conditions after fertilization and the rhizome weight at first harvest time were analyzed using all data (Table 2). There was significant negative correlation between the soil C/N ratio and rhizome yield (Figure 3). In contrast, the total N, total C, soluble P, exchangeable K, Ca, and Mg in the soil after fertilization did not significantly affect the rhizome yield. The rhizome yield was drastically influenced by the C/N ratio in the soil from the surface to 30 cm below the surface that was controlled by the application ratio of barnyard manure to leaf mold.

Barnyard manure increased fresh weight of rhizomes; however, it decreased the dl-α-tocopherol content (Figure 4). There was a significantly negative correlation between the rhizome fresh weight and α-tocopherol content ($p < 0.001$). The highest α-tocopherol content (9-10 mg 100 g^{-1} dry matter) was observed at the leaf mold application rate of 10 g m^{-2}. The nitrogen supply from the soil to plants primarily influenced the rhizome yield and their quality for use as vegetables or drugs. The vitamin E content of rhizomes under a higher soil C/N ratio was the highest among commercial vegetables in common Japanese food markets.

Table 2　Correlation between chemical components of soil and rhizome yield.

Constituent	Correlation coefficient	Significance
Total N	0.58	$p < 0.100$
Total C	0.22	
Soluble P	0.47	
Exchangeable K	0.23	
Exchangeable Ca	0.05	
Exchangeable Mg	0.04	
C/N ratio	-0.90	$p < 0.001$

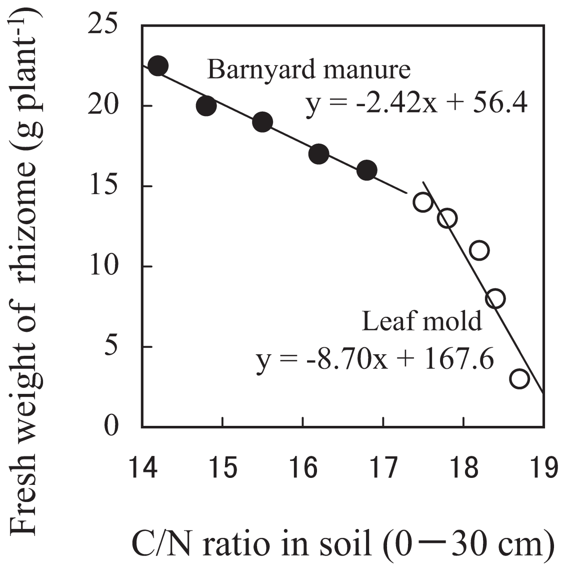


Figure 3 Relationship between the C/N ratio of soil and rhizome yield.

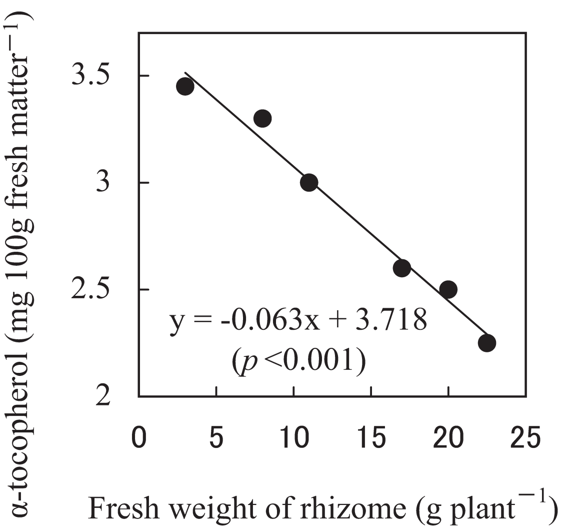


Figure 4 Relationship between the rhizome yield and α-tocopherol content.

4. Discussion

The natural habitat of *Codonopsis lanceolata* is cool, shaded forest floors in mountainous areas in Korea and Japan. In this study, the rhizome yield was high despite growing the plants for only one season and without shade. This suggested that the climate of the experimental field was favorable for vegetative growth and rhizome production. Lee et al. (1996)[9] reported that shading decreases the growth but improves the aromatic constituent content. Our results and those of Lee et al. (1996)[9] demonstrate that shading and a lower soil C/N ratio, which is obtained by use of fermented organic fertilizer, are suitable for producing high-quality vegetables.

