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Research article

Optimal fertilizer application for *Panax notoginseng* and effect of soil water on root rot disease and saponin contents

Pengguo Xia [1,☆], Hongbo Guo [1,2,☆], Hongguang Zhao [3], Jie Jiao [1], Michael K. Deyholos [2], Xijun Yan [3], Yan Liu [3], Zongsuo Liang [1,4,*]

[1] *Shaanxi Research Center of TCM Fingerprint and NP Library, College of Life Sciences, Northwest A&F University, Yangling, China*
[2] *Department of Biology, University of British Columbia Okanagan, Kelowna, Canada*
[3] *Tianjin Tasly Modern TCM Resources Co. Ltd., Tianjin, China*
[4] *College of Life Sciences, Zhejiang Sci-Tech University, Hangzhou, China*

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ABSTRACT

Background: Blind and excessive application of fertilizers was found during the cultivation of *Panax notoginseng* in fields, as well as increase in root rot disease incidence.
Methods: Both "3414" application and orthogonal test designs were performed at Shilin county, Yunnan province, China, for NPK (nitrogen, phosphorus, and potassium) and mineral fertilizers, respectively. The data were used to construct the one-, two-, and three-factor quadratic regression models. The effect of fertilizer deficiency on root yield loss was also analyzed to confirm the result predicted by these models. A pot culture experiment was performed to observe the incidence rate of root rot disease and to obtain the best range in which the highest yield of root and saponins could be realized.
Results: The best application strategy for NPK fertilizer was 0 kg/667 m^2, 17.01 kg/667 m^2, and 56.87 kg/667 m^2, respectively, which can produce the highest root yield of 1,861.90 g (dried root of 100 plants). For mineral fertilizers, calcium and magnesium fertilizers had a significant and positive effect on root yield and the content of four active saponins, respectively. The severity of root rot disease increased with the increase in soil moisture. The best range of soil moisture varied from 0.56 FC (field capacity of water) to 0.59 FC, when the highest yield of root and saponins could be realized as well as the lower incidence rate of root disease.
Conclusion: These results indicate that the amount of nitrogen fertilizer used in these fields is excessive and that of potassium fertilizer is deficient. Higher soil moisture is an important factor that increases the severity of the root rot disease.

1. Introduction

Panax notoginseng (Burk.) F. H. Chen (Araliaceae), named Sanchi in Chinese, is a highly regarded medicine in China [1]. The root of *P. notoginseng* is confirmed to have many effects on the blood system, cardiovascular system, brain, vascular system, nervous system, metabolism, and immune regulation [2]. It is currently listed as a dietary food supplement by the United States Dietary Supplement Health and Education Act [3,4]. As its popularity has increased around the world, the prices of its raw material and processed products have markedly increased. In today's markets, all Sanchi ginseng are cultivated products owing to the loss of wild species [5].

In order to obtain larger yields, large amounts of chemical, organic, and/or foliar fertilizers are applied during *P. notoginseng* cultivation, although their influence on root yield and quality is poorly understood. According to our field survey, the content of organic matter, total nitrogen, and total phosphorus in the soil after

* Corresponding author. Shaanxi Research Center of TCM Fingerprint and NP Library, College of Life Sciences, Northwest A&F University, 22 Xinong Rd, Yangling 712100, China.
E-mail address: liangzs@ms.iswc.ac.cn (Z. Liang).
☆ These authors contributed equally to this work.

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planting 1-yr-old *P. notoginseng* was 26.44%, 47.20%, and 69.52%, respectively, higher than that in soils without planting. Available nitrogen and potassium, in particular, even increased—294.55% and 47.99%, respectively. More importantly, this blind and excessive application of fertilizers can lead to an imbalance in soil nutrients, and even cause nitrate contamination. Furthermore, it has been proven that excessive use of N fertilizer reduces the concentration of naringin and rutinoside in grapefruits [6,7], anthocyanin in apples [8], and polyphenolic compounds and antioxidant activity in basil [9]. Therefore, it is urgent for us to understand the need of *P. notoginseng* for soil nutrients and to come up with an optimal strategy for fertilizer application as needed.

By contrast, Sanchi ginseng is produced in fields under artificial shade structures [5]. Farmers often plant *P. notoginseng* at a high density to decrease the cost of investment, hoping to increase their profit. In this situation, diseases such as rot root disease are found to easily occur because plant growth requires a warm and humid environment during the growing season; meanwhile, fungicide application is inhibited because of limitations on pesticide residues for medicinal materials. There is no resistant cultivar that has been bred to date, and no efficient approach has been adopted to reduce the incidence of root rot disease, although some environmental factors are known to induce the disease to become even more severe [10]. It has been found that most root diseases are favored by wet soil [11]. Therefore, we wondered if the incidence of root rot disease will decrease with the decrease in soil moisture.

