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Original Article

Effect of Different Fertilizer Ratio and Planting Dates on Growth and Acanthoside D content of *Acanthopanax divaricatus* and *Acanthopanax koreanum*

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Abstract

The cultivation methods of Acanthopanax in Korea need to be optimized. Hence, this study investigated the effect of different fertilizer ratios and planting dates on the growth and acanthoside D content of two (2) Acanthopanax species. The current recommended fertilizer rate of 10.5-8.5-8.5 kg/ha^{-} (N-P_2O_5-K_2O, respectively) produced the best plant growth of Acanthopanax. For the first year, the acanthoside D content resulting from the 2P (2x phosphate) rate was higher than that from the other fertilizer ratios, yet there were no significant differences resulting from the various treatments for either *Acanthopanax divaricatus* or *Acanthopanax koreanum*. Similarly, for the second year, there were no significant differences in the acanthoside D content resulting from the various fertilizer ratios, although for both species the acanthoside D content resulting from the 2P rate was slightly higher than that from the other treatments. Therefore, the results indicated that doubling the amount of phosphate increased the acanthoside D content. Plus, the optimum planting date with respect to growth and productivity for *Acanthopanax divaricatus* was identified as April 15.

Keywords : *Acanthopanax divaricatus, Acanthopanax koreanum*, Fertilizer, Planting date

Introduction

Suitable soil (shallow, well-drained sandy loam) and growing conditions for Acanthopanax have already been established in Korea (Kim et al.1991; Park et al. 1993; Yu 2002; Lim 2005). In fact, 2 native species of Acanthopanax namely: *Acanthopanax koreanum* and *Acanthopanax chiisanensis* have been found on Jeju island. *Acanthopanax koreanum* was found growing on Jeju island in coastal areas to elevated valleys or forest (1,400 m ASL) (Kim 1997). Plus, the cultivated variety *Acanthopanax divaricatus* grows well on the plains of mainland Korea. Information about cultivation methods for Acanthopanax are currently disseminated by the Rural Development Administration (RDA) (Han et al. 1999), where slightly acidic soils (pH 5.7) with good drainage, rich in organic matter, phosphorus, and shading (22.7~37.2%, mostly from forest trees) are the optimum growing conditions. Most researches on Acanthopanax has been focused on the effect of shading/light cover (Han et al. 2001; Kim et al. 2003). Thus, there is a lack of information about the effects of other cultivation methods.

Ovdov et al. (1965) were the first to study compounds (eleutheroside A~G) from *Eleutherococcus senticosus* (previous scientific name of Acanthopanax), and various compounds, such as lignans, coumarin, triterpenoids, and phenolics, have since been reported (Ovdov 1967; Yook 1977; Wagner et al. 1982; Hahn et al. 1984). Brekhman and Dardymov (1969) originally reported on the anti-stress and anti-fatigue effects of eleutheroside compounds isolated from *Acanthopanax senticosus*. These effective components were found to have "adaptogenic" activity and increase body immunity against stress. In particular, acanthoside D (eleutheroside E) was found to havethe highest biological effect among lignans isolated from *Acanthopanax* spp. Plus, Lee et al. (2001) refined acanthoside D from *Acanthopanax chiisanensis*, while Hong et al. (2001) did the same from domestic and imported Acanthopanax.

Accordingly, this study investigated how the cultivation method of Acanthopanax impacts the content of acanthoside D, a major component of the therapeutic and/or pharmaceutic efficacy of Acanthopanax. Specifically, the effects of the fertilizer rate and planting date on the acanthoside D content of *Acanthopanax koreanum* and *Acanthopanax divaricatus* were investigated.

Materials and Methods

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Cultivated soil

Soil samples (10 cm deep) were collected at 10 random sites in the field before the experiment and formed into 1 composite sample. The soil analysis was conducted at the Yeongcheon Agricultural Technology and Extension Center following RDA soil chemistry analysis methods. The soil pH was measured at a 1:5 soil-water diluted ratio using a pH meter. The organic matter analysis was conducted using the method of Tyurin and the available phosphate then measured using a spectrophotometer (UV-1240, SHIMADZU). The exchangeable cations were quantified using an atomic absorption spectrophotometer in accordance with the method of Brown (Table1).

The field used in this study had a mature soil, good for planting ordinary crops, with a higher acidity than mountain soil pH (pH 5.0~6.0) where the Acanthopanax tree normally grows. The available phosphorus content was also very high, yet because the cultivation soil was mostly upland soil, the experimental field was deemed similar to regular planting conditions.

