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Effects of continuous cropping on *Panax notoginseng* growth, yield and saponins content

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ABSTRACT

We investigated the effects of soils with different continuous cropping years on *P. notoginseng* seedlings growth in indoor pot experiment. The results showed that with the increase of soil continuous cropping years, the growth-related indices of *P. notoginseng* seedlings were poor. Compared with the *P. notoginseng* grown on new soil, the growth indices of plants grown on 1-year continuous cropping soil had not different. However, the dry matter and total saponin content were significantly higher. In general, the continuous cropping problem effect of *P. notoginseng* is gradual, short-term continuous cropping (1-year) had no obstacle effect. This research aimed to study the performance of *P. notoginseng* planted on continuous cropping soils, which provides a reference for the further research on other similar medicinal crops facing the continuous cropping problems.

Keywords: Continuous cropping soil, growth, NPK content, obstacle effect, *Panax notoginseng*, pot culture, saponin content, yield

INTRODUCTION

Panax notoginseng [(*Panax notoginseng* (Burk.) F.H. Chen)] belongs to Panax genus, Araliaceae family. *P. notoginseng* is widely cultivated in Yunnan Province, China, saponins being the main active ingredients in Chinese medicine containing in the roots and rhizomes of *P. notoginseng*. However, the continuous cropping obstacle of *P. notoginseng* seriously hinders the sustainable development and yields. Notoginseng Radix et Rhizoma, significantly promotes blood circulation, relieves blood stasis, reduces blood fat, heals wound, strengthens and nourishes the body (2,10). Currently, it is mostly used to treat heart and cerebral vessel diseases. *P. notoginseng* culture requires manual labour, hence, not cultivated on large scale and is mainly grown in Yunnan province, Guangxi province and other places in China. Its main cultivated area is Wenshan prefecture, Yunnan, which accounts for > 90 % of China's total production (3).

Due to specific growing environment of *P. notoginseng* and the limited planting areas, there are serious continuous cropping problems. The continuous cropping results in death of plants, low yield and poor quality; while the shortened rotation period also results in serious

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disease and low seedling survival. The continuous cropping obstacle of *P. notoginseng* is major restriction factor for the sustainable development of *P. notoginseng* industry. The causes for continuous cropping obstacles of *P. notoginseng* are mainly related to (i). Autotoxicity of *P. notoginseng* (12), (ii). allelopathic effects of pathogenic microorganisms (14) and (iii). deterioration of soil physical and chemical properties (7). However, the harmful effects of continuous cropping obstacles on the growth, yield, and even pharmacodynamics of *P. notoginseng* have not been thoroughly studied. This study aimed to explore the specific impact of continuous cropping problems of *P. notoginseng,* provide relevant reference for further research to explore the mechanism of continuous cropping obstacle and develop the mitigation measures.

MATERIALS AND METHODS

I. Experimental

The pot experiment was done in Wenshan *Panax notoginseng* Research Institute, Yanshan, Wenshan prefecture, Yunnan. The relevant parameters were studied in Beijing University of Chinese Medicine and Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing.

The test soil was collected from the *P. notoginseng* planting base in Tuozhibai Village, Panlong Yi Township, Yanshan County, Wenshan Prefecture, Yunnan Province (Latitude: 23º18'06″, longitude: 104º 23'45″, altitude: 1487 m, mean annual temperature: 16.3 ºC and annual rainfall: 996 mm). It was red soil which rich in oxide of iron and aluminium and exhibit acidity (11) developed from carbonate rocks.

The soil samples were collected in January 2008 from the plots of continuous cropping 0 year (new land), 1-year (seed bed land), 2-years and 3-years of *P. notoginseng* respectively, referred as new soil, 1-year soil, 2-year soil, and 3-year soil. The soil samples were collected upto 20 cm depth.

For transplanting, 1-year old seedlings of *P. notoginseng* were obtained from the Wenshan *Panax Notoginseng* Research Institute in Yanshan County, Wenshan Prefecture, Yunnan Province.

