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# Research Article
# Improvement of the Field Productivity in *Rehmannia glutinosa* Libosch. Crops by Application of N, P and K Fertilizers and Plant Spacing

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## Abstract
**Background and Objective:** *Rehmannia glutinosa* Libosch. is a well-known medicinal plant species thanks to its valuable catalpol content with proven pharmacological potentials. However, there was little evidence on improving the crop productivity with a high catalpol content and total root yield. The present study aimed to assess the influence of nitrogen (N), phosphorus (P) and potassium (K) fertilizers on the catalpol content of *Rehmannia glutinosa* roots sown under divergent plant spacing. **Materials and Methods:** The experiment was designed with two factors: Factor A includes five incremental levels of fertilizers (0, 60, 120, 180 and 240 kg ha$^{-1}$ for N; 0, 40, 80, 120 and 160 kg ha$^{-1}$ for P and K) and factor B includes three plant spacings of S1 = 15, S2 = 20 and S3 = 30 cm between rows and 20 cm between plants. High Performance Liquid Chromatography (HPLC) was employed to determine the catalpol content. **Results:** The results showed that a moderate level of N (180 kg ha$^{-1}$) in combination with S3 might produce the highest root yield and catalpol content. Both P and K appeared better than N in catalyzing plants to gain higher yield and accumulate maximum catalpol content at the application rates of 120 kg ha$^{-1}$. **Conclusion:** The current study confirms the positive influence of N.P.K. and plant spacing on the total yield and catalpol content in roots of Chinese foxglove and practical information to promote the mass production for medicinal materials and pharmaceutical industries.

Key words: Catalpol content, chemical fertilizer, plant density, Chinese foxglove, high performance liquid chromatography

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Rehmannia glutinosa* Libosch. (Chinese foxglove) has been listed as one of the most important medicinal plants used in oriental folk remedies[1]. The most common form of utilization for this plant for medicinal materials are dried rhizome and prepared *Rehmannia* roots, which are always chosen from acceptable market size fresh roots. Recent studies have confirmed that Chinese foxglove possessed various bioactive compounds and pharmacological functions[2]. The plant gained its high medicinal values because it is attributed to various secondary metabolites such as; iridoids, saccharides, amino acids, inorganic ions, flavonoids and other trace elements that are mainly produced in roots[1]. Therefore, Chinese foxglove was described as the principal component in folk remedies to support the functions of the blood system and other functional systems. Medicinal products of this plant species have also been used as anti-tumor and anti-senescence agents[3-5]. Catalpol is the main bioactive compound of Chinese foxglove, which has many biological functions. It was confirmed to reduce inflammation and elevated blood sugar, to promote sex hormone production and protect the liver from damage[6-8]. As increasing interest from modern medicine and the requirement for standardized pharmaceutical sources, it is essential to understand the regulation of the biosynthetic processes and accumulation of bioactive compounds in specific plant organs.

