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INFLUENCE OF NITROGEN FERTILIZER AND CULTIVAR METHODS ON YIELD AND ACTIVE COMPOUND OF ANGELICCA DAHURICA PLANT IN VIET NAM

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Abstract

In three multifactor (N, P, K) nitrogen factor (N) is one of important major influence to yield and quality of Angelica Dahurica plant. The goal of this study was to choose the nitrogen fertilizer formula that is suitable for growth and development of Angelica Dahurica plant in norther, Vietnam. The result indicated that application of 175 kg N/ha could help Angelica Dahurica plant to achieve high yield and quality than other nitrogen dosages with imperatorin content is highest at 0.35%. This nitrogen dosage also could be recommended to obtain the high-quality Angelica Dahurica medicinal material in the future.

Keywords: *Angelica dahurica Benth.et Hook F, Nitrogen dosage on growth, yield, HPLC, Imperatorin*

INTRODUCTION

Angelica dahurica (*Angelica dahurica* Benth.et Hook F.) is a precious medicinal plant in traditional medicine and a member of the Umbelliferae family, also known other name as Bai zhi, and was found in Korea, China, Japan and Russia (Li et al. 2014a, b). The plant in field and medicinal from root of *Angelica dahurica* was described as Fig 1A, B and C

A B C

Figure 1. Angelica Dahurica plant **A** Angelica Dahurica plant experiment. **B** Angelica Dahurica plant in the Nitrogen fertilizer treatment. **C** Angelica Dahurica root was used in traditional Vietnam medicine

The dried root of *Angelica dahurica* used commonly Viet Nam traditional medicine for treating wind-cold, headache, fever, toothache and rheumatic arthralgia (Vo V.C. 2012).
There are two main indicators for evaluating the medicinal quality of Angelica dahurica. One is the

traditional product shape indicators, including the shape of Angelica root, root length, thickness, section properties, powder color, physical and chemical properties and other appearance and shape indicators. The other is the active ingredient content of Angelica dahurica, that is, the content of coumarin (imperatorin, isoimperatorin), volatile oil and other components. *Angelica dahurica* compound preparations have antipyretic, analgesic and anti-inflammatory effects, which can dispel wind and cold, dry dampness and discharge pus, and relieve pain and here this reason why medicinal plants become the important medicinal plant. In addition, numerous of research studied that *Angelica dahurica* effect on photosensitization, antihypertensive, exercise and respiratory center, antibacterial anticancer and toxic (Marumoto and Miyazawa 2010).

In addition, some research also carried out the content determination and composition analysis of the volatile oils in different commercial *Angelica dahuricas*. In depth, the content of the active ingredients of Angelica dahurica begins to dominate in evaluating the quality of Angelica dahurica. Scholars have found that there are certain differences in the active components of Angelica dahurica products from different producing areas (Deng Jieyuan et al. 2004).

In recent years, the research on the standardization of production quality management of *Angelica dahuricas* (GAP) is being carried out. Fertilization is beneficial to increase the yield of Angelica dahurica (Pu Shengcai et al. 2006), and different types of fertilizers will cause nutritional differences in Angelica dahurica, resulting in different growth and yield of Angelica dahurica (Zuo Huifang et al. 2003). Previous studies have mainly focused on the effect of fertilization on the yield-increasing effect of Angelica dahurica (He Shijian et al. 2004) but there is no report on the effect of increasing the yield of Angelica dahuricae; Lai Hongliang found in the fertilization research of Angelica show that nitrogen and phosphorus fertilizers have a significant effect on the medicinal components, while potassium fertilizers have little effect. The results showed that nitrogen had a negative effect on the accumulation quality of *Angelica dahuricas*. With the increased or decreased of nitrogen application, there is no report on systematic research on the absorption and utilization of fertilizer by Angelica dahurica. In order to realize standardized planting in production area and effectively ensure the stability and controllability of *Angelica dahuricas* quality, it is necessary to control each link in the production process of Qibaizhi—especially fertilization measures (Zuo Huifang et al. 2003) on the yield and quality of medicinal materials. Liu Daguang et al. found that the effect of fertilization on the quality of white chrysanthemum under potted conditions was nitrogen > phosphorus and heptapotassium. Nitrogen and phosphorus were mixed, but it may be related to the imbalance of nutrient supply due to excessive fertilization (Li Qunxue et al. 1999).