II. Pot experiment

The experimental soil was sieved through (2 mm sieve) to remove impurities like plant residues and stone etc. Experiments began in January 2008 for 2 years (20 months). The experimental treatments consisted of one factor i.e. Duration of *P. notoginseng* continuous cropping (0,1,2,3 years). The pots size was 32 x 28 x12 cm and in each pot 10 kg experimental soil was added. One year old *P. notoginseng* seedlings of similar growth were dipped in 64 % Oxadixyl and 50 % carbendazim (diluted 500 times) for 15 min. Seventeen *P. notoginseng* seedlings were planted per pot. The various growth indices were recorded at different time periods after transplanting (Table 1).

Table 1. The time distribution of each index measurement

#	Indices	Months after *P. ginseng* planting（m）						
		3	6	7	12	15	18	20
1	Seedling survival Rate	√	√		√	√	√	√
2	Morphology		√				√	
3	Flower budding Rate		√				√	
4	Physio-biochemistry **C**haracteristics		√					
5	Nutrients			√				
6	Dry Matter		√					√
7	Saponins Content							√

III. Test Indices

(i). Growth indices

The seedling survival rate was recorded regularly after transplanting.

$$\text{Seedling survival rate (\%)} = \frac{\text{Number of existing seedlings}}{\text{Number of transplanted seedlings}} \times 100$$

To measure the plant growth, in every July (6 and 18 months after transplanting), the flower budding rate and the morphological indices (Plant height, stem diameter, and number of compound leaves) were measured as under:

(A). Flower bud: This index reflected the growth state of plants, before the reproductive growth stage.

$$\text{Flower budding rate (\%)} = \frac{\text{Number of flower budding seedlings}}{\text{Number of transplanting seedlings}} \times 100$$

(B). Plant height: The length from the base of stem above ground to the base of the compound petiole of *Panax notoginseng* (excluding flower), was measured by measuring tape.

(C). Stem diameter: Diameter of stem base above ground, was measured with vernier caliper.

Physiologicaly and biochemical indices: The following indices were measured/ calculated, in July of First year (6 months after transplanting):

(a). Leaves photosynthetic pigment: It was tested by the acetone-anhydrous ethanol extraction method (5). The absorbance of the extract for chlorophyll a, chlorophyll b, and carotenoids was tested at the wavelengths of 663 nm, 646 nm, and 470 nm respectively. Then the chlorophyll a, chlorophyll b, and carotenoids content were calculated.

(b). Soluble sugar content: It was tested using anthrone colorimetry (16).

First, a standard sugar concentration curve was made. Then the test sample was prepared and its optical density was measured by spectrophotometer at 620 nm. The sugar content in the filtrate was determined from the standard curve, and then sugar in the sample was calculated.

(c). Root volume: It was measured asper (15). The roots were excavated carefully, rinsed with water and surface moisture was dried with filter paper. Add enough water in measuring cylinder so that roots were immersed, make a mark, the reading was called A1. Then, the roots were immersed in the measuring cylinder, containing water, and the water surface raised was marked as A2.

Volume of Root = A2 Reading-A1 reading (per mL of water is calculated according to 1 cubic centimeter volume).

(d). Root vigour: It was measured by TTC method (6).

First, the TTC standard curve was made. And the test sample was prepared. Spectrophotometer was used at 485nm colorimetric, and blank test as the reference to read out the optical density. Then, check it with the standard curve, TTC reduction can be calculated.

Nutritional characteristics indices: The NPK content in ginseng plants reflected the nutritional characteristics of plants, which were tested according to (1), in August of first year (7 months after transplanting). The content was calculated from the value of measured results by standard curve.

(ii). Yield indices

Dry weight, root-shoot ratio: The harvested *P. notoginseng* plants were rinsed with clean water, and the surface moisture was blotted with filter paper. Subsequently, the plants were dried in oven at 60 ^{0}C to constant weight and the dry weights of the aboveground part and the underground part were taken.

Thereafter the root-shoot ratio were calculated as under:

$$\text{Root-Shoot Ratio (R/S)} = \frac{\text{Underground plant dry matter}}{\text{Aboveground plant dry matter}}$$

(iii). Saponins content

Content of 3 main saponins: The Ginsenoside Rg1, Ginsenoside Rb1, Notoginsenoside R1 in *Panax notoginseng* roots were determined by HPLC (4,6,9,13).

