

## Allelopathic effects of ginsenoside on soil sickness, soil enzymes, soil disease index and plant growth of Ginseng

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### ABSTRACT

Soil sickness of ginseng (*Panax ginseng* C. A. Mey.) has become a major limiting factor in ginseng cultivation. We found that Total Ginsenoside in Ginseng Root (TSPG) significantly decreased the activities of soil urease, acid phosphatase and laccase. Its high concentration of TSPG (10.00 mg L<sup>-1</sup>) significantly reduced the activity of soil sucrose. Besides, the TSPG can inhibited the growth of ginseng and increased the incidence of disease. Therefore, allelopathic effects of TSPG may be one of the main causes of Soil Sickness in ginseng.

**Keywords:** Acid phosphatase, allelopathy, disease incidence, enzyme activity, ginseng cultivation, ginsenoside, growth, incidence, laccase. *Panax ginseng*, Soil sickness, sucrose, urease,

### INTRODUCTION

Ginseng is a perennial herb found mainly in tropical areas of North America and Asia (9). It is widely cultivated in China, Japan, Korea and other countries in the region (10). However, allelopathy of ginseng leads to deterioration of soil physical and chemical properties, nutrients imbalance, decrease the enzyme activity and increase the disease incidence (30). Allelochemicals are produced in the roots, stems, leaves and fruits of plants, and released into the soil through root secretion, leaching, volatilization and decomposition, thus producing Auto-toxic effects and inhibiting the normal growth, development and metabolism of plants (1,7,17). Allelopathy is found in *Panax quiquefolium* L. (11), *Panax notoginseng* Burkill. (15), *Rehmannia glutinosa* Gaertn. (6,14), *Pseudostellaria davidii* Franch. (26), *Atractylodes macrocephala* Koidz. (32) and other medicinal plants. According to their chemical structure and properties, allelopathic substances can be classified into 10 categories, namely (i). Water-soluble organic acids, linear alcohols, aliphatic aldehydes and ketones; (ii). Simple unsaturated lactone; (iii) long-chain fatty acids and polyacetylene; (iv) quinone substances; (v). Phenolic substances; (vi). Cinnamic acid and its derivatives; (vii). Coumarins; (viii). Flavonoids; (ix). Tannins; (x). Steroids and terpenoids (21). Ginsenoside is the main active ingredient of ginseng (3,25). The ginsenoside was detected in soil methanol extract, ginseng water extract and ginseng root exudates (15). Ginsenoside R1, Rg1, Re, Rg2 and Rd inhibits seedling emergence, growth and root cell activity (26). High concentration of Rg1 are inhibitory to seed germination and seedling growth, while its low concentration are stimulatory (13). Like monomer saponins, the ginsenoside is also self-toxic, stimulates growth of pathogenic bacteria and changes the microbial balance the soil, that is, it has allelopathic potential (19,31).

Studies on allelopathy of ginsenoside mainly focus on soil microbial diversity, seed germination, seedling growth and root activity. While, there are few studies on its allelopathic effects on soil enzyme activity and ginseng growth. From the perspective of

improving the soil quality, the effects of ginsenosides on soil enzyme activity and growth of ginseng may have profound impacts, especially when there is less land available to for ginseng cultivation. In previous studies, we found that ginsenoside Rg1 can cause soil diseases, hence, we thought whether Total Ginsenoside in Ginseng Root (TSPG) also cause soil diseases. To verify this, we artificially added 0.10 mg L<sup>-1</sup>, 1.00 mg L<sup>-1</sup> and 10.00mg L<sup>-1</sup> of TSPG in the soil of new woodland soil (Control soil from forest without ginseng culture), to study their effects on soil enzyme activities and ginseng quality. These results will be helpful to know the effects of ginseng allelochemicals on the soil enzymes activities in ginseng cultivated soil and to understand the mechanism of development of continuous cropping problems in ginseng.

