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Research article

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

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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

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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			

Table 2
The 3414 application design of NPK fertilizer for 3-yr-old *Panax notoginseng* in Yunnan Province

Treatment No.	No. assignment	Urea (kg/667 m^2)	No. assignment	P_2O_5 (kg/667 m^2)	No. assignment	K_2SO_4 (kg/667 m^2)
1	0	0	0	0	0	0
2	0	0	2	22.5	2	45
3	1	11.3	2	22.5	2	45
4	2	22.5	0	0	2	45
5	2	22.5	1	11.3	2	45
6	2	22.5	2	22.5	2	45
7	2	22.5	3	33.8	2	45
8	2	22.5	2	22.5	0	0
9	2	22.5	2	22.5	1	22.5
10	2	22.5	2	22.5	3	67.5
11	3	33.8	2	22.5	2	45
12	1	11.3	1	11.3	2	45
13	1	11.3	2	22.5	1	22.5
14	2	22.5	1	11.3	1	22.5

NPK, nitrogen, phosphorus, and potassium

were also recorded during a 6-mo period of water control. The incidence percentage = (the number of root rot plants/50) × 100%.

2.5. Determination of root weight

In the field experiment of fertilizer application, roots of *P. notoginseng* were harvested on November 20. Ten individuals were randomly harvested from each plot, and 10 plots were randomly selected to obtain fresh roots of 100 individuals. They were dried in an oven at 40°C for about 15 d to have a constant weight after cleaning.

For the water control experiment, six plants from three pots in each treatment were collected to observe fresh weight of aboveground and underground parts. The dried weight of aboveground and underground parts was determined after oven drying, and the drying rate was then obtained, respectively, for each part. The dried weight of both aboveground and underground parts for another 12 plants from six pots in each treatment were also determined, and then they were used to detect the content of four effective saponins.

2.6. Content of four active saponins

The samples were prepared by following the method provided by China Pharmacopeia [15]. The chromatographic condition followed the guidelines set by the Standard Administration of the People's Republic of China [16]. A Waters (Milford, MA, USA) chromatography system equipped with 1525 Binary HPLC pump, 2487 dual λ absorbance detector, and 2707 autosampler was used to perform the HPLC analysis and to determine the content of four active saponins. The column configuration consisted of a Waters SYMMETRY C18 reserved-phase column (5 μm, 250 mm × 4.6 mm) and a SYMMETRY C18 guard column (5 μm, 200 mm × 4 mm). The column temperature was maintained at 30°C. UV absorption was measured at 203 nm.

Table 3
Orthogonal L_{16} (4^5) test design used in application of four mineral fertilizers to 3-year-old *Panax notoginseng* at Shilin County, Yunnan Province, China$^{1)}$

Treatment No.	Zn (kg/667 m^2)	Mg (kg/667 m^2)	Ca (kg/667 m^2)	B (kg/667 m^2)
1	0	0	22.5	0.030
2	0.4	0	0	0
3	0.8	0	15	0.045
4	1.2	0	7.5	0.015
5	0	0.6	15	0.015
6	0.4	0.6	7.5	0.045
7	0.8	0.6	22.5	0
8	1.2	0.6	0	0.030
9	0	1.2	0	0.045
10	0.4	1.2	22.5	0.015
11	0.8	1.2	7.5	0.030
12	1.2	1.2	15	0
13	0	1.8	7.5	0
14	0.4	1.8	15	0.030
15	0.8	1.8	0	0.015
16	1.2	1.8	22.5	0.045

B, boron; Ca, calcium; Mg, magnesium; Zn, zinc

Methyl alcohol (MeOH) and acetonitrile (MeCN) (HPLC grade) were purchased from Tedia Co., Inc. (Fairfield, OH, USA). Ultrapure water was generated with an ultrapure water system (Shanghai Ultrapure Technology, Shanghai, China). Standard ginsenosides Rg1 (110703-201027), Rb1 (110704-201122), Rd (111818-201001), and notoginsenoside R1 (110745-200617) were purchased from the National Institute for the Control of Biological and Pharmaceutical Products (Beijing, China). The contents of these four active saponins were determined from those dried roots that were harvested from the pots with different water stresses.

3. Results

3.1. Fitting model between NPK fertilizer application and dried root weight

The dried root weight of 100 plants was determined from 10 field plots in each treatment of NPK fertilizer application (Table 4). A three-factor quadratic regression model was developed

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1^2 + b_5X_2^2 + b_6X_3^2 + b_7X_1X_2 + b_8X_1X_3 + b_9X_2X_3,$$

where Y represents the dried root weight of 100 plants (g) as a dependent variable, and X_1, X_2, and X_3 are independent variables representing N, P, and K fertilizers, respectively. When taking the data of Table 4 into this model, Equation 1 was obtained,

$$Y = 1{,}353.544 - 2.339X_1 + 12.186X_2 + 10.521X_3 - 0.040 \times \underline{X}_1^2 - 0.203X_2^2 - 0.057X_3^2 + 0.012X_1X_2 - 0.056X_2X_3 - 0.143X_1X_3 \quad (1)$$

Through the analysis of variance of Equation 1, the R^2 value (0.982 in Table 5) showed good fitting between this regression model and the field data, exhibiting its potential for fertilizer application. When the significance of these indices from b_1 to b_9 was estimated, only b_3 was significant, exhibiting that K fertilizer had a significant effect on root weight.