The fresh *P. notoginseng* plants were rinsed with clean water to remove dirt, dried and sliced. Then, these were dried to constant weight at 40 ^{0}C and powdered to pass through 100-mesh sieve. The 0.200 g powder was transferred to 10 mL volumetric flask and added 70 % methanol to scale mark. Ultrasonic extraction was applied at frequency of 20 kHz for 30 min, the loss of methanol was made up and shaken well. The solution was filtered through 0.45 μm filter membrane to get the filtrate as the test solution.

Ginsenoside Rg1 (110703-200424), Rb1 (110704-200424), and Notoginsenoside R1 (110745-200312) were purchased from the National Institutes for Food and Drug Control in China, and their purity were no less than 98.0%. The appropriate amount of reference substance was dried to constant weight, which was accurately weighed, and then, 70 % methanol was added respectively to prepare reference solutions containing ginsenoside Rg1 (1.2 mg/mL), Rb1 (1.2mg/mL), and notoginsenoside R1 (0.45 mg/mL).

The reference solution was injected into HPLC according to the serial injection volume. The sample was passed through Shim-Pack VP-ODS C18 (250 mm × 4.6 mm, 5 μm) column, and the detection wavelength and column temperature were 213nm and 40 ^{0}C, respectively. With the injection volume as the abscissa (X) and the chromatographic peak area as the ordinate (Y), the linear regression equations of 3-reference substances were calculated. The results are shown in Table 2. There was good linear relationship among the three components in their respective concentration range.

Shimazu LC-10ATVP high performance liquid chromatograph, SPD-10AVP UV Detector, Class-VP workstation control processing, and ShimadzuAX200 electronic analytical balance were used for determination.

Table 2. Linear relationships among the 3-main saponins in *P. notoginseng* roots

Saponins	Regression Equation	r	Linearity range /μg
Ginsenoside Rg1	Y=578274X+482548	0.9992	3.6-21.6
Ginsenoside Rb1	Y=441086X+423713	0.9996	3.6-21.6
Notoginsenoside R1	Y=389885X+85467	0.9995	1.35-8.1

The test powdered *P. notoginseng* sample was weighed and the test solution was prepared according to the above method. 20 μL of both the reference solution and the test solution were used to determine the content of ginsenoside Rg1, Rb1 and notoginsenoside R1 according to the above chromatographic conditions. The determination was repeated thrice and the average value was obtained.

IV. Data analysis: After deleting the abnormal values the test data were subjected to computer. According to the corresponding statistical analysis methods of each test design, Excel, SPSS 11.5 and DPS 7.05 software. The linear regression equation was used to fit the linear relationship of the content of the saponins. The content change data of *P. notoginseng* saponin was seen in mean ± SD and the rest were presented in the form of a graph or table.

RESULTS AND DISCUSSION

I. Growth of *Panax notoginseng* seedlings

(i). Seedling survival State

The effect of continuous cropping on the survival rate of *P. notoginseng* seedlings, showed that as the time goes by, there was gradual decline in the survival of *P. notoginseng* seedlings (Fig. 1). Comparing the survival rates of *P. notoginseng* seedlings on different continuous cropping soils, the survival rates of *P. notoginseng* seedlings on new soil and 1-year soil were similar at 15 months after transplanting. There was no significant differences between the seedlings survival in new soil and 1-year soil groups. However, the survival rate of *P. notoginseng* planted on 2-year soil and 3-year soil decreased significantly. The survival rate of *P. notoginseng* seedlings transplanted on 2-year soil was only 1.33 % after 6 months and dropped to zero after 12 months. And the survival rate of *P. notoginseng* on 3-

year soil was 13.06 % after 12 months but compared with the seedlings planted on new soil and 1-year soil, the survival rate of seedlings was significantly low. After 18 months of transplanting, the survival rate of seedlings growing on 3-year soil was only 1.67 %. These results indicated that the continuous cropping obstacle effects continued throughout the growth of *Panax notoginseng*. The obstacle effect in 1-year soil was little on the survival of *P. notoginseng* seedlings, yet 2-year soil collected showed the strongest obstacle effect. In general, with the extension of continuous cropping years, the impact of continuous cropping obstacles on the survival rate of *P. notoginseng* seedlings became more pronounced.