In our survey in South Korea (Sakamoto et al., 1998)[15], we found that organic farmers in Kangwon Province, Korea, had developed an appropriate organic fertilizer that was mixed and fermented over 1 year with saw dust, rice bran, and rice chaff. This fertilizer was applied at a rate of 6 g m^{-2} before seeding. In the present study, we determined that the key element in organic manure production is control of the soil C/N ratio at around 17% (in the case of Andosol soil).

Wang et al. (1995 ; 1996)[18,19] reported that *C. lanceolata* is chemotaxonomically similar to *C. pilosula* and has many polysaccharides with immunomodulatory effects. In addition, Maeng and Lee (1991)[10] reported that ethanol extracts of the *C. lanceolata* rhizome have effective anti-oxidant activity that is stronger than the anti-oxidant activity of extracts of *Panax ginseng* CA Meyer. The *C. pilosula* rhizome also has protective action against experimentally-induced gastric ulcer in rats (Wang *et al*., 1997)[20]. On the basis of these reports and our own results, we conclude that these rhizomes are healthy food materials. Although there is a trade-off relationship between the rhizome yield and quality, farming using an organic fertilizer that inhibits soil nitrogen supply and rhizome growth is considered valuable in the food market.

C. lanceolata is a traditional wild vegetable for the Ainu population in northern Japan. Analysis of the chemical composition of wild plants used by the Ainu population revealed that among 67 species, this species has the highest vitamin E content (Annetai et al., 1996)[1]. Annetai et al. (1996)[1] also reported that the vitamin E content of edible wild plants used by the Ainu population was <0.5 mg 100 g^{-1} fresh matter, whereas that of the *C. lanceolata* rhizome was 2.38 mg 100 g^{-1} fresh matter. We found that the dl-α-tocopherol content of small rhizomes produced by fertilization with fermented leaf mold, which increased the soil C/N ratio, was increased. The highest vitamin E content was obtained by applying leaf mold at a rate of 10 g m^{-2}, which resulted in the vitamin E content of 9–10 mg 100 g^{-1} dry matter. The vitamin E content was the highest among commercial vegetables in common Japanese food markets. We believe that C. lanceolata will gradually be accepted as a health food.

Many wild species of the family Campanulaceae are potential sources of functional foods and useful drugs for maintaining health in Asian people. For example, many species of *Platycodon, Campanula,* and *Adenophora* are popular sources of traditional crude drugs and foods. Recently, *Codonopsis lanceolata* has gained attention as a potential functional food with anti-cancer activity in the Korean population and researchers of Korean herbal medicines. Hata et al. (1998)[3] showed that edible wild plants found in Akita, Japan, have differentiation-inducing activity of the human leukemia cell line HL60 and may be used as model systems for drug screening. However, Inoue (2003)[7] reported that the ethanol-soluble fraction from the *C. lanceolata* rhizome has neither the nitroblue tetrazolium reduction activity to the human promyelocytic leukemia cell line HL60 nor neutrophil activity. They also demonstrated that the fraction had no inhibiting effect to melanine synthetic activity of the mouse melanoma cell line B16. Further investigation of other food functional aspect is required in relation to anti-cancer activity for establishing *C. lanceolata* as a functional food in the future.