Table 4
Dried root weight of 100 *Panax notoginseng* plants in 14 treatments of NPK fertilizer application

Treatment No.	N (kg/667 m^2)	P_2O_5 (kg/667 m^2)	K_2SO4 (kg/667 m^2)	Dried root weight of 100 plants (g)[1)]
1	0	0	0	1,358 ± 42.04^a
2	0	22.5	45	1,821 ± 25.63^f
3	11.3	22.5	45	1,762 ± 54.74^e
4	22.5	0	45	1,503 ± 15.88^b
5	22.5	11.3	45	1,572 ± 24.00^c
6	22.5	22.5	45	1,605 ± 46.16^c
7	22.5	33.8	45	1,568 ± 26.00^c
8	22.5	22.5	0	1,446 ± 28.16^b
9	22.5	22.5	22.5	1,576 ± 27.84^c
10	22.5	22.5	67.5	1,592 ± 17.58^c
11	33.8	22.5	45	1,478 ± 21.63^b
12	11.3	11.3	45	1,672 ± 20.08^d
13	11.3	22.5	22.5	1,616 ± 36.10^c
14	22.5	11.3	22.5	1,493 ± 7.17^b

NPK, nitrogen, phosphorus, and potassium

[1)] Means with same lowercase letters denote no significant difference at $p = 0.05$, as determined using one-way analysis of variance analysis in SPSS 19.0 statistics (SPSS Inc., Chicago, IL, USA)

When N fertilizer application was fixed at the level of 22.5 kg/667 m^2, the effect of P and K fertilizer application on root weight was fitted using the two-factor quadratic regression model, $Y = b_0 + b_2X_2 + b_3X_3 + b_5X_2^2 + b_6X_3^2 + b_9X_2X_3$, where Y represents the dried root of 100 plants (g) as a dependent variable, and X_2 and X_3 are the independent variables representing P and K fertilizers, respectively. When inputting the data of treatments 4–10 and 14 (Table 4) into this regression model, Equation 2 was obtained,

$$Y = 1{,}161.83 + 18.15X_2 + 10.42X_3 - 0.236X_2^2 - 0.065X_3^2 - 0.178X_2X_3. \quad (2)$$

The analysis of variance and R^2 value (0.982) also showed good fitting between this regression model and field data (Table 6). The indices of b_2, b_3, b_5, and b_6 in the two-factor quadratic regression model were all significant except b_9. When dried root weight reached the highest level (1,612.45 g), the best application amount for P_2O_5 and K_2SO_4 was 17.01 kg/667 m^2 and 56.87 kg/667 m^2, respectively.

If the fertilizer application of P_2O_5 and K_2SO_4 were simultaneously fixed at the level of 22.5 kg/667 m^2 and 45 kg/667 m^2, respectively, the one-factor quadratic regression model was constructed, $Y = b_0 + b_1X_1 + b_4X_1^2$, where Y is a dependent variable and X_1 is an independent variable of N fertilizer. When taking the data of treatments 2, 3, 6, and 11 into this model, Equation 3 was obtained:

$$Y = 1{,}827.47 - 6.01X_1 - 0.134X_1^2 \quad (3)$$

Similarly, the one-factor quadratic regression model was constructed for P_2O_5 fertilizer, $Y = b_0 + b_2X_2 + b_5X_2^2$, when fixing the application level of N and K fertilizers at 22.5 kg/667 m^2 and 45 kg/667 m^2, respectively. After taking the data into this model, Equation 4 was obtained:

$$Y = 1{,}501.29 + 9.07X^2 - 0.208X_2^2 \quad (4)$$

The last one-factor quadratic regression model ($Y = b_0 + b_3X_3 + b_6X_3^2$) was constructed for K fertilizer, when the application levels of N and P fertilizers were both fixed at 22.5 kg/667 m^2. Equation 5 was also obtained:

$$Y = 1{,}448.95 + 6.842X_3 - 0.071X_3^2 \quad (5)$$

The analysis of variance was performed to assess the fitness between field data and regression models, and the R^2 values for Equations 3–5 were 0.988, 0.989, and 0.989, respectively, showing good fitness.