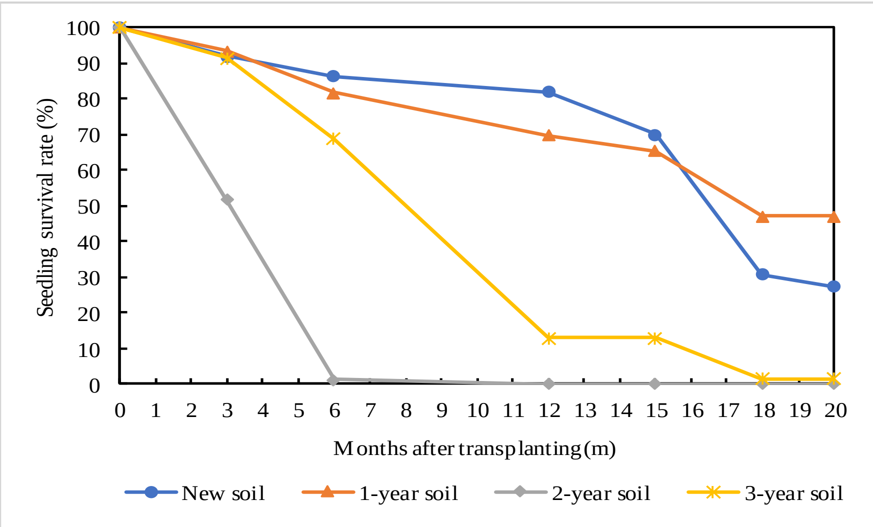

Figure 1. Effects of continuous cropping soil on the survival rate of *P. notoginseng* seedlings

In these experiments, we observed that growth of *P. notoginseng* cultivated on the 2-years soil was worst and all plants died 12 months after transplanting. The situation was worse even than the 3-years soil. In this regard, we conducted a traceability investigation and found that the fore crop on 2-year soil suffered from pests and diseases, which may have left residual pathogens in the soil. Therefore, we speculated that the effects of continuous cropping on *P. notoginseng* depended not only on the continuous cropping years, but also on the pests and diseases infestations in the previous crop. It is known that the occurrence of pests and diseases is one of the factors causing the formation of continuous cropping obstacles of *P. notoginseng*. We will monitor the phytotoxic allelochemicals and Microbes types and their population in future research to find the reasons for seedlings death. Because on 2-year soil, all seedlings died within 12 months after transplanting and the on 3-year soil only 1.67 % seedlings survived after 20 months of transplanting, hence, the growth data of plants on 2-year soil and 3-year soil could not be obtained in subsequent experiments. Hence, the partial indices of *P. ginseng* plants on 2-year soil and 3-year soil will not be considered in subsequent sections.

(ii). Morphological characteristic of *Panax notoginseng*:

The morphological changes in *P. notoginseng* plants under different continuous cropping years conditions were studied in the flower budding stage in first year (6 months after transplanting) and second year (18 months after transplanting) in July (Table 3).

Table 3. Changes in *P. notoginseng* plant morphology in different continuous cropping years conditions

Cropping years	Plant height (cm)		Stem diameter (cm)		Compound leaves Number	
	First Year	Second year	First Year	Second year	First Year	Second year
New soil	8.88 ab	13.10 a	2.00 a	3.45 a	2.25 ab	2.92 a
1-year soil	12.75 a	17.15 a	2.75 a	3.83 a	3.25 a	2.98 a
2-year soil	4.25 b	0.00 b	1.88 a	0.00 c	1.50 b	0.00 c
3-year soil	7.25 b	4.13 b	1.63 a	1.50 b	1.50 b	1.50 b

First Year : 6 months after transplanting, Second Year: 18 months after transplanting. Different lower case letters (a,b,c) indicate significant differences ($P < 0.05$) in cropping years.

The plant height, stem diameter, compound leaves number of *P. notoginseng* on 1-year soil were similar to new soil, but the differences in four groups were non-significant. However, with the further increase in continuous cropping years, these indices of four groups showed significant differences.