Materials and management

The experiment used two-year-old saplings of *Acanthopanax divaricatus* and *Acanthopanax koreanum* obtained from an Acanthopanax farm (Cheonan, Korea). The saplings were planted during the first year after pinching (30 cm), soil tillage, soil preparation, and soil compost treatment (1500 kg/10a). The recommended fertilizer was used ($N-P_2O_5-K_2O$: 21-17-17, 50 kg/10a). The growth characteristics of the plants (length and fresh-weight) were examined. Additional fertilizer ($N-P_2O_5-K_2O$: 21-17-17, 50 kg/10a) was applied during the second year. The harvest was performed except for the pinching part. Harvested stems were divided to lower part and upper part with central stem as the center. After stems were collected, stems were dried at room temperature, and ground into powder for acanthoside D analysis

Cultivation factors

(1) Fertilizer rate

Standard treatments were used in this experiment (10.5-8.5-8.5, $N-P_2O_5-K_2O$, kg/10a), treatment 1 (21.0-8.5-8.5, $2N-P_2O_5-K_2O$, kg/10a), treatment 2 (10.5-17.0-8.5, $N-2P_2O_5-K_2O$, kg/10a), treatment 3 (10.5-8.5-17.0, $N-P_2O_5-2K_2O$, kg/10a), and treatment 4 (21.0-17.0-17.0, $2N-2P_2O_5-2K_2O$, kg/10a).

(2) Planting date

The planting was performed 3 times (March 30, April 15, and April 30). The standard spots were 3m×3m with a 1m space between the plants and 18 plants in 1 spot. The experiment design was split plots and replicated 3 times.

Acanthoside D content analysis

(1) Purification of acanthoside D reference material

The acanthopanax stem powder was extracted three times with methanol and suspended in water : n-butanol solvent. Acanthoside D was then isolated from the supernatant layer using open column chromatography (7 X 60 cm, No. 7734). The structures of the isolated compounds were identified by means of FAB-MS, ^{1}H-NMR, and ^{13}C-NMR spectrometry using Acanthoside D as the reference standard (HPLC-grade >97% purity).

Acanthoside D: FAB-MS: m/z 743 [M + H]+; ^{1}H-NMR (500 MHz, DMSO-d6): δ6.67 (4H, s, H-2',6'), 4.88 (2H, d, J = 7.3 Hz, glucosyl H-1), 4.67 (2H, d, J = 3.6 Hz, H-2), 4.28 (2H, dd, J = 8.5, 6.6 Hz, H-4eq), 4.20 (2H, dd, J = 8.5, 3.0 Hz, H-4ax), 3.76 (12H, s, 4 × OMe), 3.19 (2H, m, H-1); ^{13}C-NMR (125 MHz, DMSO-d6): δ153.2 (C-3',5'), 138.1 (C-4'), 134.1 (C-1'), 104.6 (C-2',6'), 103.3 (Glc C-1), 85.7 (C-2), 77.5 (Glc C-5), 76.7 (Glc C-3), 74.5 (Glc C-2), 72.1 (C-4), 70.2 (Glc C-4), 61.2 (Glc C-6), 57.0 (OMe), 54.2 (C-1) (Figure 1).

(2) Production of standard solutions

Different standard concentrations of acanthoside D were prepared in 50% methanol. Acanthoside D standards were used to establish a calibration curve based on an equation of Y=128.24+165436X (n=6), R^2=0.9998 (Figure 2).

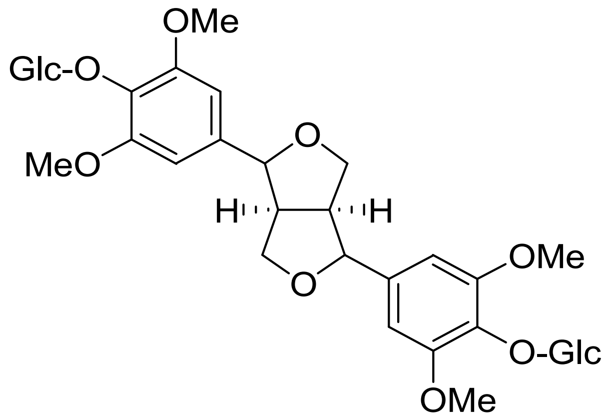

Figure 1. Chemical structure of acanthoside D.