The basic raw material for the production of *Angelica dahuricas* is the root extract and Fertilizer and cultivar play a major role to yield and quality of *Angelica dahuricas* medicinal content, study on *Angelica dahuricas* plant are very limited and *there are still* no scientific reports *yet* in Northwest provinces in Viet Nam. Therefore, how to applica of N, P, K fertilization are appeared to be quite important for the production of high amount of quality raw material is necessary to improve the commercial cultivation process in the future for higher yields and qulity of leaves and steviol glycosides in the norther VietNam conditions. In this research, we study the effect of the Nitrogen fertilizer amount *of Angelica dahuricas* plant medicinal Norther Viet Nam province and the formula level of fertilizer had select 175 kg Nitrogen (N) per hectare was suitable for quality of *Angelica dahuricas* plants.

MATERIALS AND METHODS

Plant material and Experimental Site

This study was performed from October 2020 to September 2021 at Research centre of Medicinal plants (20°53 N, 105°44E, 20 m above mean sea-level) Ha Noi, Viet Nam. The experiment soil was selected from 0 to 40 cm soil depth with pH 6.62 before design and was analyzed in laboratory of Soils and Fertilizers Research Institute (SFRI), Viet NAM (table 1).

Table 1: The soil basic nutrient content in the *Angelica dahurica* experimental filed

Depth of Soil (cm)	Oganic matter (mg/100g)	Total N (N%)	Total P - (P2O5%)	Total K- (K2O%)	Available N (mg/100g)	Available P (mg/100g)	Available K (mg/100g)
0-40	13,9	0,15	0,17	0,79	0,63	1,93	4,18

Seedling of Angelica dahurica was provided by National Institute of Medicinal Materials, Quang Trun, Hoan Kiem, Ha Noi, it was regarding productive performance in the Ha Noi climatic conditions of the North region of Viet Nam.

EXPERIMENTAL METHODS

The experimental design for Nitrogen fertilizer experiment was in randomized blocks to evaluating the effect of fertilizers formula with three replications, which was included: (N0) 0 kg N as control; (N1) 50 kg N; (N2) 75 kg N; (N3) 100 kg N (N4) 125 kg N; (N5) 150 kg N; (N6) 175 kg N; (N7) 200 kg N (The combination was supported after Analyze the nutrient content of stevia plants were token from the soil in Norther region of Viet Nam) respectively for each hectare. Fertilizers: pre plant application (100 Kg of superphosphate P2O5 ha-1) + (40 kg KCl ha-1); Tillering application, 40kg KCl ha-1for two times; plant density were specified as 30cm × 30cm for all experiment. Three managements were set for each level and the formula application was adopted follow as: Basal fertilization were applied at the time of planting: 25% N + 50% K20 + 100% P205; Top-dressing fertilizer: phase 1 was applied at 60 days after planting (Nitrogen solution 1%N); phase 2 (90 DAP) 25% N; phase 3 (120 DAP): 25% N + 25% K20; phase 4 (150 DAP) 25% N + 25% K20. A half dose of N and full dose of P and K as per treatment were applied at the time of planting, while remaining half dose of N was applied at 45 days after transplanting. Area of each plot was set 10 m^2. The total area of experiment was 240 m^2.

GROWTH AND DEVELOPMENT ANALYSIS

Growth indexes included Plant height and root stem diameter of *Angelica dahurica* plants were recorded at harvest. The plant height was measured with a meter ruler from ground to the base of the fully opened leaf

YIELD ANALYSIS

The roots stem diameter was measured with slide calipers up to 0.01 mm accuracy. Other hand, Biomass yield (total fresh tuber), fresh tuber yield, and dry tuber yield were determined in each plant. We estimated the fresh biomass, fresh and dry tuber yield per plant using one digital scale with precision of 0.01 g. Roots were dried at 45°C temperature in hot air dryer for 24 hours and stored in clean gunny bags. The total weight of dry root of each treatment were dry weight. At this temperature, the quality of dried roots produced was prepared for next experiment.