The accumulation of plant secondary metabolites is strongly affected by environmental factors that cause variations in their content and distribution[9,10]. Currently, the chemical and pharmacological properties of Chinese foxglove were widely studied. However, there is very little knowledge and understanding of the interrelation between secondary metabolite accumulation and fertilizing application[2]. It is well-known that nutrient uptaking is a prerequisite for plant development, function and metabolism. Chemical fertilizers such as; N, P and K are the three most essential mineral elements in plants as they are the main components in almost critical metabolic and physiological processes[11]. For instance, Bernstein *et al.*[12] reported that the nutritional supplements affected cannabinoid content and its organ-specific and spatial-specific responses in cannabis (*Cannabis sativa*). Higher amounts of carbohydrate, phenolic and flavonoid content were observed in drumstick trees (*Moringa oleifera*) treated with an enhancing application of NPK[13]. Emami Bistgani *et al.*[14] revealed that the fertilization regimes improved the growth and medicinal properties of thyme (*Thymus daenensis*). However, there still exist conflicting results regarding the influence of inorganic fertilizers on numerous secondary metabolite accumulation in various medicinal plant crops[10,15]. Plant spacing plays a key role in establishing the micro-environment for the Chinese foxglove and other medicinal plant crops. Therefore, building an optimal spacing between plants can ensure crops to gain better yield and some desired quantitative traits such as; the content of bioactive compounds[16-18]. Some studies have developed biotechnological based-methods to increase the content of secondary metabolites in Chinese foxglove over the last decades[19,20]. Moreover, seed propagation was traditionally accepted but not preferred recently due to reduced seed availability and low propagation rate. Therefore, root cutting propagation with the application of plant regeneration, micropropagation and plant transformation have been selected for high-efficiency propagations and plant bioactive compound improvement[21,22]. Although, these biotechnological applications have been developed and played important roles in the manipulation of plants for improvement of medicinal plant crops and the production of bioactive compounds, their feasibility in agricultural production is more complex and still needs further optimization. Moreover, there has been very little work clarifying the effect of fertilization management and cultivation techniques on this plant's crops for optimal production of catalpol and the total yield, especially in Vietnam. Therefore, field experiments of the present study were designed to examine the effects of N, P, K application doses and plant spacing on the catalpol content and the total yield in roots of Chinese foxglove crops.

## MATERIALS AND METHODS

**Study area:** The field experiments were carried out on alluvial soil areas along the Red River Delta in Phu Tho province (21°14'N, 105°20'E) of the Northern Vietnam during the dry season from Winter, 2018 to Spring, 2019. The region has a hot and humid monsoon-type climate, which is distinguished by dry and rainy seasons. Other climatic parameters were recorded as 1,800 mm of annual rainfall, mean temperature from 22.2-26.5°C, relative humidity from 75- 88%[23].

**Experimental design:** Three independent experiments were established with the dose and spacing-combination of each fertilizer with plant spacings. The treatments of each experiment included five doses of one of N, P or K. While, any of two fertilizers and organic biofertilizers were added at their recommended doses for the other fertilizer experiment. The doses under test were 0, 60, 120, 180, 240 kg $ha^{-1}$ N (in the form of ammonium nitrate, 33.5% N), 0, 40, 80, 120, 160 kg $ha^{-1}$ P (in the form of superphosphate) and 0, 40, 80, 120, 160 kg $ha^{-1}$K (in the form of potassium chloride). N, P, K and organic biofertilizers were added to all treatments at the recommended doses of 180, 130, 90, and10.000 kg $ha^{-1}$, respectively, to ensure the normal growth and development of plants. Each fertilizer application rate was applied to plant spacing S1, S2, S3 defined as 15, 20 and 30 cm spaced rows with a plant to plant distance of 20 cm in a plot size of 10 $m^2$. All 45 treatments replicated three times were placed in a completely randomized block design.

Total 100% of P fertilizer and organic biofertilizer had been spread once as basal application. The fertilization of N and K was applied into splits as a top dressing, including ½N and ¼K at the 30th day of the initial stage of vegetative growth, ½N and ½K was added after 20 days from the first one, the fertilization was completed by adding the rest of ¼ K at 90 days after planting.

**Planting procedure and data measurement:** Chinese foxglove roots suitable for planting were selected after harvesting. Explants cut from roots (1-2 cm) were placed in all treated plots and the control. Competition between weeds and the experimental plant were manually eliminated.

The roots were harvested after 170 days from planting. Post-harvest parameters such as; the number of tuber/plant, average fresh weight of root (g) and fresh weight of roots/plant were once recorded to calculate the total yield of roots/ha (kg).