Based on the one-factor quadratic model, it was not necessary to apply N fertilizer, which could yield the highest root weight (1,827.47 g; Table 7). After a comprehensive evaluation of the application level recommended by these models, the best application levels for N, P, and K fertilizers were 0 kg/667 m^2, 17.01 kg/667 m^2, and 56.87 kg/667 m^2, respectively, which produced the highest root weight of 1,861.90 g (Table 7).

3.2. *Effect of absence of NPK fertilizer on root yield loss in current experimental fields*

The dried root weight of 100 individuals was calculated for evaluating yield loss, when N, P, or K fertilizer is absent. The equation used for this calculation was as follows:

percentage of relative yield = (dried root weight of 100 individuals when N, P, or K fertilizer was absent/dried root weight of 100 individuals with abundant N, P, and K fertilizer) × 100%.

The percentage of relative yield for N, P, and K fertilizers was 113.46%, 93.64%, and 90.09%, respectively. This indicates that the application of N fertilizer in experimental fields is redundant (Table 8). The dried root yield in the field without N fertilizer was higher (34.09%) than that in the field without fertilizer application. However, the additional N fertilizer application of 11.3 kg/667 m^2, 22.5 kg/667 m^2, and 33.8 kg/667 m^2 reduced dried root yield by 3.24%, 11.86%, and 18.85%, respectively. Meanwhile, K fertilizer had a positive effect on yield to some extent. When applying 22.5 m^2, 45 m^2, and 67.5 kg/667 m^2 K fertilizer in fields, the percentage of dried root weight increased by 8.99%, 11.00%, and 10.01%, respectively.

3.3. *Effects of application of mineral fertilizations on root yield and the content of four active saponins*

The dried root weight of 100 individuals and the content of four saponins in each treatment of application of mineral fertilizers were determined as shown in Table 9. After calculation using Wolfram Mathematica 8 software (Wofram Research Inc., Champaign, IL, USA), both K value and range (R) were obtained. As

Table 5
Analysis of variance of Equation 1 that contained a three-factor quadratic regression model for measuring the effect of NPK fertilizer application on dried root yield of *Panax notoginseng*

Model		SS	*df*	MS	*F*	Sig.	R^2	Standard estimation error
3-factor quadratic regression model	Regression	189,300.01	9	21,033.33	23.63	0.004	0.982	29.83
	Residual	3,559.71	4	889.93				
	Total	192,859.71	13					

df, degree of freedom; MS, mean square; NPK, nitrogen, phosphorus, and potassium; Sig., significance; SS, sum of squares of deviations from mean

Table 6
Analysis of variance of Equation 2 that contained a two-factor quadratic regression model for measuring the effect of PK fertilizer application on dried root yield of *Panax notoginseng* if N fertilizer was fixed at the level of 22.5 kg/667 m^2

Model		SS	*df*	MS	*F*	Sig.	R^2	Standard estimation error
2-factor quadratic regression model (PK)	Regression	21,886.69	5	4,377.34	21.50	0.045	0.982	14.27
	Residual	407.19	2	203.59				
	Total	22,293.88	7					

df, degree of freedom; MS, mean square; N, nitrogen, PK, potassium phosphate; Sig, significance; SS, sum of squares of deviations from mean

shown in Table 10, the *K* value was largest when Zn and Mg fertilizers were at the second level, whereas Ca and B were at the fourth level. The increase in *K* values for each of Zn, Mg, and B fertilizers was not accordance with the increase in application amount. However, the *K* value of Ca fertilizer increased with the increase in application amount. The range (*R*) for four fertilizers decreased as follows: Ca > Mg > B > Zn, which indicates that Ca fertilizer has a larger effect on root weight compared with the other three.

Both *K* value and range (*R*) were calculated for four fertilizers when evaluating their effect on the content of four effective saponins in roots. The *K* value reached its highest when Zn and Ca fertilizers were applied at the first level, and Mg and B fertilizers were at the fourth and the second levels, respectively (Table 11). The *K* values for Zn, Ca, and B fertilizers did not increase with the increase in application amount of each. Only the *K* value of Mg fertilizer showed the same tendency to application amount. The range (*R*) also showed the same tendency, Mg > Ca > Zn > B, also indicating that Mg fertilizer has a greater effect on the accumulation of saponins (Table 11).

The analysis of variance further exhibited that Zn, Mg, and B fertilizers had no significant effect on dried root weight, but Ca had a positive significance (Table 12). When compared with dried root weight among the four application levels, a significant difference was found between the first and the fourth levels. As for the effect of these four fertilizers on the accumulation of four active saponins, only Mg had positive significance, and a significant difference was found between the first and the fourth application levels (Table 13).

3.4. *Effect of mineral fertilizer absence on root yield loss in current fields*

The yield of four active saponins (dried root weight of 100 plants × saponin content) was calculated for evaluating yield loss, when Ca, Mg, Zn, or B fertilizer was absent. The equation used was as follows:

% of relative yield = (yield of 4 saponins when Ca, Mg, Zn, or B fertilizer was at the first level (Table 9)/highest yield of four saponins in Ca, Mg, Zn, or B fertilizer application) × 100%.