REAGENTS AND CHEMICAL

For determination of furanocoumarin content (Imperatorin) of *Angelica dahurica*, Instrument and Reagent were used include: Waters Acquity UPC2 USA, HPLC, EmPower Color Spectrum Management System, PL203 electronic balance, KQ-5200DE numerical control ultrasonic cleaner, 5µl microliter syringe and 0.45 micro-perforated filter membrane as described (Shiau et al. 2011). Acetonitrile and dichloromethane were both chromatographically purified. The Imperatorin (98%) reference was provided by Solar Energy Co, Taiwan.

CHROMATOGRAPHIC ANALYSIS CONDITION

ODS hypersil (Thermo scientific) column (UPC²2-EP 250 mm×4,6 mm, 5 µm), acetonitrile and ultrafilterred water were the mobile phase (70:30 v/v), flow rate was 1.0 ml min^{-1}, column temperature was 28^0C; ELSD parameters: drift tube temperature 70^0C, spray tube temperature 25^0C, nitrogen gas flow rate was 1.5mL min^{-1}.

SOLUTION PREPARATION

Test article solution preparation: 0.5 g powder of *Angelica dahurica root* was crushed and transferred to bolt containing 50 ml of 70 % ethanol and then filtered by Sieve 710 through precise weighing, and added 50 ml mobile phase through precise weighing, soaked the powder of root *of Angelica dahurica* for 2 hours and placed it in ultrasonic treatment for 30 minutes and then taken out and cooled and weighed. After all, compensated the weight loss using mobile phase, filtered with 0.45 micro-perforated filter membrane, and finally took the subsequent filtrate as the finish solution. And then, the supernatant was selected as the sample.

ROOT EXTRACTS AND MEASUREMENT OF FURANOCOUMARINS (IMPERATORIN) CONTENT (USP- HTTP://HMC.USP.ORG)

The logarithms of peak responses were plotted to compare with the logarithms of imperatorin content in mg mL^{-1} from the standard solutions and the regression line was determined using a least-squares analysis; or, a linear regression equation was established using a least-squares analysis according to the logarithms of the peak responses versus logarithms of imperatorin in mg mL^{-1} from the standard solutions.

The concentration, C, in mg mL^{-1}, was determined by regression line of the relevant analyze in the sample solution or linear regression equation. Imperatorin percentages in *Angelica dahurica* were calculated separately follow as:

$$X\ (\%) = \frac{C\ X\ 100\ X\ 10\ X\ 100}{w\ X\ 1000\ X\ (100-A)} X \quad 100 = \frac{C\ X\ 10000}{w\ X\ (100-A)}$$

Explanation:

X= Imperatorin content (%)

C= Concentration of the relevant analyze in the sample solution as determined above (mg mL^{-1})

A= volume of the sample solution (oC)

W= weight of *Angelica dahurica* root taken to prepare the sample solution (mg)

STATISTICAL ANALYSIS

Statistical data analysis using IRRISTAT5.0 and Excel 2007 software, and then strain and variety fertilizing amount were included as experimental factor to calculate the significant difference of

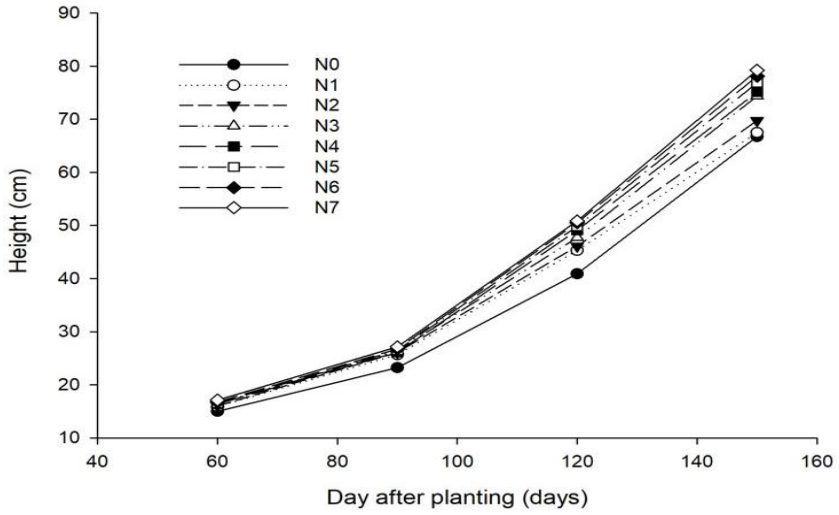
imperatorin content in *Angelica dahurica*.