## MATERIALS AND METHODS

Ginseng soil was collected from Baixi Forest Farm, Fusong County, Baishan City, Jilin (689 m above sea level, 42°34'198"N, 127°28'03"E) in August, 2019. The parent material of soil in the study area was classified as dark brown earth. All the soils were new woodland soils that had not been planted with ginseng. Ginseng seedlings were purchased from Ginseng trading market, Wanliang Town, Fusong County, Jilin Province (512.2 m above sea level, 42°44'38"N, 127°30'98"E). The ginseng cultivar was horse teeth'. All chemicals and solvents (DNS, phenol 2,6-Dibromoquinone-4-chloroimide, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), disodium phenyl phosphate) used were of analytical grade.

### Site Description and test design

The studies were done in our shaded greenhouse (4 m long, 3.5 m wide and 3 m in height), Changchun Agricultural Exposition Park, Jilin Province (43°8'177" N, 125°44'08" E), East China. The climatic of Changchun is temperate, annual mean temperature: 7.7 °C, mean annual precipitation: 746.7 mm. The experiment was done in plastic pots (length 37 cm × width 37 cm × height 21 cm). The soil was sieved through 2 mm size to removed rocks and other impurities. Two years old seedlings of similar size were transplanted into pots on November 11, 2019. Nine seedlings were cultivated in each pot, treatments were replicated thrice in complete randomized design. One litre water was added per pot every 3d to maintain the maximum water capacity in pots. To keep soil moisture and temperature stable, the soil was covered with 2 cm peat layer of organic matter (rich in nitrogen, phosphorus, potassium, calcium, manganese and other nutrients).

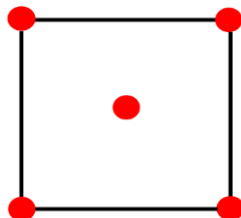


Figure 1. Schematic diagram of soil 5-point sampling method. The red circles are sampling points.

### Soil samples collection

The rhizosphere soil from pots was collected from 10 cm depth from the root system in November and December 2019. The soil around the root system of ginseng was collected by 5-point sampling method (Fig. 1). The collected soil is mixed to produce a uniform sample. The soil was dried naturally and ground, roots etc. from these soil samples were removed by sieving through 2 mm sieve. The soil properties and enzyme activity were determined.

### Soil properties

Soil properties were analyzed using standard soil test methods per the agriculture protocols for China (33). Soil pH was determined by pH meter (soil to water ratio was 1:5), Soil Conductivity by conductivity meter (soil to water ratio was 1:5), Soil Organic Matter (SOM) by potassium dichromate volume-dilutive heat method, Total Nitrogen (TN) by Kjeldahl digestion method, Total Phosphorus (TP) by Mo-Sb anti spectrophotometric method, Available Nitrogen (AN) using the alkali-hydrolyzed diffusing method, Available phosphorus (AP) was extracted with  $\text{NH}_4\text{F-HCl}$  solution, and then determined by ultraviolet visible spectrophotometer (2).

### Soil enzyme activity

Soil enzymes are the most active biological component in soil for the nutrients transport and energy flow in the soil ecosystem (12,19,29). Important enzymes urease (S-UE), sucrase (S-SC), catalase (S-CAT), acid phosphatase (S-ACP) and laccase (SL) timely and accurately reflects the intensity and direction of soil organic matter conversion and indicates the soil fertility status. These enzymes were measured using a kit produced by the company Solarbio. Enzyme activity was determined using 96-well microtiter plates and followed the product manual provided by Solarbio. Each sample was repeated five times.