The percentage of relative yield for Ca, Mg, Zn, and B fertilizer was 95.3%, 86.6%, 98.9%, and 92.3%, respectively. This indicates that both Ca and Zn fertilizers used in experimental fields are excessive (Table 8). The percentage of relative yield in Mg fertilizer fields was in the lowest range of abundance (Table 8), which might increase the yield to some extent if additional fertilizer is applied.

3.5. *Effect of soil water content on dried root weight, drying rate, and root/shoot ratio*

The dried root weight increased with the increase in soil water content up to 0.60 FC, with a dried root weight of 13.28 g/plant, and then decreased thereafter, showing a dried root weight of 12.35 g/plant and 7.97 g/plant at 0.70 FC and 0.85 FC, respectively (Table 14). The largest dried root weight at 0.6 FC was 1.29-, 1.07-, and 1.67-folds of 0.45 FC, 0.70 FC, and 0.85 FC, respectively, among which the differences between 0.6 FC and 0.45 FC and between 0.6 FC and 0.85 FC were significant (Table 14). Different from the dried root weight, the drying rate and the root/shoot ratio decreased in accordance with the increase in soil water. The drying rate at 0.45 FC was significantly higher than that at 0.70 FC and 0.85 FC. Obviously, the root/shoot ratio was easily affected by the soil water content, and a significant difference was found between any two of them (Table 14).

3.6. *Effect of soil water on content of four active saponins*

The effects of soil water on the accumulation of four active saponins were different. Both ginsenosides Rb1 and Rd decreased with the increase in soil water. The content of ginsenoside Rb1 at 0.45 FC was significantly higher than those at 0.60 FC, 0.70 FC, and 0.85 FC, with a significant difference noticed also between 0.60 FC and 0.85 FC. For ginsenoside Rd, the content at 0.45 FC was significantly higher than that at 0.85 FC. The other two ginsenosides, R1 and Rg1, however, were not sensitive to the changes in soil water content, and no significance was found between any two soil water contents for these saponins (Fig. 1; Table 15).

3.7. *Effect of soil water on the incidence of root rot disease*

The incidences of root rot disease increased with the increase in soil water content, showing that the disease incidence rates at 0.45 FC, 0.60 FC, 0.70 FC, and 0.85 FC were 4%, 10%, 12%, and 32%, respectively. For the occurrence rate at 0.85 FC, in particular, it was

Table 7
Dried root weight of 100 *Panax notoginseng* plants in different application levels of NPK fertilizer fitted by different regression models

Model	N (kg/667 m^2)	P_2O_5 (kg/667 m^2)	K_2SO_4 (kg/667 m^2)	Dried root weight of 100 individuals (g)$^{1)}$
3-factor quadratic model	0	22.50	45.00	1,826.28 ± 5.59^c
	22.50	21.80	45.00	1,614.03 ± 7.51^b
	22.50	45.00	48.18	1,528.26 ± 9.25^a
	0	17.01	56.87	1,861.90 ± 14.93^d
2-factor quadratic model	22.50	17.01	56.87	1,612.45 ± 11.17^b
1-factor quadratic model	0.00	22.50	45.00	1,827.47 ± 13.01^c
	22.50	21.80	45.00	1,600.17 ± 7.48^b
	22.50	45.00	48.18	1,608.43 ± 7.70^b

$^{1)}$ Means with same lowercase letters denote no significant difference at $p = 0.05$, as determined using one-way analysis of variance analysis in SPSS 19.0 statistics (SPP Inc., Chicago, IL, USA)

Table 8
Effect of fertilizer on yield increase in different soil nutrients

Soil nutrient	% of relative yield	Effect of fertilization
Lacking	< 50	The most significant effect on yield increase
Scarce	50–75	Significant effect on yield increase
Medium	75–85	Increasing yield at some extent
Abundant	85–95	No obvious effect on yield increase
Redundant	> 95	No effect, even negative on yield increase

20% higher than that at 0.70 FC. During the growing season, the root rot disease easily occurred in August.