**Reagents and standard solution preparation:** High Performance Liquid Chromatography (HPLC) grade acetonitrile (A.C.N.), phosphoric acid (0.1%), dichloromethane, methanol and n-hexane were purchased from Merk (Germany). The standard catalpol ($\geq$98%) and other reagents of analytical grade were obtained from the National Institute for Food and Drug Control (China). The concentration of catalpol in the mixed working stock standard solution was 1.0 mg in mL of methanol. The make calibration curves, five series concentrations including 10, 20, 40, 80 and 120 µg $mL^{-1}$ were prepared by diluting the stock working solution. A calibration curve was constructed by plotting the logarithm of the HPLC-UV peak areas vs. the logarithm concentration of each standard.

**Extraction and assay of catalpol in *R. glutinosa* roots:** The frozen samples were homogenized with liquid nitrogen and 0.8 g of each sample powder was extracted with 50 mL of methanol in an ultrasonic water bath at boiling temperature for 90 min and cooled down until room temperature. Methanol was then added to compensate the volume which had been lost during the previous boiling. The extract (10 mL) was centrifuged at 12000 rpm for 5 min. The supernatant was evaporated to dryness in a rotary evaporator at 50°C under the reduced pressure, re-dissolved in the isocratic mobile phase solution contained acetonitrile and phosphoric acid (99:1 ratio) and then filtered with 0.45 µm cellulose acetate membrane filter before HPLC analysis. For HPLC-UV quantification, 10 µL of filtered extracts and standards were run at 30°C on a Shimadzu system equipped with a C18 column (5 µm, 250×4.6 mm), SPD-20A UV/Vis detector, SIL-20A autosampler (Japan). The isocratic mobile phase solution contained acetonitrile and phosphoric acid (99:1 ratio) and was delivered at 1.0 mL/min. Catalpol was monitored at $\lambda$ 210 nm. The presence of catalpol in Chinese foxglove roots were determined by comparing both retention time and spectral data with those of their corresponding authentic standards. In contrast, the concentration was determined by linear standard calibration curves. Their content was shown in dry weight. Three replicates of each stage of root sample were measured.

**Statistical analysis of data:** The collected data were subjected to statistical analysis by using IRRISTAT 5.0 program. The means of the treatments were compared by using the Least Significant Difference (LSD) test at the 0.05 level.

## RESULTS

**Determination of catalpol by HPLC:** The dried roots of Chinese foxglove extracts were monitored at $\lambda$ 210 nm for catalpol (Fig. 1). The content of catalpol was calculated for standardization. Figure 1 showed that the standard of catalpol and the sample extract were detected at the same time (17.9 min on average) with precision of 0.17% (based on Relative Standard Deviation, (RSD) (%)). The RSD for repeatability was 1.14% (<2%). These two parameters indicated that the instrument and extraction method were suitatble for reproducible results. The linear regression equations obtained were as follow:

$$Y = 2.10^6X+2847(R^2 = 0.999)$$

where, Y is the catalpol/IS (international standard) peak area ratio and X is the catalpol concentration. As the results, the concentrations of catalpol in response to fertilizers were