### Disease severity, disease index and development of Ginseng root diseases

The disease severity index, the root disease index and root growth were calculated for all pots as under:

$$\text{The disease severity index (DSI)} = \frac{(Xa \times 1) + (Xb \times 2) + (Xc \times 3) + (Xd \times 4) + (Xe \times 5) + (Xf \times 6)}{Xa + Xb + Xc + Xd + Xe + Xf}$$

$$\text{Root disease index} = \frac{\text{Number of infected plants}}{\text{Total amount of plants}} \times 100\%$$

$$\text{Root weight} = \frac{\text{Root weight (after harvest)} - \text{Root weight (original not planted)}}{\text{Root weight (original not planted)}} \times 100\%$$

Where, “root weight (original not planted)”: Mean weight of 2-year old, 100- ginseng seedlings of similar size before transplanting. Xa, Xb, Xc, Xd, Xe, and Xf: Numbers of plants roots with rot severity of grades A, B, C, D, E, and F, respectively (18,21).

The conditions of ginseng roots were Graded as under at harvest:

- Grade A:** No visible lesions at the root,
- Grade B:** Showed lesions up to 0.9 mm in diameter (brown),
- Grade C:** Showed lesions 1-4.0 mm diameter (dark brown),
- Grade D:** Showed lesions 4-7.0 mm diameter (black),
- Grade E:** Showed mutual fusion of the lesions, and
- Grade F:** Showed complete lesions.

### Statistical analyses

All results were presented as means  $\pm$  standard deviation (S.D.). All data were statistically analyzed by SPSS software (ver. 20.0; IBM, New York, USA). In statistical analysis, the results were considered significant when  $p < 0.05$ . If the results of One-way ANOVA showed the significant difference at the 0.05, we used Duncan's new multiple range test of variance performed to compare the different treatments.

## RESULTS AND DISCUSSION

### Basic characteristics of soil

The soil of new forest land was acidic (pH 5 and 6) and the Conductivity was 101.03. As per China's soil fertility classification standards, the experimental soil was rich in Organic Matter and Total Nitrogen but poor in Total Phosphorus, Available Nitrogen, Available Phosphorus (Table 1). The soil was suitable for normal growth of ginseng.

Table 1. Soil properties of new forest soils

Soil Physical Property	New Forest Soil
pH	5.12±0.67
conductivity (us/cm)	101.03±0.5
Soil organic matter (g/kg)	98.23±9.24
Total nitrogen (mg/g)	11.23±1.38
Total phosphorus (mg/g)	0.43±0.02
Available nitrogen (mg/g)	0.095±0.015
Available phosphorus (mg/g)	0.035±0.03
C/N	8.36±0.72

### Soil Enzyme activity

Different concentrations of TSPG had variable effects on soil enzyme activities at different stages (Fig. 2). In soil samples collected in November and December, the enzyme activities of urease, catalase and laccase were lower than control. This showed that TSPG at different concentrations had variable allelopathic effects on urease, catalase and laccase. In urease, high concentration of TSPG (10.00 mg L<sup>-1</sup>) in the soil collected in November and December was most inhibitory to enzyme activity. In catalase, low concentration (0.10 mg L<sup>-1</sup>) TSPG in the soil collected in November and December was most inhibitory to enzyme activity. In laccase, the low concentration (0.10 mg L<sup>-1</sup>) TSPG in the soil collected in November was most inhibitory to enzyme activity, while the high concentration (10.00 mg L<sup>-1</sup>) TSPG in the soil collected in December was also inhibitory to enzyme activity. However, in sucrase, soil enzyme activity was higher TSPG at low concentration (0.10 mg L<sup>-1</sup>) and high concentration (10.00 mg L<sup>-1</sup>) than control, indicating that ginseng had positive self-protection effects at low concentration and high concentration, its medium concentration (1.00 mg L<sup>-1</sup>) was also inhibitory. In acid phosphatase, the soil enzyme activity treated with high concentration (10.00 mg L<sup>-1</sup>) was significantly higher than control, indicating that TSPG has a protective effect at high concentration. The soil enzymes are one of the main components of soil and include their activities in soil. The soil enzymes play an important role in the soil microecological environment through highly dynamic nutrients exchanges (5,24). Therefore, TSPG can lead to the occurrence of soil sickness by adversely affecting the soil enzymes activities. The TSPG concentration in ginseng rhizosphere soil, proved that secondary phytochemicals affects the composition of soil microbial community and leads to occurrence of ginseng soil-borne diseases (19). This is consistent with our experimental results.