In second year, the *P. notoginseng* on new soil and 1-year soil made good growth compared with the data of 2-year and 3-year soil groups. Plants growth on 2 and 3-year soil groups was worst, as the plants withered and died on 2-year soil.

Compared to first year, effects of continuous cropping contributed to the worse phenotypic indices of plants on 2-year soil and 3-year soil significantly. The growth of *P. notoginseng* on 2 and 3-year soil was almost stagnant than first year. Plants on new and 1-year soil attained good growth. This showed that, influence of continuous cropping soil slowly appeared on the development of *P. notoginseng* with time, but the short term cropping did not have adverse impact.

(iii). **Flower bud State**

The budding rate of *P. notoginseng* plants under different continuous cropping years of soil conditions is shown in Figure 2. As an important index of the plants entering the reproductive growth stage, it shows the growth state of the plant. In first year of *P. notoginseng* planting, the budding rate of *P. notoginseng* planted on different soil were about 50 %. There was no statistical difference among the groups. However in second year of planting, the rate of the flower budding on the three soil groups followed the order: the new soil > 1-year soil > 3-year soil. The budding rate of plants on the new soil was highest (87.5 %). The budding rate of plants on 3-year soil was lowest (50 %).

The above flower budding rate showed the laws that the continuous cropping obstacle effect had little effect on plant development in short term planting. There were no significant differences among the *P. notoginseng* planted on new soil, 1-year soil and 3-year soil. However, in second year of *P. notoginseng* planting, the differences in the flower budding rate of three groups occurred. The flower budding rate showed a downward trend with the

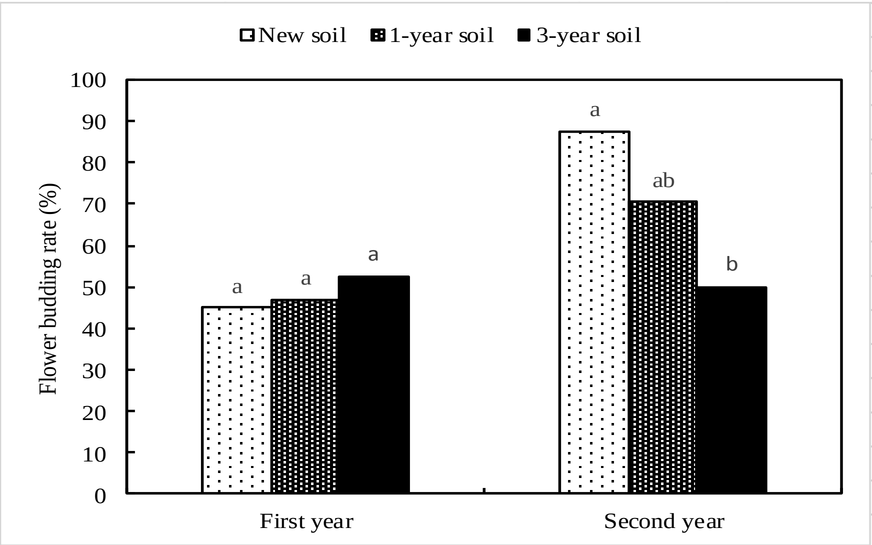

Figure 2. Effects of continuous cropping on flower budding rate in *P. notoginseng* plants
Different lower case letters (a,b,c) indicate significant differences ($P< 0.05$) in cropping years.

increasing number of years of continuous cultivation of soil. This showed that, the influence of continuous cropping soil slowly appeared on development of *P. notoginseng* with time.

(iv). Physiology and biochemistry characteristics of *Panax notoginseng* seedlings

The effects of continuous cropping were studied on soluble sugar content, photosynthetic pigments, root volume and root vigour of *P. notoginseng* planted on soils (Table 4). Compared with the new soil treatment, the content of photosynthetic pigments and soluble sugar, root volume and root vigour of *P. notoginseng* on 1-year and 3-year soil were higher. The growth trend of *P. notoginseng* planted on soil for 2-years was significantly poor and the above physiological indices were also reduced. The above indices also showed the worst growth state of plants on 2-year soil group.