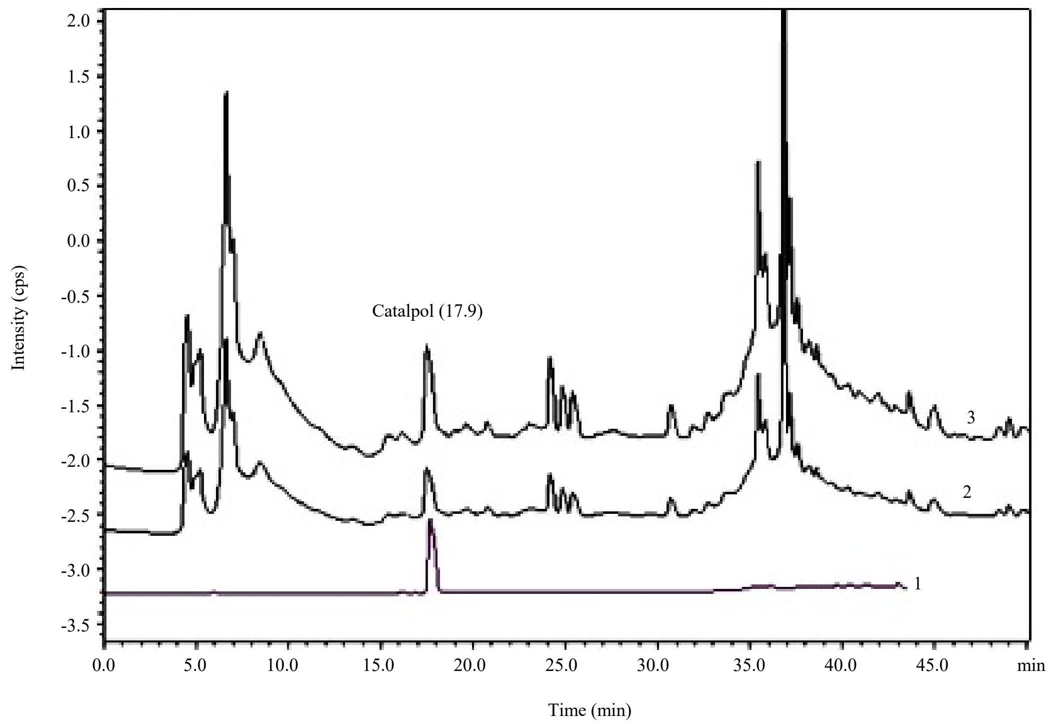


Fig. 1: HPLC-UV chromatographs of standard (1), Chinese foxglove extract (2) and a mixture of standard and Chinese foxglove extract (3)

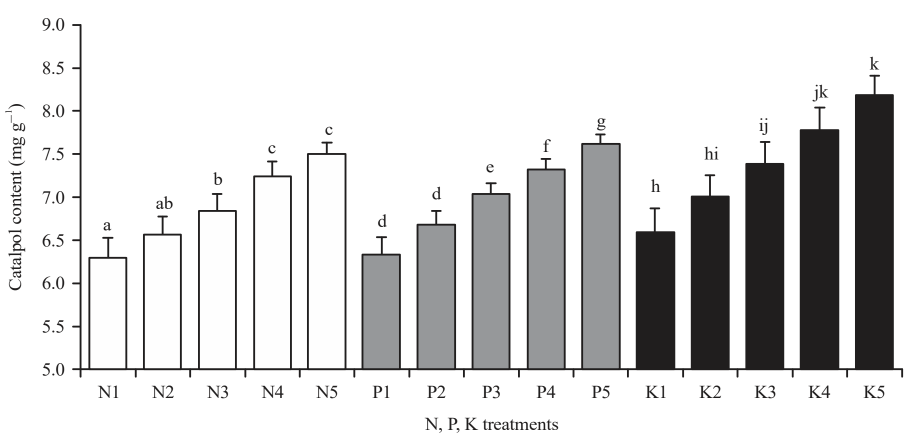


Fig. 2: Catalpol content in roots of *Rehmannia glutinosa* as affected by N, P, K supplementation

N. fertilization: N1 = 0, N2 = 60, N3 = 120, N4 = 180 and N5 = 240 kg N ha$^{-1}$, P. fertilization: P1 = 0, P2 = 40, P3 = 80, P4 = 120 and P5 = 160 kg P ha$^{-1}$; K. fertilization: K1 = 0, K2 = 40, K3 = 80, K4 = 120 and K5 = 160 kg P ha$^{-1}$. Presented data are averages ±SE (n = 9). Different letters above the bars represent significant differences between treatments. The means of the treatments were compared by using the Least Significant Difference (LSD) test at the 0.05 level

determined and presented in comparison between experimental formula in Fig. 2, 3 and Table 1.