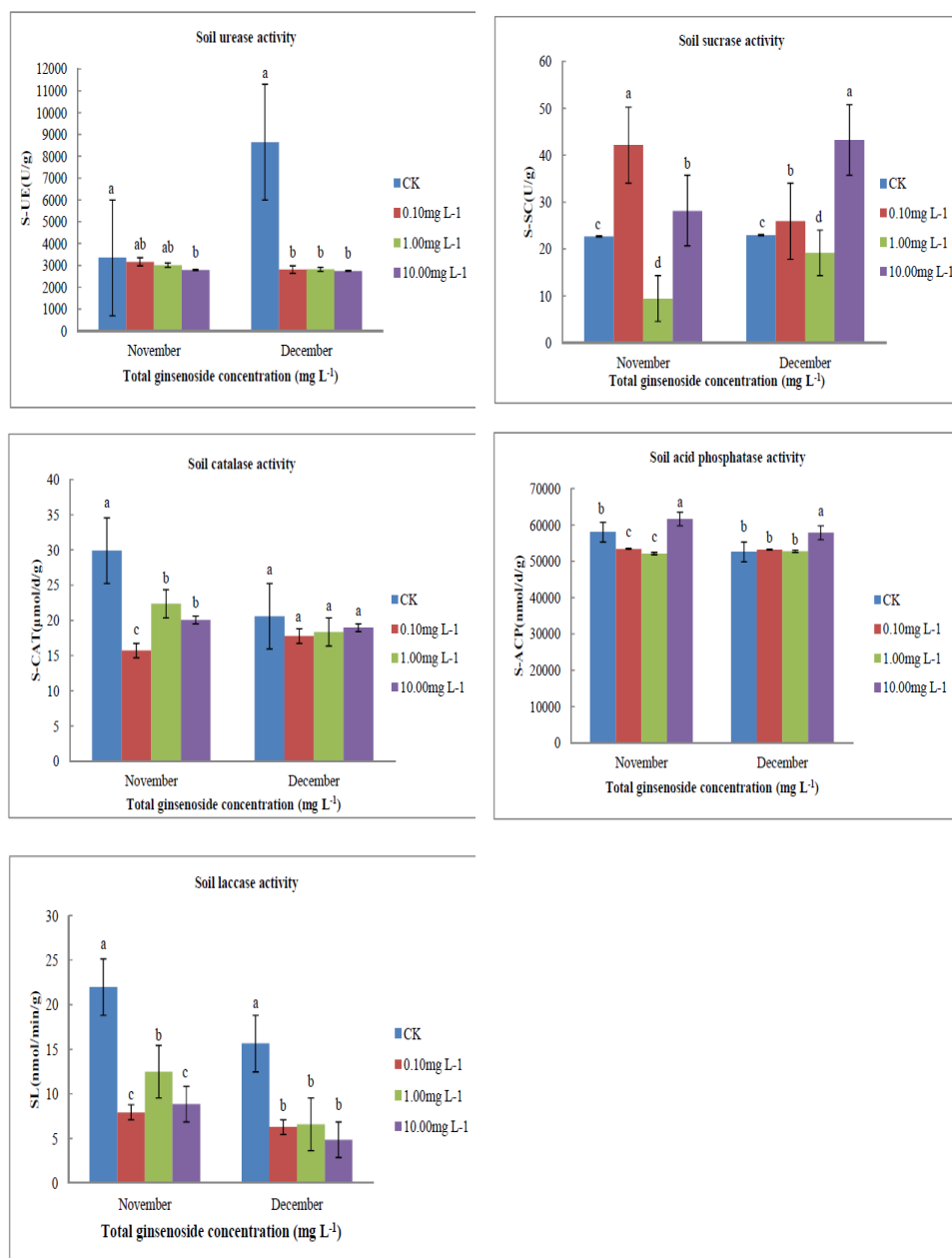


Figure 2. Effects of different concentrations of TSPG on soil enzyme activities. Each value of extract represents means  $\pm$ SE (standard errors) (n=5). Means with similar letters are not significantly different at  $p < 0.05$ .