RESULTS AND DISCUSSION

Effect of nitrogen fertilizer doses on growth indexes of *Angelica dahurica*.

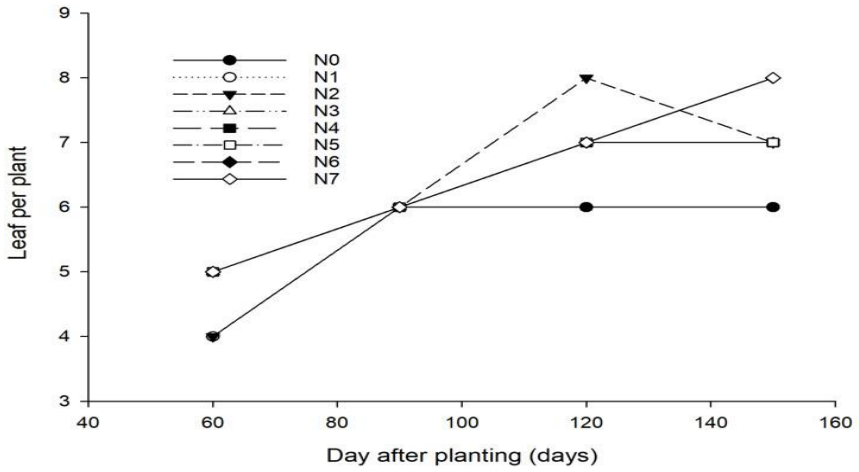
The growth indexes include height, number of leaf in plant. As shown in figure 2, 3 the results of *Angelica dahurica.* plants were described under different nitrogen fertilizer treatments. After treatment, there was no interaction to leaf between formula at significant wit 95% confidence but the leaves are heighted in N6, N7 nitrogen dosage.

Figure 2. Effect of nitrogen doses on height index of Angelica dahurica.



In contrast, the result in figure 2 indicated that plant height showed different between nitrogen treatment. It can be seen that, if increase nitrogen dosage the plant growth is bestter than other dosage. Afer 150 DAP, The plant heighest reached 79.25 cm at N7 and heigh second reached 78.12 cm. However, plant height between the treatments was not statistically significant with 95% confidence. Three biological experiments were performed, which produced similar results.

Figure 3. Leaves of Angelica dahurica after 8 treatment



EFFECT OF DIFFERENT NITROGEN DOSAGE ON YIELD IN *ANGELICA DAHURICA*

The Yield of the *Angelica dahurica* production is the goal of this research, and its also affects the economic efficiency and quality of plant in cultivation. In this study, the results in Figure 4 showed that *Angelica dahurica plant* responded differently with different nitrogen dosage. All data are means ± SD calculated from three replicates. Three biological experiments were performed, which produced similar results.

The biomass yield of *Angelica dahurica plant* was high at application at N5, N6, N7 and was not significant different between three treatments. So N6 (175kg N/ha) was maybe suitable for *Angelica dahurica plant* cultivated in norther Viet Nam.

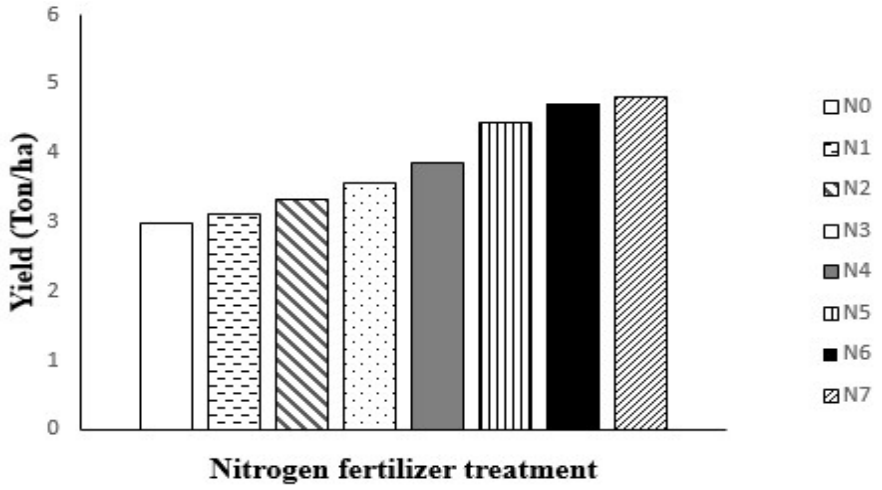


Figure 4: Effect of nitrogen fertilizer treatment on yield of ***Angelica dahurica***