Table 4. Effects of different continuous cropping years on physiological and biochemistry characteristics of *P. notoginseng* plants (6 months after transplanting)

Continuous cropping years	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Carotenoid (mg/g)	Leaves soluble sugar content (%)	Root volume (cm^3)	Root vigour (μg/g·h)
New soil	2.267 ab	1.142 a	0.819 a	22.25 a	5.51 b	0.685 b
1-year soil	2.352 a	1.235 a	0.874 a	23.37 a	5.84 ab	0.795 b
2-year soil	0.096 c	0.013 b	0.261 c	8.35 b	1.36 c	0.147 c
3-year soil	2.127 b	1.077 a	0.760 b	25.76 a	6.12 a	0.923 a

Different lower case letters (a,b,c) indicate significant differences ($P< 0.05$) in cropping years.

(v). Nutritional characteristics of *P. notoginseng*

To explore the effects of continuous cropping on the nutritional characteristics of *P. notoginseng* plants*,* the N, P*,* and K content in the aboveground parts (stems and leaves) and underground parts (roots) of *P. notoginseng* plants on new soil and 1-year soil were determined. The results (Figure 3), showed that NPK content in stem and leaves of *P. notoginseng* were higher than in roots. There were no significant differences in the NPK content in *P. notoginseng* planted on new soil or 1-year old soil. The experimental data indicated that short-term continuous cropping had limited impact on the NPK absorption by plants.

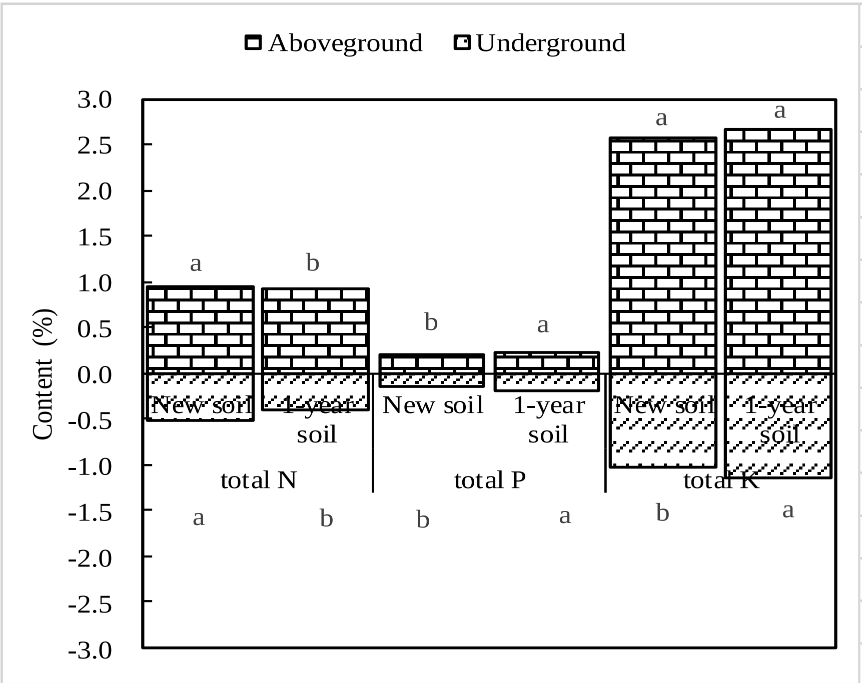

Figure 3. Effects of continuous cropping on NPK content in aboveground and underground parts of *P. notoginseng* plants (6 months after transplanting）

Different lower case letters (a,b,c) indicate significant differences (P< 0.05) in cropping years.

II. *P. notoginseng* Yield

The accumulation of dry matter in *P. notoginseng* was studied under continuous cropping conditions (Table 5). The dry matter accumulation and the root-shoot ratio of *P. notoginseng* planted on new, 1 and 3-year soil were higher in first year (6 months after transplanting). Only the growth of *P. notoginseng* planted on 2-year soil was the worst as plants almost withered and died. Thus, the dry matter accumulation and the root-shoot ratio of *P. notoginseng* seedlings on new soil, 1-year soil and 3-year soil, were not influenced by the continuous cropping obstacles at 6-months after transplanting.