### Ginseng growth

There were no significant differences in shoots growth of ginseng plants treated with low concentration ( $0.10 \text{ mg L}^{-1}$ ) of TSPG and control. With the increase in TSPG concentration, ginseng plants growth was decreased (Fig 3). Growth rates of treated ginseng plants were measured as root weight harvest index. The CK (89.33 %) growth rate was significantly higher than  $1.00 \text{ mg L}^{-1}$  and  $10.00 \text{ mg L}^{-1}$  TSPG, concentration but was similar to  $0.10 \text{ mg L}^{-1}$  (Fig 4). These results indicated that TSPG can induce allelopathy and affects the growth of experimental plants. The higher the concentration of TSPG, the stronger was the allelopathy of ginseng.

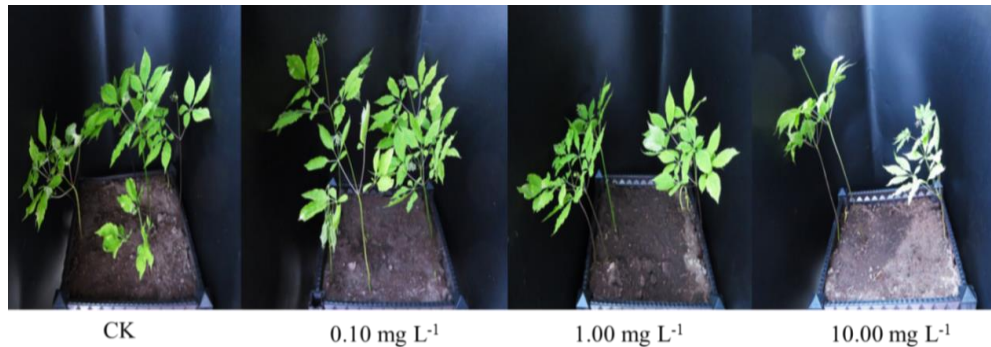


Figure 3. Effects of different concentrations of TSPG on shoot (aboveground growth) of ginseng at 45 days after transplanting.

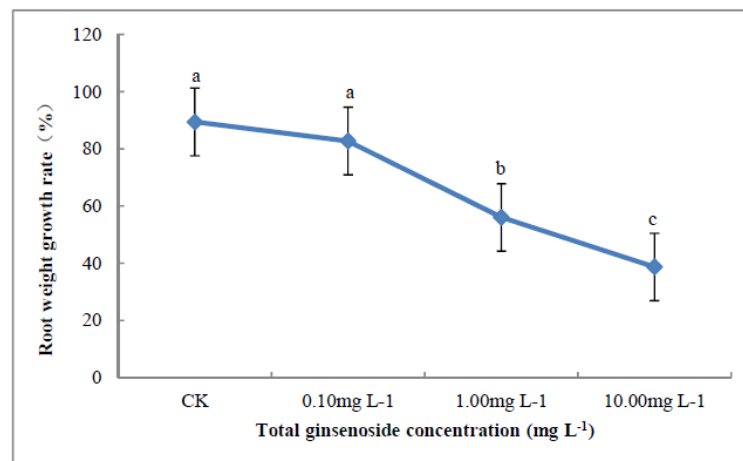


Figure 4. Growth rate of root weight of four treatments. Each value of extract represents means  $\pm$ SE (Standard Errors) ( $n=5$ ). Means with similar letters are not significantly different at  $p < 0.05$ .