3.8. *Fitting model construction for obtaining appropriate soil water*

The data on dried root weight, four saponins' content, root rot disease incidence, and soil water content were used for regression-fitted analysis, and three equations were obtained:

$$Y_1 = -98.36X^2 + 121.8X - 24.60 \ (0.45 \le X \le 0.85, R^2 = 0.996),$$

$$Y_2 = -3.592X + 9.802 \ (0.45 \le X \le 0.85, R^2 = 0.980),$$

and

$$Y_3 = 0.431e^{5X} \ (0.45 \le X \le 0.85, R^2 = 0.974).$$

Y_1, Y_2, and Y_3 represent dried root weight (g), the yield of four saponins (g) in dried root of 100 plants, and the number of plants with root rot in a total of 100 individuals, respectively. X represents the ratio of real field capacity of water in soil to the largest field capacity of soil water. Considering that soil water content affected the dried root weight, contents of four saponins, and root rot disease incidence, two more equations between dried root weight (g, using 100 plants) and soil water content [$F_y(X)$], and between the content of four saponins (g, using 100 individuals) and soil water content [$F_s(X)$] were constructed:

$$F_y(X) = (100 - Y_3) \times Y_1$$

and

$$F_s(X) = (100 - Y_3) \times Y_1 \times Y_2/100.$$

When taking the obtained Y_1, Y_2, and Y_3 values from the above three equations into these two equations, the new two equations were obtained:

$$F_y(X) = (100 - 0.431e^{5X}) \times (-98.36X^2 + 121.8X - 24.60),$$

$$0.45 \le X \le 0.85,$$

and

$$F_s(X) = (100 - 0.431e^{5X}) \times (-98.36X^2 + 121.8X - 24.60) \times (-3.592X + 9.802)/100,$$

$$0.45 \le X \le 0.85.$$

Both curves of $F_y(X)$ and $F_s(X)$ increased and then decreased in the range of $0.45 \le X \le 0.85$ with peak coordinates of (0.59, 1,195.05) and (0.564, 92.39), respectively (Fig. 2), indicating that the

Table 9
Dried root weight of 100 *Panax notoginseng* plants harvested from fields applied with different mineral fertilizers as well as the content of four active saponins in roots

Treatment No.	Zn (kg/667 m^2)	Mg (kg/667 m^2)	Ca (kg/667 m^2)	B (kg/667 m^2)	Dried root weight of 100 individuals (g)	Content of four saponins (%)[1]
1	0	0	22.5	0.03	1,905 ± 14.00f	6.634 ± 0.135^{a}
2	0.4	0	0	0	1,666 ± 16.64b	7.751 ± 0.126^{e}
3	0.8	0	15	0.045	1,769 ± 17.58d	6.620 ± 0.125^{a}
4	1.2	0	7.5	0.015	1,724 ± 19.08c	7.244 ± $0.116^{b,c}$
5	0	0.6	15	0.015	1,897 ± 25.87f	8.146 ± $0.110^{f,g}$
6	0.4	0.6	7.5	0.045	1,943 ± 11.27g	7.432 ± $0.376^{b,c,d}$
7	0.8	0.6	22.5	0	1,811 ± 12.12e	7.168 ± 0.076^{b}
8	1.2	0.6	0	0.03	1,674 ± 22.34b	7.328 ± $0.111b^{c,d}$
9	0	1.2	0	0.045	1,559 ± 8.72a	8.789 ± 0.148^{h}
10	0.4	1.2	22.5	0.015	1,834 ± 15.62e	7.606 ± $0.102^{d,e}$
11	0.8	1.2	7.5	0.03	1,664 ± 12.00b	7.562 ± $0.159^{c,d,e}$
12	1.2	1.2	15	0	1,711 ± 10.82c	7.391 ± $0.078^{b,c,d}$
13	0	1.8	7.5	0	1,637 ± 16.52b	8.027 ± 0.062^{f}
14	0.4	1.8	15	0.03	1,745 ± 19.29cd	8.305 ± $0.095^{f,g}$
15	0.8	1.8	0	0.015	1,655 ± 24.76b	8.447 ± 0.093^{g}
16	1.2	1.8	22.5	0.045	1,924 ± 16.09fg	8.333 ± $0.108^{f,g}$

B, boron; Ca, calcium; Mg, magnesium; Zn, zinc

[1] Means with same lowercase letters denote no significant difference at $p = 0.05$, as determined using one-way analysis of variance analysis in SPSS 19.0 statistics (SPSS Inc., Chicago, IL, USA)

Table 10
K value and range calculated from the data listed in Table 9 to measure the application effects of four mineral fertilizers on root yield using Wolfram Mathematica 8 Software (Wolfram Research Inc., Champaign, IL, USA)

Fertilizer	Application level	*K* value	Range	Fertilizer	Application level	*K* value	Range
Zn	1	1,749.5	72.3	Mg	1	1,766.0	139.3
	2	1,797.0			2	1,831.3	
	3	1,724.8			3	1,692.0	
	4	1,758.3			4	1,740.3	
Ca	1	1,638.5	230.0	B	1	1,706.3	92.5
	2	1,742.0			2	1,777.5	
	3	1,780.5			3	1,747.0	
	4	1,868.5			4	1,798.8	

B, boron; Ca, calcium; Mg, magnesium; Zn, zinc

Table 11
K value and range calculated from the data listed in Table 10 to measure the application effects of four mineral fertilizers on the content of four saponins using Wolfram Mathematica 8 Software