(3) Analysis of acanthoside D

The acanthopanax stem powder was extracted with 50% methanol. The extracts were then filtered through a 0.45 membrane filter (PALL Pall Corporation, Washington, NY, USA) and analyzed using a Gilson 305 HPLC system (Middleton, UK).

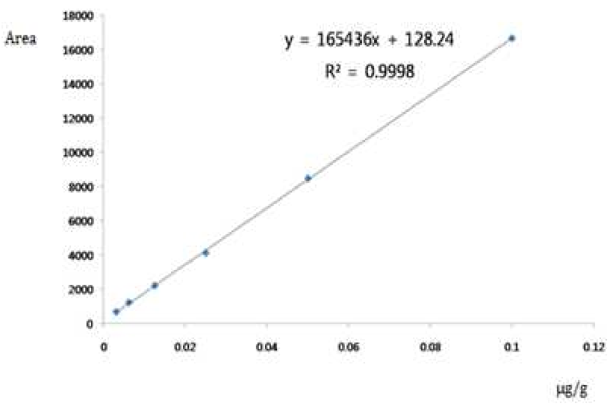

Figure 2. Acanthoside D standard calibration curve

Table 1. Soil conditions in experimental field.

pH	O.M(%)	P_2O_5 (mg/kg)	Ex. Cation ($cmol^+$/kg)			EC (ds/m)
			K	Ca	Mg	
7.0	3.4	435	0.36	3.0	1.2	0.07

The separation of the extracts was performed using a Nucleodur 100-5 C_{18} column (4.6×150mm, 5㎛). The detection wavelength was 210 nm (Gilson UV 119, Middleton, UK). A gradient elution of water and acetonitrile (H_2O : ACN) was used starting at 100 : 0, followed by 75 : 25 for the next 25 mins, with a flow rate of 1 mL/min. The injection volume was 10㎕(Kim 2006).

Results

Effect of different fertilizer ratios on growth and acanthoside D content of *Acanthopanax divaricatus* and *Acanthopanax koreanum*

The response to the fertilizer treatments varied, mainly due to varietal differences between *A. divaricatus* and *A. koreanum*. Superior agronomic characteristics were observed when applying the standard or recommended fertilizer rate (N-P-K) to *A. divaricatus* in both planting years (Table 2). However, treatment with 2x the rate of nutrients (2N-2P-2K) resulted in low stem height and weight values, while doubling the rate of potassium application (N-P-2K) resulted in a significantly lower mean stem weight (124.8 g) than the standard (143.2 g). For the first year of *A. koreanum* planting, the standard fertilizer rate resulted to highest mean stem height and weight, while the values from the N-P-2K treatment were the lowest. For the second year of planting, the 2N-2P-2K treatment resulted in the highest stem height (1078.1 cm), while the standard treatment produced the lowest stem height(890.2 cm). The stem weight was the highest with the N-2P-K treatment (321.0 g) and the lowest with the 2N-P-K treatment (295.4). In general, there were no significant differences among the treatments based on the response of *A. divaricatus* and *A. koreanum* to different fertilizer ratios. The standard fertilizer rate produced in a good agronomic response from *A. divaricatus* for both years, whereas it only showed favorable results from *A. koreanum* during the first year.

The different fertilizer treatments caused statistically-insignificant differences in the acanthoside D content for the first year of cultivation (Table 3). However, thedoubled-rate of phosphorus (N-2P-K) produced the highest acanthoside D content in *A. divaricatus* and highest content in the lower part of *A. koreanum*. The standard fertilizer rate produced the lowest content in both parts (2.49 mg/g upper, 2.72 mg/g lower parts) of *A. divaricatus*. In case of *A. koreanum*, the lowest acanthoside D content was observed in the upper part (1.09 mg/g) when treated with 2N-2P-2K and the lower part(1.08 mg/g) at 2N-P-K

Similarly, for the second year, the different fertilizer ratios resulted to no significant differences with the standard rate in terms of acanthoside D content (Table 4). However, the 2N-2P-2K treatment produced the lowest acanthoside D content in both parts of *A. divaricatus*. The highest acanthoside D content was obtained from the upper part of *A. koreanum* after treatment with 2N-P-K (1.22 mg/g), while it was significantly reduced to ~50% with the N-2P-K treatment (0.61 mg/g). The lower part of *A. koreanum* produced the highest acanthoside D content with the N-2P-K treatment (1.37 mg/g) and the lowest with the 2N-2P-2K treatment (0.88 mg/g).

