https://doi.org/10.26651/allelo.j/2021-53-2-1336 0971-4693/94 Euro 20.00
Allelopathy Journal 53 (2): 177-188 (July, 2021) *International Allelopathy Foundation* 2021
Table: 2, Figs : 5

Effects of soils on release and accumulation of highly active ginsenoside allelochemicals in soil

X.R. Meng, Y. Zhan, M. Tang, X. Chen, E.P. Wang, Z. Zhang, L.Y. Du, Y.H. Xu[1] Q. Li* and C.B. Chen*
National High-tech Industrial Development Zone, Jilin Ginseng Academy,
Changchun University of Chinese Medicine, Postal Address: No. 1035,
Bosuo Road, Jingyue Changchun , 130117, China.
E. Mail: ccb2021@126.com,wode17k@163.com

(Received in revised form: May 30, 2021)

ABSTRACT

Allelopathy is the main cause of soil sickness of ginseng (*Panax ginseng* C. A. Mey.) crop, but the source of very effective allelochemicals and their possible physiological damage have not been determined. In this study, we used 5-types of soils viz., (i). Healthy soil (HS), (ii). Soil with stems and leaves extracts (SEL), (iii). Soil + stems and leaves (BS), (iv). Rhizosphere soil (RS) and (v). Continuous cropping problem soil (CCS) in pot culture. We simulated the different release pathways of allelochemicals from ginseng plants and the contents of ginsenoside Rg_1, Rb_1, Re and Rd were determined in these soils. We determined the activity of soluble protease and antioxidant enzymes in ginseng soil and also monitored the growth and development of ginseng plants. The results showed that the effects of the single allelochemicals release pathway of *Panax ginseng* followed the order: (RS) > (BS) > (SEL). The ginsenosides Rg_1, Rb_1, Re and Rd were mainly released as root exudates. The interactions occurred between these ginsenosides in soil, which seemed to be one of the key inducements to cause the oxidative stress injury. This study may provide a theoretical basis to explain the release pathways and accumulation patterns of ginsenoside allelochemical in soil.

Keywords: Allelochemicals, allelopathy, antioxidant enzymes, continuous cropping problem, ginseng, ginsenoside, growth, *Panax ginseng,* protease

INTRODUCTION

Ginseng (*Panax ginseng* C.A.Mey.) of Panax family is perennial herb. Modern pharmacological research have shown that it resist & ageing, anti-depressant, anti-senile dementia, anti-neoplastic and cure other diseases (7,8,13). Its long-term planting causes soil sickness, which reduces the plants growth, increases plant diseases and decreases quality of product. The soil sickness is the main problem prohibiting the sustainable development of ginseng industry in China. Therefore, it is necessary to find the mechanism of soil sickness to improve the ginseng quality and production.

The main pathways of plants to release the allelochemicals are plant leaching, root exudation and decomposition of stem and leaf residues. The allelopathic autotoxicity is recognized as one of the important inducements of continuous cropping problem (5,17). Early studies on continuous cropping problem of ginseng mainly focused on soil-borne diseases, physical and chemical properties of soil and nutrients imbalance. Recent studies

*Correspondence author, [1]State Research Centre of Ginseng Breeding, Jilin Agricultural University, Changchun, 130118, China.

have shown that allelochemicals secreted from ginseng inhibits its own seed germination and seedlings growth, while promotes spore germination and mycelial growth of *Cylindrocarpon destructans* pathogen of ginseng (2,10). Allelopathy plays an increasingly prominent role in continuous cropping problem. However, the present research on *Panax ginseng* mainly focus on the identification of allelochemicals and the mechanism of action, but not on the pathways of release of ginseng allelochemicals.

The saponin allelochemicals viz., Rg_1, Rb_1, Re, and Rd causes autotoxicity and has strong biological activity (9,19). Under environmental stress, plants produce more reactive oxygen species (ROS), while the antioxidant enzymes system in plants resist ROS harmful effects. Therefore, the antioxidant enzymes are the key to evaluate the autotoxicity of allelochemicals (6). In this study, 5-soils were (i). Healthy soil (HS), (ii). Soil with stems and leaves extracts (SEL), (iii). Soil + stems and leaves (BS), (iv). Rhizosphere soil (RS), (v). Continuous cropping problem soil (CCS). Details of experimental soils used are shown in Table 1. These treatments simulated 4-allelochemicals releasing pathways: (a). healthy group, (b). Eluviation, (c). Decomposition, (d). Exudation, (e). Continuous cropping, to monitor the growth and antioxidant indexes of *Panax ginseng*. The contents of four key ginsenoside allelochemicals (Rg_1, Rb_1, Re and Rd) were detected in different soils.

MATERIALS AND METHODS

Chemicals and reagents

Acetonitrile and methanol (HPLC grade, Tedia) and formic acid (HPLC grade, Sigma-Aldrich). Ultrapure water (18 MΩ/cm, Milli-Q water system). Reference ginsenosides Rb_1, Rb_2, Rd, Re, Rg_1 (≥ 98 %, Baoman Bio Ltd.). Coomassie brilliant blue solution. Catalase (CAT), Superoxide dismutase (SOD), Peroxidase (POD) and Malondialdehyde (MDA) assay kits (Shanghai Solarbio Bio Ltd.).