Fertilizer	Application level	*K* value	Range	Fertilizer	Application level	*K* value	Range
Zn	1	7.899	0.450	Mg	1	7.062	1.216
	2	7.773			2	7.519	
	3	7.449			3	7.837	
	4	7.574			4	8.278	
Ca	1	8.079	0.643	B	1	7.584	0.403
	2	7.566			2	7.861	
	3	7.616			3	7.458	
	4	7.435			4	7.794	

B, boron; Ca, calcium; Mg, magnesium; Zn, zinc

largest dried root weight (1,195.05 g/100 plants) was obtained at 0.59 FC. If the soil water content was at 0.564 FC, the yield of four saponins of 100 plants could reach the largest amount of 92.39 g.

4. Discussion

In China, progress in food production resulted from the rapid increase in the use of chemical fertilizer nitrogen with a mean application rate of about 180 kg/ha y^{-1} or 120 kg/ha $crop^{-1}$ in the year 1998 [17]. *P. notoginseng*, a medicinal crop that offers high profit, also has a high fertilizer demand. Farms often blindly apply more fertilizers (especially urea), hoping to obtain the largest yields and thus maximize their profits. Our fitting model result and the analysis of relative yield loss all showed that use of N fertilizer in fields is excessive and that it has no or negative effect on yield increase (Table 7). In conventional vegetable production, excessive N fertilizer use has led to low N fertilizer use efficiency in most intensive vegetable production regions [18–20]. Furthermore, for the higher application rates of 112.5 kg N/ha and 150 kg N/ha, in the field without irrigation, the net residual rates are equivalent to 14.6% and 30.8% of N applied, respectively [21]. These data are accordance with the percentage (25.34%) of available nitrogen in the clearings when compared with that in the nearby experimental fields (Table 1). A field survey of Anguo, Hebei province, China, has shown that the nitrate contents of the 0–20 cm, 20–40 cm, and 40–60 cm soil layers in those fields planted with medicinal crops were 37.96–436.86 mg/kg, 26.27–435.64 mg/kg, and 12.91–383.23 mg/kg, respectively, which were obviously higher than 3.71–184.65 mg/kg, 8.14–198.11 mg/kg, and 6.04–145.81 mg/kg found in the corresponding soil layer of cereal fields [22]. Therefore, accurate estimates of N input, output, and N balance in soil system are very important because nitrate will enter the hydrological system or denitrify in deep soil layers when unused NO_3^- is transported below the root zone, resulting in nitrate pollution of groundwater [20,23–26].

Table 12
Analysis of variance was used to measure the significance of effects of four mineral fertilizers on root yield of *Panax notoginseng*

Source of variance	*df*	SS	MS	*F*	$F_{0.05}$	$F_{0.01}$
Zn	3	10,789.25	3,596.42	<1	3.29	5.24
Mg	3	40,396.25	13,465.42	1.64		
Ca	3	109,004.75	36,334.92	4.43*		
B	3	19,353.25	6,451.08	<1		
Error	3	24,608.25	8,202.75			
Total variation	15	204,151.75				

B, boron; Ca, calcium; *df*, degree of freedom; Mg, magnesium; MS, mean square; SS, sum of squares of deviations from mean; Zn, zinc
The asterisk means the significance at $p<0.05$

Table 13
Analysis of variance was used to measure the significance of effects of four mineral fertilizers on content of four active saponins in roots of *Panax notoginseng*

Source of variance	*df*	SS	MS	*F*	$F_{0.05}$	$F_{0.01}$
Zn	3	0.484	0.161	<1	3.29	5.24
Mg	3	3.159	1.053	3.39*		
Ca	3	0.944	0.315	1.01		
B	3	0.416	0.139	<1		
Error	3	0.932	0.311			
Total variation	15	5.935				

df, degree of freedom; MS, mean square; SS, sum of squares of deviations from mean
The asterisk means the significance at $P<0.05$

Our results also found that application of calcium fertilizer could significantly increase the root yield of *P. notoginseng* (Table 12). The significant yield increase with the application of Ca was also confirmed in potato, wherein the tuber yield increased by 10.2% [27]. After application of 450–600 kg/ha Ca–Si fertilizer (available CaO 30%, SiO_2 35%) in the sandy soil of Puyang county, Henan, China, the peanut yield increased by 20.03–24.71% [28]. The result of a previous survey on soil characteristics in fields planted with *P. notoginseng* showed that the average of pH value, cation exchange capacity, and the percentage of base saturation in 14 experimental sites of the three main production counties of Yunnan province were 5.13 m.e/kg, 241.5 m.e/kg, and 35.90%, respectively [29]. These survey data indicate that these soils are acidic, wherein protons and aluminum ions are abundant, but calcium and magnesium ions are deficient. Therefore, the application of calcium and magnesium fertilizers to acidic soil will balance the ions and thus increase the root yield of *P. notoginseng*, as theoretically revealed by our fitting model; moreover, this hypothesis has been confirmed in peanut [28]. However, if a larger amount of magnesium fertilizer is applied,