**Content of catalpol changed with the application of chemical fertilizers and plant spacing:** N, P, K fertilizers and plant spacing significantly affected the content of catalpol ($p<0.00$). The interaction between N and spacing were positive ($p<0.00$). However, plant spacing was negatively correlated with P and K (Fig. 2). The higher amount of catalpol was produced as increasing levels of N.P.K. fertilization and the differences between formula are significant ($p<0.00$). The highest level of catalpol were 7.24, 7.62 and 8.18 mg g$^{-1}$ in plants fertilized with 240 kg N ha$^{-1}$, 160 kg P ha$^{-1}$ and 160 kg K ha$^{-1}$, respectively, while the lowest values were

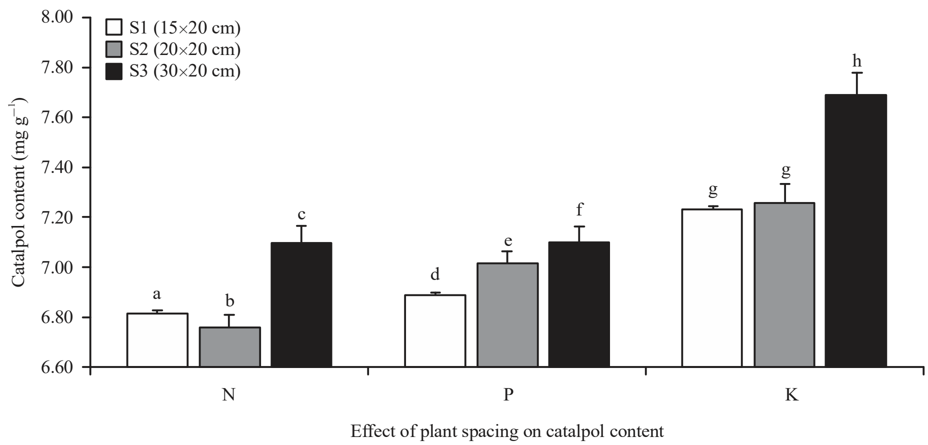


Fig. 3: Catalpol content in roots of *Rehmannia glutinosa* as affected by plant density

S1: 15×20 cm, S2: 20×20 cm, S3: 30×20 cm. Presented data are averages $\pm LSD_{0.05}$ (n = 9). Different letters above the bars represent significant differences between treatments. The means of the treatments were compared by using the Least Significant Difference (LSD) test at the 0.05 level

Table 1: Effect of N, P, K and plant spacing on catalpol content (mg $g^{-1}$) and total yield (kg $ha^{-1}$) in roots of *Rehmannia glutinosa*

| Treatments | Catalpol content (mg $g^{-1}$) | | | Root yield (kg $ha^{-1}$)* | | |
|---|---|---|---|---|---|---|
| Fertilizer/spacing | S1 | S2 | S3 | S1 | S2 | S3 |
| N1 | 0.61 | 0.62 | 0.66 | 109.1 | 107.7 | 146.5 |
| N2 | 0.64 | 0.65 | 0.68 | 139.7 | 140.2 | 172.9 |
| N3 | 0.68 | 0.67 | 0.71 | 168.8 | 176.3 | 207.9 |
| N4 | 0.73 | 0.71 | 0.74 | 211.7 | 211.9 | 261.7 |
| N5 | 0.75 | 0.74 | 0.76 | 251.1 | 249.7 | 254.5 |
| $P/LSD_{0.05\ (N)}$ | 0.00/0.007 | | | 0.05/8.99 | | |
| $P/LSD_{0.05\ (S)}$ | 0.00/0.005 | | | 0.05/7.13 | | |
| $P/LSD_{0.05\ (NxS)}$ | 0.00/0.01 | | | 0.05/15.57 | | |
| P1 | 0.62 | 0.63 | 0.65 | 114.6 | 113.1 | 153.8 |
| P2 | 0.65 | 0.67 | 0.68 | 146.7 | 147.2 | 181.6 |
| P3 | 0.69 | 0.70 | 0.71 | 177.2 | 185.1 | 218.3 |
| P4 | 0.72 | 0.73 | 0.74 | 222.2 | 222.5 | 274.8 |
| P5 | 0.75 | 0.77 | 0.77 | 263.7 | 262.1 | 267.2 |
| P/LSD 0.5 (P) | 0.00/0.007 | | | 0.05/6.93 | | |
| P/LSD 0.5 (S) | 0.00/0.007 | | | 0.05/5.50 | | |
| P/LSD 0.5 (PxS) | 0.66/0.01 | | | 0.05/12.01 | | |
| K1 | 0.64 | 0.65 | 0.69 | 120.00 | 118.50 | 161.10 |
| K2 | 0.69 | 0.69 | 0.73 | 153.70 | 154.20 | 190.20 |
| K3 | 0.72 | 0.72 | 0.77 | 185.70 | 193.90 | 228.70 |
| K4 | 0.76 | 0.76 | 0.81 | 232.80 | 233.10 | 287.80 |
| K5 | 0.80 | 0.80 | 0.85 | 276.30 | 274.60 | 279.90 |
| P/LSD 0.5 (K) | 0.00/0.009 | | | 0.05/7.27 | | |
| P/LSD 0.5 (S) | 0.00/0.007 | | | 0.05/5.76 | | |
| P/LSD 0.5 (KxS) | 0.75/0.01 | | | 0.05/12.59 | | |