Overall, the agronomic response of *A. koreanum* was relatively varied/ unstable compared to that of the cultivar *A. divaricatus*. The acanthoside D content was slightly influenced, yet not significantly affected by the fertilizer treatments.

Effect of different planting dates on growth and acanthoside D content of *Acanthopanax divaricatus* and *Acanthopanax koreanum*

The different planting dates resulted in significant differences for both *A. divaricatus* and *A. koreanum* (Table 5). For *A.*

Table 2. Effect of different fertilizer ratios on mean stem height and weight of *Acanthopanax* spp. (n=27)

Fertilizer	*A. divaricatus*				*A. koreanum*			
	First year		Second year		First year		Second year	
	Height (cm)	Stem Weight (g)	Height (cm)	Stem Weight (g)	Height (cm)	Stem Weight (g)	Height (cm)	Stem Weight (g)
2N-P-K	47.3a	74.8a	259.9a	131.3ab	263.5a	237.0a	909.3a	295.4a
N-2P-K	65.9a	79.7a	270.0a	136.8ab	267.0a	258.0a	977.2a	321.0a
N-P-2K	58.5a	82.7a	244.7a	124.8b	240.9a	233.7a	925.0a	302.4a
2N-2P-2K	43.5a	68.7a	243.3a	137.9ab	281.3a	256.5a	1078.1a	310.7a
N-P-K	68.3a	82.2a	278.1a	143.2a	294.3a	263.7a	890.2a	301.7a

Means within same column with same letter are not significantly different at 5% level by Duncan's New Multiple Range Test (DNMRT).

Table 3. Effect of different fertilizer ratios on acanthoside D content of *Acanthopanax* spp. in first year. (n=6)

Fertilizer	Acanthoside D (mg/g)			
	A. divaricatus		*A. koreanum*	
	Upper part	Lower part	Upper part	Lower part
2N-P-K	2.50 ± 0.03a	2.85 ± 0.09a	1.32 ± 0.58a	1.08 ± 0.32b
N-2P-K	2.96 ± 0.03a	3.14 ± 0.27a	1.31 ± 0.42a	1.54 ± 0.32a
N-P-2K	2.68 ± 0.40a	3.00 ± 0.28a	1.07 ± 0.30a	1.42 ± 0.15ab
2N-2P-2K	2.83 ± 0.74a	3.01 ± 0.59a	1.09 ± 0.16a	1.42 ± 0.26ab
N-P-K	2.49 ± 0.68a	2.72 ± 0.58a	1.31 ± 0.46a	1.49 ± 0.32a

Means within same column with same letter are not significantly different at 5% level by Duncan's New Multiple Range Test (DNMRT).

Table 4. Effect of different fertilizer ratios on acanthoside D content of *Acanthopanax* spp. in second year. (n=6)

Fertilizer	Acanthoside D (mg/g)			
	A. divaricatus		*A. koreanum*	
	Upper part	Lower part	Upper part	Lower part
2N-P-K	1.63 ± 0.66a	3.53 ± 0.47a	1.22 ± 0.20a	1.13 ± 0.08ab
N-2P-K	1.90 ± 0.80a	3.39 ± 0.51a	0.61 ± 0.10b	1.37 ± 0.24a
N-P-2K	1.60 ± 0.33a	4.30 ± 0.42a	1.21 ± 0.10a	1.04 ± 0.31ab
2N-2P-2K	0.80 ± 0.11a	3.20 ± 0.39a	0.75 ± 0.14b	0.88 ± 0.21b
N-P-K	1.12 ± 0.32a	3.46 ± 0.79a	0.98 ± 0.41ab	1.18 ± 0.11ab

Means within same column with same letter are not significantly different at 5% level by Duncan's New Multiple Range Test (DNMRT).

divaricatus, early planting (March 30) resulted in inferior agronomic characteristics in both cultivation years. However, planting on April 15 (mid) produced the highest stem height and weight in the first year, and the same response was observed for late planting (April 30) in the second year. Meanwhile, for *A. koreanum*, late planting caused a poor agronomic response in both years. Yet, good agronomic characteristics were roduced with early planting in the first year, while mid-planting (April 15) produced the highest stem height (716.0 cm) and early planting (March 30) produced the highest stem weight (277.1

Table 5.Effect of different planting dates on growth of *Acanthopanax* spp. (n=27)

Planting date	*A. divaricatus*				*A. koreanum*			
	First year		Second year		First year		Second year	
	Height (cm)	Stem Weight (g)	Height (cm)	Stem Weight (g)	Height (cm)	Stem Weight (g)	Height (cm)	Stem Weight (g))
March 30	46.7b	81.7b	164.3b	99.9b	254.0a	276.4a	579.2b	277.1a
April 15	94.6a	105.0a	239.3a	110.6ab	242.0a	244.2ab	716.0a	272.4a
April 30	67.8b	101.3a	246.3a	120.4a	173.4b	225.6b	565.7b	259.1a

Means within same column with same letter are not significantly different at 5% level by Duncan's New Multiple Range Test (DNMRT).