In second year (20 months after transplanting), there were significant differences in dry matter accumulation of *P. notoginseng* (Table. 5). At this time, large number of *P. notoginseng* plants died on 2-year soil and 3-year soil. In this experiment, the dry matter of *P. notoginseng* on new soil was 6.78 g/pot and on 1-year soil and 3-years soil was 21.24 g/pot, and 2.14 g/pot, respectively. It can be found that the total dry matter of *P. notoginseng* from high to low in the soils and followed the order: 1-year soil > new soil > 3-year soil > 2-year soil, clearly showing the continuous cropping obstacles.

Table 5**.** The dry matter accumulation in *P. notoginseng* plants in different continuous cropping years conditions

Continuous cropping years	Underground DW		Above ground DW		Total DW		Root shoot ratio	
	6 Months	20 months	6 Months	20 months	6 Months	20 months	6 Months	20 months
New soil	0.85 b	3.81 b	0.60 a	2.97 b	1.45 b	6.78 b	1.43 b	1.28 b
1-year soil	1.18 a	14.36 a	0.62 a	6.88 a	1.80 a	21.24 a	1.90 a	2.08 a
2-year soil	0.15 c	0.00 c	0.16 b	0.00 c	0.31 c	0.00 c	0.94 c	-
3-year soil	1.29 a	2.14 bc	0.64 a	0.00 c	1.93 a	2.14 c	2.02 a	-

6 Months: 6- Months after transplanting (Unit : g/plant), 20 Months: 20- Months after transplanting (Unit : g/pot). Different lower case letters (a,b,c) indicate significant differences ($P< 0.05$) in cropping years.

Besides, the dry matter of *P. notoginseng* on 1-year soil was almost 3-times higher than plants on new soil in second year. The experimental results showed that the Notoginseng Radix et Rhizoma gave higher yield, when planted for a short time. And the underground dry weight of *P. notoginseng* on 1-year soil was greater than aboveground. Because the development of underground part of plant should be ensured to facilitate the uptake of water and nutrients by the plants (8).

The above experiments showed that with the increase of continuous cropping time obstacle effect increased. Although continuous cropping obstacles adversely affected the yield of *P. notoginseng* plants, but not in short-term continuous cropping.

(III). ***Saponins*** **content in** ***P. notoginseng***

The content and composition of saponins of *P. notoginseng* changes under continuous cropping conditions as shown in Table 6.

Table 6. Effects of continuous cropping on the content and components of saponins (%) in *P. notoginseng* (20 months after transplanting）

Cropping years	Ginsenoside Rg1 (%)	Ginsenoside Rb1 (%)	Notoginsenoside R1 (%)	Total saponins (%)
New soil	3.34±0.07	2.12±0.02	0.46±0.03	5.93±0.03
1-year soil	2.87*±0.04	2.59*±0.03	0.82*±0.02	6.27*±0.01

The total saponins content of *P. notoginseng* on 1-year soil was 6.27 %, which was higher than on new soil (5.93 %), and there were statistically significant differences. Further analysis showed the changes in saponins content of *P. notoginseng* that the ginsenoside Rg1 content of *P. notoginseng* planted on 1-year soil was significantly decreased, than on new soil. The content of ginsenoside Rb1 and notoginsenoside R1 were significantly improved which increased the total saponins content.

CONCLUSIONS

(i). The extension of continuous cropping years of *P. notoginseng* deteriorated the plants growth, resulted in low yield and poor quality of medicinal materials.
(ii). Short-term continuous cropping (1-year) had little or no obstacle effects on the growth state of *P. notoginseng*.
(iii). The growth of *P. notoginseng* on the 2-year soil was worst and all plants died. It may due to the harmful effects of preceding crop heavily infested with insect pests and diseases, which showed that the soil environment become worse without appropriate cultivation.

The continuous cropping obstacles always affects the *P. notoginseng* growth and yield and depends on the environmental conditions of plants. Therefore, the continuous cropping period was not the only factor that caused the continuous cropping obstacle.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest : The Authors declare no conflict of Interest.

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