### Disease index and disease severity of Ginseng

The disease indexes of root and disease severity of roots in different treatments were measured at ginseng harvest (Fig. 5 and Table 2). Statistically there was no significant differences between the roots (underground parts) of TSPG treated with low concentration ( $0.10 \text{ mg L}^{-1}$ ) and control. With the increase in TSPG concentration, root diseases of ginseng

were aggravated, root tubers could not expand normally, and the number of fibrous roots was significantly reduced. The root disease index of CK was significantly lower than 1.00 mg L<sup>-1</sup> and 10.00 mg L<sup>-1</sup>, but there was no significant difference between CK and 0.10 mg L<sup>-1</sup>. The root disease indexes of soil treated with TSPG (except 0.10 mg L<sup>-1</sup>) were significantly higher than those treated with 1.00 mg L<sup>-1</sup> and 10.00 mg L<sup>-1</sup>. Similarly, the disease severity of CK was lower than other treatments. The severity of CK was significantly lower than 1.00 mg L<sup>-1</sup> and 10.00 mg L<sup>-1</sup>, but not significantly different from that of 0.10 mg L<sup>-1</sup>. This indicated that higher the concentration of TSPG, the stronger was the allelopathic effects of ginseng, which increased the incidence of ginseng root rot. Because the TSPG promotes the degradation of plant litter and organic matter, increasing the allelochemicals, thus leading to disease severity (28).

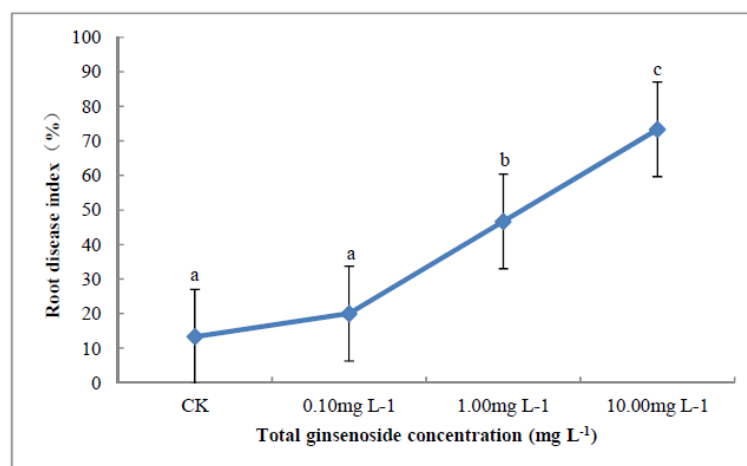


Figure 5. Root disease indexes of four treatments. Each value of extract represents means  $\pm$ SE (Standard Errors) (n=3). Means with similar letters are not significantly different at  $p < 0.05$ .

Table 2. Severity of root disease under four treatments

TSPG Dose (mg L <sup>-1</sup> )	Ginseng Root Disease Severity
CK	1.20a
0.10	1.40a
1.00	2.27a
10.0	2.53a

Each value of extract represents means  $\pm$ SE (standard errors) (n=3). Means with similar letters are not significantly different at  $p < 0.05$ .

## CONCLUSIONS

The allelopathy significantly affects soil enzyme activity, plant growth and crop quality. TSPG had variable allelopathic effects on urease, catalase and laccase enzymes. In sucrose, soil enzyme activity was higher at low (0.10 mg L<sup>-1</sup>) and high concentration (10.00 mg L<sup>-1</sup>) than control. Thus ginseng had positive self-protection effects at low and high concentrations, and had inhibitory effects at medium concentration (1.00 mg L<sup>-1</sup>). In acid phosphatase, the soil enzyme activity at high concentration (10.00 mg L<sup>-1</sup>) was significantly higher than control, indicating that ginseng had protective effects at high concentration. Contrarily, the TSPG (except 0.10 mg L<sup>-1</sup>) stimulated the root morbidity, aggravated disease,

reduced root growth and inhibited the plant growth. Therefore, we speculated that TSPG is one of the possible factors leading to the soil sickness of ginseng, in continuous monoculture, which decreased the ginseng roots yield and quality. Hence, further in-depth studies are needed on the allelopathic potential of TSPG.

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