Table 14
Effects of soil moisture on dried root weight of *Panax notoginseng*, and on drying rate and root/shoot ratio$^{1)}$

Soil water content (FC)	Dry root weight of single plant (g)	Drying rate	Root/shoot ratio
0.45	10.282 ± 1.060^b	0.391 ± 0.022^b	3.156 ± 0.055^d
0.60	13.278 ± 0.279^c	$0.335 \pm 0.0233^{a,b}$	2.876 ± 0.038^c
0.70	12.352 ± 1.569^c	0.260 ± 0.077^a	2.440 ± 0.035^b
0.85	7.971 ± 0.823^a	0.256 ± 0.044^a	2.280 ± 0.070^a

1) Different lowercase letters indicate significant difference ($n = 6$, $p < 0.05$). FC represents the original field capacity of water in soil (36.8%)

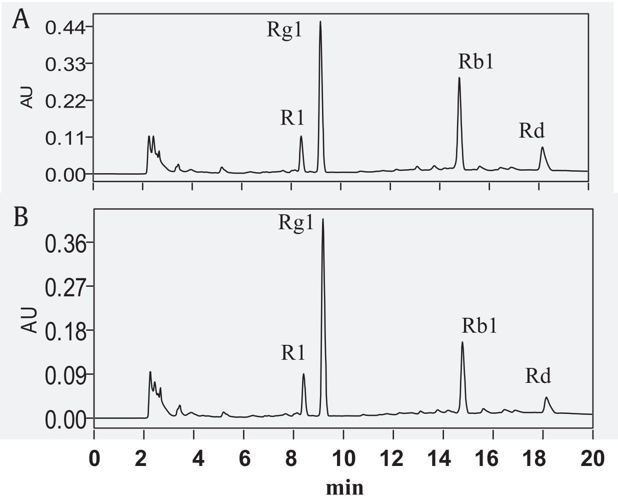

Fig. 1. High performance liquid chromatography (HPLC) fingerprint of four active saponins in dried roots of *Panax notoginseng*. (A) *P. notoginseng* grown in soil with 0.45 FC (field capacity of water of 36.8%). (B) *P. notoginseng* grown in soil with 0.85 FC.

Table 15
Effect of soil moisture on the content of four active saponins in dried root of *Panax notoginseng*[1)]

Soil water content (FC)	R1 (%)	Rg1 (%)	Rb1 (%)	Rd (%)	Content of 4 saponins (%)
0.45	1.213 ± 0.243^{a}	2.555 ± 0.235^{a}	3.628 ± 0.283^{c}	0.778 ± 0.176^{b}	8.175729
0.60	0.950 ± 0.342^{a}	2.875 ± 0.259^{a}	3.208 ± 0.319^{b}	$0.698 \pm 0.107^{a,b}$	7.732433
0.70	1.079 ± 0.131^{a}	2.564 ± 0.460^{a}	$2.965 \pm 0.141^{a,b}$	$0.563 \pm 0.074^{a,b}$	7.172518
0.85	0.959 ± 0.227^{a}	2.688 ± 0.381^{a}	2.643 ± 0.505^{a}	0.497 ± 0.220^{a}	6.788908

[1)] Different lowercase letters indicate significant difference ($n = 18$, $p < 0.05$). FC represents the original field capacity of water in soil (36.8%)

the magnesium ions would produce antagonistic action to potassium ions [30], but calcium ions would not. Hence, in field production, application of calcium fertilizer often shows a better yield increase compared with magnesium fertilizer, especially for crops with higher demand for potassium (e.g., peanuts) [28].

It has been found that the amount of magnesium ions absorbed by plants is lower than that of calcium and potassium because of the antagonistic action between them [31]. As revealed by the survey of Jin et al [29], the soils planted with *P. notoginseng* are all acidic, which is weak in retaining magnesium ions, thus leading to its leaching from soil. Application of magnesium fertilizer (225 kg/ha^2) in acidic or slightly acidic soil in Yunnan was found to increase plant growth, contents of chlorophyll and carotene, and photosynthetic and transpiration intensity in tobacco [31]. Through a pot culture experiment, He et al [30] found that application of 40 mg/kg and 80 mg/kg magnesium fertilizer could attain the highest root yield and the highest biomass of whole plant of *Paris polyphilla* var. *yunnanensis*, respectively, but further application would result in a significant decrease of both. These reports confirm our results as predicted by the fitting model that magnesium fertilizer can increase the root yield of *P. notogingseng* within the level of 9 kg/ha (0.6 kg/667 m^2) (Table 10). In fact, magnesium fertilizer can also change the contents of secondary metabolites and/or their quality. In the pot culture experiment, He et al [30] detected the highest content of total saponins in new roots at the application level of 80 mg/kg magnesium fertilizer. The roots of *P. notoginseng* are all newly produced, and they ought to have a strong ability to absorb more magnesium, thus enhancing the accumulation of saponins. However, if excessive magnesium fertilizer is applied, it would decrease the absorption of phosphorus, potassium, and calcium in plants, as well as the K/Mg ratio in leaves, causing leaf roll and wilt [30,32]. In this study, the highest application level of magnesium used in the model is 1.8 kg/667 m^2, which is obviously lower than that of 80 mg/kg in the pot culture experiment. Therefore, it also induced a significant increase in the content of four active saponins, accompanied by the increased application level of magnesium fertilizer. However, owing to the negative effect of the excessive amount of magnesium on other ions, the proper application level should be restricted to a suitable range.