To address serious concerns on the negative effect of excessive fertilizer application on the root yield and quality of *P. notoginseng*, and the need to reduce the incidence of root disease in the field, this investigation was performed to (1) construct a fitting model between NPK (nitrogen, phosphorus, and potassium) fertilizer and dried root yield, as well as the abundance analysis of each fertilizer in experimental fields; (2) measure the effects of mineral fertilizers on dried root yield and on the accumulation of four active saponins; (3) evaluate the effects of soil moisture on the accumulation of four active saponins and on the incidence of root rot disease to propose the best soil moisture for root yield and the content of active saponins following the most fitted model.

2. Materials and methods

2.1. Plant and soil

Three-yr-old *P. notoginseng* seedlings were used to study the effect of fertilizer application on root yield and the content of saponins. The experimental site is located in the town of Guishan in Shilin county, Yunnan, China (24°44′14″ N, 103°38′54″ E, altitude 2,128 m). The soil type was red clay soil with dense viscosity. Only those fields with uniform topographic and geomorphic conditions were selected for use. The soil nutrients of both the experimental field and the clearing beside it were determined prior to the field experiment (Table 1).

2.2. Application design of NPK fertilizer

The “3414” application design of NPK fertilizer was followed (Table 2), because it has been applied in many crops including medicinal plants, such as winter wheat [12], *Chrysanthemum indicum* [13], and Smooth Cayenne pineapple [14]. Both N (urea) and K (K_2SO4) fertilizers were applied four times during the growing season—on April 15, June 15, August 15, and October 15. The amounts used in the first application were 4/16 N and 4/16 K, and in the second application were 6/16 N and 6/16 K. In the third application we used 5/16 N and 4/16 K, and in the last application we used 1/16 N and 2/16 K. The P fertilizer (P_2O_5) was applied half on April 15 and half on August 15. The area of the soil plot was 2 m × 1.5 m, and each treatment comprised 15 plots. Prior to application, these fertilizers were mixed with 200 g organic fertilizer and 300 g dry soil, and then they were placed in a plastic bag for 3 d.

2.3. Application of mineral fertilizer

An orthogonal test design was adopted, and the field experiment was carried out based on the L_{16} (4^5) method. Four levels were designed for each fertilizer used: Zn ($ZnSO_4$), B (Borax), Ca ($CaSO_4$), and Mg ($MgSO_4$) (Table 3). All four fertilizers were applied twice during the growing season, on April 15 and August 15, with each of half. The area of each field plot was 2 m × 1.5 m, and each treatment comprised 15 plots. Similar to NPK application, these fertilizers were mixed with 33.48 g urea, 93.80 g K_2SO_4, 47.85 g ammonium phosphate, 200 g organic fertilizer, and 300 g dry soil, and then they were placed in a plastic bag for 3 d until application.

2.4. Effect of field capacity of water on root weight

The soil sample was taken from the plowed soil of the idle field with field capacity of water (FC) of 36.80%. A total of 200 3-yr-old individuals with uniform morphology were randomly selected for the drought stress experiment, which were planted on March 12, 2012, in plastic pots with dimensions of 22 cm (height), 27 cm (mouth diameter), and 18 cm (bottom diameter). The total weight of stones and pot was 1.7 kg, and each pot was then filled with 6.3 kg of filtered soil sample. The pots were put under rain shelter after covering 150 g dried pine needle on potted soil. The water control experiment was performed on April 20, when the seedling leaves were sprouting. Four stress levels were designed—0.45 FC, 0.60 FC, 0.70 FC, and 0.85 FC—with a control of FC. Each level had 25 pots with two plants in each. The water loss was supplemented at 17:00 daily. The water control ended on October 20, when the roots were harvested to observe dried weight and to detect the content of four active saponins.

To build the relationship between soil moisture and the incidence of root rot disease, those plants exhibiting root rot disease

Table 1
Soil nutrient of experimental field and the clearing beside it at Guishan, Shiling County, Yunnan Province

Soil nutrient	Clearing	Experimental field	Soil nutrient (mg/kg)	Clearing	Experimental field
Organic content (%)	3.290	4.160	Available potassium	166.58	246.52
Total nitrogen (%)	0.125	0.184	Available calcium	886.45	1,488.25
Total phosphorus (%)	0.111	0.188	Availability magnesium	75.39	217.08
Total potassium (%)	0.697	0.628	Available boron	0.11	0.41
Available nitrogen (mg/kg)	9.376	36.993	Available zinc	0.67	1.11
Available phosphorus (mg/kg)	35.966	91.901			