Means of the treatments were compared using the Least Significant Difference (LSD) test at 0.05 level. *Effect of N and P on the total yield of Chinese foxglove was positively confirmed by Pham *et al.*[18]. N fertilization: N1: 0, N2: 60, N3: 120, N4: 180 and N5: 240 kg N $ha^{-1}$, P. fertilization: P1: 0, P2: 40, P3: 80, P4: 120 and P5: 160 kg P $ha^{-1}$, K. fertilization: K1: 0, K2: 40, K3: 80, K4: 120, K5: 160 kg P $ha^{-1}$, Plant spacing: S1: 15×20 cm, S2: 20×20 cm, S3: 30×20 cm

observed in none-fertilized treatments (N1 = 6.2, P1 = 6.33 and K1 = 6.60 mg $g^{-1}$). However, there were insignificant variations between the highest levels of N4 and N5, K4 and K5. Therefore, N4 and K4 would be considered as the optimal doses for catalpol content production.

Plant spacing also greatly affected the accumulation of catalpol in the roots of *Rehmannia glutinosa* (Fig. 3). Plants in the largest spacing (S3) produced the highest amount of catalpol at all three fertilizer experiments and were significantly different from ones of other spacing arrangements ($p<0.00$, Table 1). While, catalpol produced from S1 and S2 settlements were significantly different when fertilized with N and P, these two values were insignificant as plants treated with K fertilization. It was notable that catalpol content fertilized with K was always higher than that in plant treated with N, P and within each plant spacing mode (Fig. 2 and 3).

The interaction between N and plant spacing significantly impacted the catalpol content ($p<0.00$) and reach the highest at the combination of N5 or N4 with S3. However, there was no correlation between either P or K fertilizers with plant spacing ($p<0.66$ and $p<0.75$, respectively).

Data in Table 1 reported that all three fertilizers, plant spacing and their interaction significantly affected the total root yield ($p<0.05$). Chinese foxglove plants fertilized with 180 kg N $ha^{-1}$and 120 kg P $ha^{-1}$ produced the highest fresh yield and marketable root quality when being spaced at $30\times20$ cm distancing. Of these three fertilizers, K significantly promoted the growth and development of this Chinese foxglove roots and reached the peak by K4 dose (120 kg $ha^{-1}$). This dose in combination with plant spacing, also brought a positive influence on the total root yield with an optimum at S3 spacing.