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References

1) Annetai, M., Ogawa, H., Hayashi, T., Aoyagi, M., Chida, M., Muraki, M., Yasuda, C., Yabunaka, T., Akino, S. and Yano, S. (1996) Studies on wild plants traditionally used by the Ainu people. Part I. Contents of vitamin A, C and E in edible plants. Report of Hokkaido Eisei Kenkyushyo, **46** : 34–39

2) Fukuoka, I. and Sato, S. (1995) Botanical Memories of Ainu. Sofukan, Tokyo. p.177–180

3) Hata, K., Ishikawa, K. and Hori, K. (1998) Differentiation-inducing activities of human leukemia cell line (HL60) by extracts of edible wild plants in Akita. Natural Medicines **52** : 269–272

4) Hayashi, Y. (1968) Foods and life of Ainu people. In Education Board of Hokkaido ed., Ethnological research note of Ainu. 92–113

5) Inoue, N. (1998a) Traditional use of wild plants in Okumibu, Shinshu. Agricultural Technology and Culture, **21** : 159–191

6) Inoue, N. (1998b) Turu-ninjin (*Codonopsis lanceolata*). In Fujimaki, H. ed., Dictionary of biological resources in Japan. Noubunkyo, Tokyo. p.215–216

7) Inoue, N. (2003) Traditional use and quality as food of edible wild plant *Codonopsis lanceolata* Trautv. in the mountainous area of Japan and Korea. The Annals of Environmental Science, Shinshu University, **25** : 29–44

8) Iwai, M., Komatsu, K. and Namba, T. (1992) Pharmacognostical Studies on the *Codonopsis* plants. (3) On the botanical origin of Chinese crude drug "Danshen" derived from sect. *Electae* plant (I). Shoyakugaku Zassi, **46** : 217–223

9) Lee, S., Kim, S., Nam, M., Choi, B. and Lee, S. (1996) Effects of shading and organic matter applications on growth and aromatic constituents of *Codonopsis lanceolata*. Korean Journal of Crop Science, **41** : 496–504

10) Maeng, Y. S. and Park, H. K. (1991) Antioxidant activity of ethanol from Dodok (*Codonopsis lanceolata*). Korean Journal of Food Science and Technology, **23** : 311–316

11) Namba, T., Komatsu, K., Iwai, M. and Xu, G. F. (1992a) Pharmacognostical Studies on the *Codonopsis* plants. (1) On the anatomical characteristics of the root of sect. botanical origin of Chinese crude drug "Danshen" derived from sect. *Codonopsis* plants. Shoyakugaku Zassi, **46** : 156–164

12) Namba, T., Komatsu, K. and Iwai, M. (1992b) Pharmacognostical Studies on the *Codonopsis* plants. (2) On the botanical origin of Chinese crude drug "Danshen" and its related crude drugs derived from sect. *Codonopsis*. Shoyakugaku Zassi, **46** : 165-173. (*Shoyakugaku Zassi = Natural Medicines*)

13) Park, C. and Lee, K. (1991) Production of edible wild plants in Korea. Sun Jin Mum Hwa Sa, Seoul.

14) Pemberton, R. and Lee, N. (1996) Wild food plants in South Korea ; market presence, new crops, and exports to the United States. Economic Botany, **50** : 57-70.

15) Sakamoto, S. (1998) Comparative ethnobiological studies in the area surrounding the Japan Sea, especially in Korea and Japan. Bulletin of the interdicipinary Research Institute of Environmental Sciences, **17** : 79-95

16) Shiraoi Ainu Museum (1989) Chiru-Muck. In Shiraoi Ainu Museum ed., Ainu people and plants. Shiraoi Ainu Museum, Hokkaido 21

17) Shirado, S. (1993) Tou-Do. In Field note. Shogakukan, Tokyo. 46-50

18) Wang, Z. T., Ma, G. Y., Tu, P. F., Xu, G. J. and Ng, T. B. (1995) Chemotaxonomic study of *Codonopsis* (family *Campanulaceae*) and its related genera. Biological Systematics and Ecology, **23** : 809-812

19) Wang, Z. T., Ng, T. B., Yeung, H. W. and Xu, G. J. (1996) Immunomodulatory effect of a polysaccharide -enriched preparation of *Codonopsis pilosula* root. General Pharmacology, **27** : 1347-1350

20) Wang, Z., Du, Q., Xu, G., Wang, R., Fu, D. and Ng, T. (1997) Investigation on the protective action of *Codonopsis pilosula* (Dangshen) extract on experimentally-induced gastric ulcer in rats. General Pharmacology, **28** : 469 -473