INFLUEANCE OF NITROGEN DOSE ON QUALITY OF *ANGELICA DAHURICA*

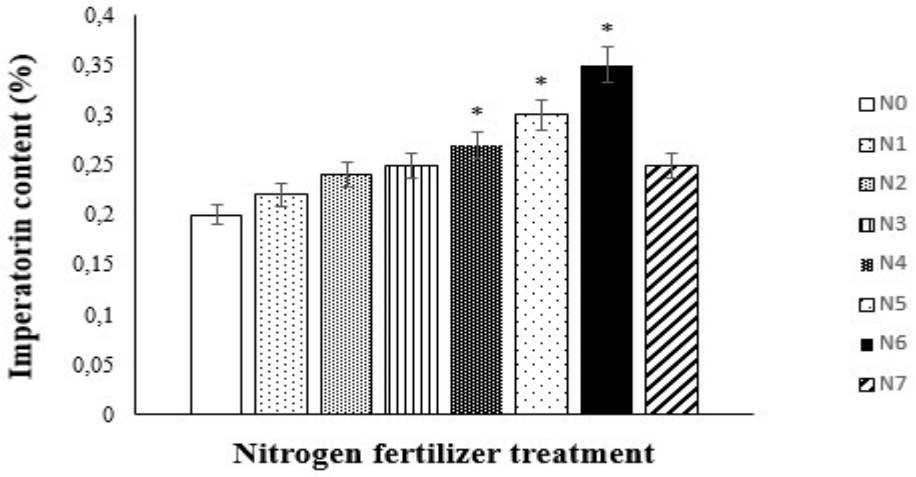


Figure 5: Effect of nitrogen fertilizer treatment on quality of ***Angelica dahurica***

As shown in figure 5 *Angelica dahurica* plants under eight nitrogen fertilization treatment have

clearly different in quality. After treatment, *Angelica dahurica* tube were collected for measurements and analyzation of quality content (imperatorin content). The means and SD were calculated from three replicates.

At the treatment N8 yield was highest (figure 4). However, the application of N in treatments N5, N6, N7 was not significant different (at LSD0.05). And addition, N6 formula obtained the highest impratorin content.
The result in figure 5, 6 indicated that the applying nitrogen fertilizer could increase yield and quality in *Angelica dahurica* buts it is adding over quality of tube maybe decrease. Hence, application of N6 was more suitable for *Angelica dahurica* crop.

CONCLUSION AND SUGGESTION

In recent studies have mainly focused on the effect of fertilization on growth parameters and the yield-increasing effect of Angelica dahurica (He Shijian et al. 2004). Lai Hongliang. 1992 in the fertilization research of Angelica sinensis indicated that nitrogen and phosphorus fertilizers have a significant effect on the medicinal components, while potassium fertilizers have little effect. Han Jianping et al. studied the effects of nitrogen and phosphorus fertilizers on the secondary metabolites of *Angelica dahurica*. The results showed that nitrogen had a negative effect on the accumulation of Angelica dahurica. If it increase of nitrogen application, the mass fraction of Angelica dahurica gradually decreased ; Phosphate fertilizer had a positive effect on the accumulation of *Angelica dahurica* and the application of phosphorus fertilizer can decline of Angelica dahurica caused by nitrogen. Different types of fertilizers have different effects on increasing the content of active ingredients.

In the or the hand, Differences fertilizer uptake influent in growth may be because of the higher absorption of water and mineral nutrients due to extensive colonization of roots (Harrier LA & Sawczak J. 2000).

In this research, all treatments increased growth parameters and yield of Angelica dahurica as compared to CK experiment. The results show that, the low range of fertilizer level may be decreases the yield of *plant*, however Over fertilizing can even lead to disease resulting in severe crop loss. In this study also indicate that maximum plant yield and quality of *Angelica dahurica* plant were support as 175kg Nitrogen per hectare.

In the future, under the threat of global cultivar methods and nutrient treatment may be became the best choice, it can be targeted for the development to enhance the imperatorin content as functional in the traditional medicine. In addition, their ability in the medical treatment need further studies in the future.

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