## DISCUSSION

Catalpol content in roots was significantly improved as the increment of fertilizer and reached the peak at the highest doses of all N, P and K. Due to N4 and K4 were not statistically different from N5 and K5, respectively, these dosages should take consideration into effects for economical use of fertilizers. The results are believed to be the attribution of the synergistic effect with N.P.K., which accelerated the biochemical activities[26]. The impact of plant fertilization on the production of secondary metabolites have been studied in some medicinal plants[13,24,25]. The results suggested the principal role of N, P and K in secondary metabolites biosynthesis and accumulation in plants. A balanced supply of N.P.K. via fertilizer application can enhance the nutraceutical value of globe artichoke[27]. The use of N.P.K. 40:30:40 kg $ha^{-1}$ observed the highest asiaticoside and total secondary metabolites content[25]. The root of forest anchomanes (*Anchomanes difformis*) had their highest level of saponin content[28] when being fertilized with N.P.K. at a dose level of 100 kg $ha^{-1}$. Catalpol contents in Chinese foxglove roots increased with the increment of N application at 200 kg $ha^{-1}$; however, this event was not observed as the effect of P and K[29]. In this early study, P and K only increase the yield of Chinese foxglove roots at the application level of 400 and 200 kg $ha^{-1}$, respectively, which were much higher than that in the present study.

The result also indicated that higher application than N4 level maybe not essential to achieve the optimal accumulation of secondary metabolites. Some studies showed that secondary metabolites such as; flavonoids concentration in plants are negatively correlated with N concentration[9,10,30]. Stevioside accumulation in the leaf of sugar leaf (*Stevia rebaudiana*) was significantly improved by the moderate level of N[26], which also suggested the synergistic effect of other nutritional elements or environmental factors. The increment of $K^+$ and $Mn^+$ could increase the accumulation of iridoid glycosides. These compounds, however, were suppressed when ammonium salt or nitrate was used in the culture medium alone as an N source[31]. These events were also supported by the positive interaction between plant spacing and N on catalpol content in the current study. In a recent study by Duan *et al.*[32], some key enzymes were identified to involve in the synthesis of iridoid, but their regulation upon catalpol content was not recognized. It was believed that the activity of phenylalanine lyase, the critical catalytic enzyme in the flavonoid synthesis pathway, in plants suffered from N deficiency is more active than that in the plant with sufficient N[33]. As a result, N5 dose maybe excessive and not as effective as N4 dose in inducing the accumulation of secondary metabolites. N4 (180 kg $ha^{-1}$) and P4 (120 kg $ha^{-1}$) were found to produce the highest yield and quality of roots in our previous study in similar experiments[18]. However, for the optima of commercial cultivation of Chinese foxglove in both terms of the total yield and catalpol content, it is suggested to apply N4, P4 with S3 ($20\times30$xm) rather than N5 and P5.

According to Sahebi *et al.*[34], NPK treated doses, especially K fertilizing might act as elicitor triggering the production of glucanase, chitinase, POX and PAL activities. These are the key steps of secondary metabolite biosynthesis. The relatively higher catalpol content found in all K doses compared to N and P, in the present study supported the hypothesis that K fertilizing is better than other macronutrients in facilitating the accumulation of catalpol in roots. The result was in line with other studies, which confirmed the critical role of P on improving the yield and quality of crops, especially ones for medicinal purposes. P application significantly increased the grain yield of wheat and rice by 13 and 50% over the control, respectively[35]. The quality of sugar beet and total root yield were improved as K fertilizers increased[36]. These significant responses may be responsible for the beneficial effect of the K element, which activated many catalytic enzymes in almost all metabolic processes and biosyntheses[37–39]. It was noted that plants treated in K experiments were observed with relatively higher performance in growth and development of roots compared with those in N and P fertilizer experiments (Table 1). K treated plants also produced higher catalpol content compared to N and P indicated the primary role of this nutrient in Chinese foxglove crops for mass production of medicinal materials.