P. notoginseng, a shady plant with a C_3 cycle, prefers warm and humid environments for its growth. Its cultivation fields are all covered with adumbral and branches with residual leaves of *Metasequoia glyptostroboides* to maintain such an environment [5]. Under this type of environment, the root rot disease easily occurs based on our field survey. If the relative humidity of air reaches 95% and air temperature hits 20°C, the root rot disease would severely occur and spread quickly [10]. It has been found that most root diseases are favored by wet soil [11], and that warm and wet conditions hamper complete disease suppression, although frequent fungicide applications are used to prevent disease spread [33]. In fact, when growing *P. notoginseng*, it is prohibited to apply any fungicide because its roots are used as medicine. Fortunately, lower temperature and humidity can reduce the incidence of the disease [33]. Our pot culture experiment of soil moisture also confirmed that lower soil moisture could reduce the development of root rot disease, for which the optimum range of the soil water content varied from 0.564 FC to 0.59 FC, when considering the effect of soil moisture on root yield and the content of active saponins (Fig. 2). However, for certain crops such as *Salvia miltiorrhiza* (in our previous work), the root yield will significantly decrease under medium water stress (0.5 FC) when compared with that in 0.7 FC [34]. The contents of all six active components increased under the condition of drought stress except rosmarinic acid [34]. A field survey showed that the common incidence rates for root rot disease of *P. notoginseng* are 5–20%, with a severity higher than 70% in the 1980s [35]. In fact, this disease incidence has been increasing in the past two decades, owing to the limitation of farmlands, unavoidable continuous mono-cropping, and the significantly positive effect of continuous mono-cropping on the incidence of root rot disease [4]. The pathogens are not fully understood to date, and it is expected that the residues will be retained in the soils for many years after harvest. Our results confirmed that the presence of more

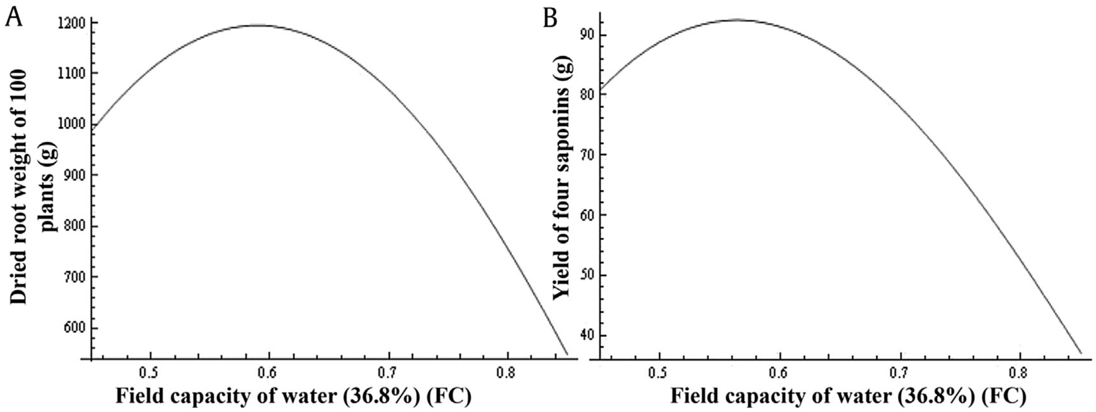

Fig. 2. Fitting models. (A) Fitting models showing the relationship between field capacity of water and dried root weight of 100 plants of *Panax notoginseng*. (B) Fitting models showing the relationship between FC (field capacity of water) and yield of four saponins.

soil water will exacerbate the impact of these pathogens and thus cause the disease to become even more severe. In this situation, controlling soil water by using pot culture to produce the root of *P. notoginseng* may prove to be our sole choice in the future. Our pot culture experiment has provided the theoretical evidence for this and the corresponding fitting model (Fig. 2).

Conflicts of interest

All authors declare no conflicts of interest.

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