Table 6.Effect of different planting dates on acanthoside D content of *Acanthopanax* spp. in first year. (n=6)

Planting date	Acanthoside D (mg/g)			
	A. divaricatus		*A. koreanum*	
	Upper part	Lower part	Upper part	Lower part
March 30	2.22 ± 0.16a	3.10 ± 0.01a	0.93 ± 0.29a	1.30 ± 0.23a
April 15	2.53 ± 0.14a	2.66 ± 0.30a	0.87 ± 0.29a	1.16 ± 0.24a
April 30	2.58 ± 0.07a	2.78 ± 0.12a	0.97 ± 0.27a	1.32 ± 0.30a

Means within same column with same letter are not significantly different at 5% level by Duncan's New Multiple Range Test (DNMRT).

Table 7.Effect of different planting dates on acanthoside D content of *Acanthopanax* spp. in second year. (n=6)

Planting date	Acanthoside D (mg/g)			
	A. divaricatus		*A. koreanum*	
	Upper part	Lower part	Upper part	Lower part
March 30	1.42 ± 0.08a	3.06 ± 0.15a	0.79 ± 0.19a	1.18 ± 0.09a
April 15	1.80 ± 0.40a	3.51 ± 0.63a	0.77 ± 0.14a	0.93 ± 0.06b
April 30	1.37 ± 1.01a	3.29 ± 0.47a	0.83 ± 0.20a	1.15 ± 0.12a

Means within same column with same letter are not significantly different at 5% level by Duncan's New Multiple Range Test (DNMRT).

g) in the second year.

For the first year of evaluation, the acanthoside D content was not significantly influenced by the planting date (Table 6). The highest content in the upper part (2.58 mg/g) of *A. divaricatus* was observed when planted late (April 30). Early planting (March 30) resulted in the lowest acanthoside D content (2.22 mg/g) in the upper part and the highest in the lower part (3.10 mg/g) of *A. divaricatus*. Late planting (April 30) resulted in the highest acanthoside D content in both parts of *A. koreanum*, while mid-planting (April 15) produced the opposite response. Similarly, the evaluation after the second year of cultivation only showed a slight influence of the planting date on *A. koreanum* (Table 7). Planting on April 15 (mid) produced the lowest acanthoside D content (0.93 mg/g) in the lower part, while the highest content (1.18 mg/g) was obtained with early planting (March 30). Late planting produced the highest acanthoside D content in the upper part (0.83 mg/g),while mid-planting resulted in the lowest acanthoside D content (0.77 mg/g). Mid-planting (April 15) resulted in the highest acanthoside D content in both parts of *A. divaricatus*.

Thus, the overall effect of the planting date was more stable on *A. divaricatus* than on *A. koreanum*. In general, early planting and late planting resulted in a less favorable agronomic response from *A. divaricatus* and *A. koreanum*, respectively. The effect of the planting date to *A. koreanum* only had a slight effect on the acanthoside D content in the second year, yet not in the first year of cultivation.

Discussion

The present study attempted to provide basic information necessary for the effective growth of *Acanthopanax divaricatus* and *Acanthopanax koreanum*, two commonly grown varieties of *Acanthopanax* spp. on the Korean peninsula and Jeju Island. Thus, the acanthoside D content, a major ingredient for the growth and efficacy of *Acanthopanax*, was investigated relative to the amount of fertilizer applied and the date of planting. No significant differences were found in the growth of *Acanthopanax divaricatus* and *Acanthopanax koreanum* after treatment with different ratios of fertilizer, while the conventional fertilization provided different results. In the case of *Acanthopanax divaricatus*, the amount of acanthoside D was higher with the phosphate double ratio treatment. Thus, increasing the phosphate fertilizer can increase the acanthoside D content in the plant. In addition, planting on April 15 produced a higher plant height and fresh weight. Thus, when considering the conventional planting date for *Acanthopanax divaricatus* in late April, planting a little earlier will help improve the plant growth.

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