The main bioactive compound of Chinese foxglove was identified to be catalpol, which is the most important active iridoid glycosides and was used for quality control marker for this medicinal plant in the Vietnamese and Chinese Pharmacopoeia[40]. This content quantified in the present study ranged from 6.2-8.2 mg $g^{-1}$, which was roughly three times more than the current national standards of Vietnam and China. This study's catalpol amount was similar to a recent verification by Kim *et al.*[22], which used the same method of determination and standard rootstock seedling for cultivation. The dry root of Chinese foxglove was standardized to contain 5.4 mg $g^{-1}$ catalpol, which was determined by the same method of HPLC with the present study[41]. Few other studies also identified the catalpol contents; however, those levels are various due to different organs of the plant, development stages, cultivars and determination methods[42-45]. These studies appeared to not able to demonstrated the optimal fertilizer and cultivating establishments to achieve the acceptable field productivity of Chinese foxglove cultivation.

Secondary metabolites are often induced in plants as a mechanism of defense in response to biotic or abiotic stresses, which lead to the production of reactive oxygen species. In the current study, the least density plants (S1: 30×20 cm) from all three types of fertilizers significantly promoted the production of catalpol. Plants in these plots obtained more nutrients for growth and development, on the other hand, they may be under UV solar radiation as significantly exposed to sunlight, which can increase the secondary metabolite biosynthesis as a protective mechanism[46]. A high concentration of flavonoids, anthocyanins and tannins and the activities of phenylalanine ammonia-lyase (PAL) were observed in Indian ginseng (*Withania somnifera*) in response to UV-B radiation[47]. The total phenol content, POD and PAL activities also enhanced in leaves and roots of *Curcuma longa* under light exposure[48]. As a result of UV radiation stress, maximum thymol content was observed at the beginning of blooming and the largest spacing 45×50 cm[16]. Kim *et al.*[22] established a field experiment in which plants were arranged in much higher density (15×15 cm) to create stresses for obtaining higher accumulation of secondary metabolites. However, this cultivating establishment failed to produce marketable size roots. Therefore, the product might be neither accepted by the market to be used in traditional medicines nor pharmaceutical companies. The only positive interaction between spacing with N on catalpol content, but not applied with P or K, which indicated the regulating role of N in plant secondary metabolism. Meng *et al.*[49] also found that N fertilizer and plant density significantly produced the positive interaction between auxin, cellulose and hemicellulose contents, which subsequently promoted the quality of stem fiber and the yield of maize.

The application of NPK fertilizers had determined a substantial increase of catalpol content and root yield, especially K fertilizer. Either closer or wider plant spacing than 30 × 20 cm is not recommended as it may reduce the productivity of the Chinese foxglove cultivation for medicinal materials. Besides, it is worth to carry out further investigation to understand the molecular mechanism of the correlation between desirable traits, especially pharmaceutical property, of this crop plant and related environmental factors.

## CONCLUSION

The significant influence of N, P and K, in combination with plant spacing on catalpol content and total root yield were confirmed by the field experiments. The production of catalpol and root yield were observed as the increment of each fertilizer and reached a peak at their rates of 180 kg N $ha^{-1}$, 120 kg P $ha^{-1}$ and 120 kg K $ha^{-1}$. A moderate rate of N is recommended to gain the optimal cropping, especially in combination with the maximum spacing of 20×30 cm. P and K were independent with plant spacing in influencing catalpol content, but their interaction was positive in improving the total fresh yield of Chinese foxglove roots. It can be concluded that for the optimal production of catalpol content and total root yield, Chinese foxglove plants may be densely cultivated at a spacing of 20×30 cm and fertilized with 180 kg N $ha^{-1}$, 120 kg P $ha^{-1}$ and 120 kg K $ha^{-1}$.

## SIGNIFICANCE STATEMENT

The application of N, P and K with optimal plant spacing can improve the productivity of Chinese foxglove crops, which are promissing to provide substaintial medicinal materials from this plant to the market with dedicated quality. This study will help the researchers to uncover the critical areas of cultivation techniques that many researchers were not able to carry out. This also consults agricultural managers and farmers for an optimal fertilizer application, therefore, increase productivity and profit in commercial production of Chinese foxglove for medicinal plant materials in Vietnam.

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