Pot Culture Experiment

The studies were done in our shaded greenhouse (4 m long, 3.5 m wide and 3 m height), Changchun Agricultural Exposition Park, Jilin Province (43º8177" N, 125º4408" E. The experiment was done in plastic pots (length 37 cm × width 37 cm × height 21 cm). All the plastic pots were placed in the shaded greenhouse in natural sunlight, (temperature: 20 ℃, Relitive humidity: 70 - 75 %). The soil was sieved through 2 mm size to removed rocks and other impurities. Each plastic pot contained 15 kg soil. Two years old ginseng seedlings of similar size were transplanted into pots on November 11, 2019. Nine seedlings were planted per pot, treatments were replicated thrice in complete randomized design. One litre distilled water was added per pot every 3 days to maintain field capacity moisture. After 5-months growth, ginseng roots samples were harvested. Five point sampling method was used to collect the soil around ginseng roots from 3-sites per pot and at 3-growth stages [leaf spreading stage (November 21, 2019), flowering stage (December 3, 2019) and harvest stage (April 11, 2020)]. After natural air drying, the samples were powdered and sieved through 0.425 mm size for testing.

Table 1. Details of Experimental soils.

Soil Type	Details
HS	Soil was collected from Baixi forest farm, Fusong County, Baishan City, Jilin Province.
SEL	Soil was collected from the same site of healthy soil site. The leaves and stem (washed in water) of 6-years old greenhouse grown ginseng plants were washed in water to simulate rain leachates. One liter of leachate was added every 3 days.
BS	Soil was collected from the same site of healthy soil site. The whole plants (Leaf + stem + roots) of 6-year-old greenhouse grown ginseng plants were washed, cut in 2 cm pieces, air dried and buried to decay in meshed bags for 90 days. The decayed residue were mixed in soil in 1:30 ratio.
RS	During the harvest period, soil was shook off around ginseng roots of 6-years old continuous cropped ginseng.
CCS	Soil was collected from 6- years old continuously cropped ginseng field, near the new forest land.

Soil solution preparation

Reference solutions for 4-ginsenosides were prepared in 80 % methanol-water (4:1 ratio) (HPLC grade) and the final concentration of each solution was 1 mg/mL. Each reference sample was diluted with 80 % methanol-water (HPLC grade) to make 8-concentrations (0.01 0.05, 0.1, 0.5, 1, 2, 5, 10 ppm) for UPLC-QQQ-MS analysis.

Twenty g soil of each treatment was extracted overnight with 200 ml of 80 % methanol-water. The samples were extracted twice with ultrasonic wave at 40 ºC for 60 min each time. The extracts were centrifuged at 10000 rpm at 4 ºC for 10 min to get supernatant. The methanol was volatilized at 50 ºC on water bath, leaving the water phase, which was extracted thrice with chloroform and water-saturated n-butanol (1:1). The n-butanol phase was evaporated by dry nitrogen, then made volume to 200 µL with 80 % HPLC grade methanol, filtered through 0.22 µm membrane and analyzed by UPLC-QQQ-MS.

Determination of ginsenosides in soil by UPLC-QQQ-MS

Ginsenosides were separated on a Thermo Scientific Syncronis C_{18} column (50 mm×3 mm, 1.7 µm). The mobile phase was solvent A, containing 0.1 % formic acid aqueous solution and acetonitrile was solvent B. The gradient elution program was set as under : 0-5 min at B; 5-29 min at 19 %- 25 % B; 29-72 min at 25 %-40 % B; 72-77 min at 40%-90% B; 77-80 min at 90 % B; 80-83 min at 90 %- 19% B; 83-88 min at 19 % B with a flow rate of 200 µL/min. The injection volume was 5 µL and the column temperature was set at 35 ºC. The sampler temperature was set at 4 ºC.

Mass spectrometry was detected via electrospray ionization in negative ion mode and multiple reaction monitoring (MRM), mass scanning range: m/z 100~1500, spray voltage: 2500 V, sheath gas pressure: 35 arb; auxiliary gas pressure: 10 arb; transmission capillary tube temperature: 350 degrees; atomizer temperature: 300 ℃.

Determination of ginseng growth and antioxidant enzymes

(i). Determination of soluble protease: The soluble protein was determined by Coomassie brilliant blue method and was defined as 1g protein per L of plant tissue homogenate.

(ii). Determination of antioxidant enzymes: Catalase (CAT), Superoxide dismutase (SOD), Peroxidase (POD) and Malondialdehyde (MDA) activities were determined with the kit produced by Shanghai Solarbio Biotechnology Co., Ltd. CAT activity was defined as the amount of 1μ mol H_2O_2 decomposed per second per mg tissue protein; SOD was defined as the amount of SOD corresponding to 50 % inhibition rate of SOD in the reaction system; POD was defined as the amount of enzyme catalyzing 1 μg substrate per minute per mg tissue protein at 37 ℃; MDA content was defined as the amount of MDA produced in per mg tissue protein.

(iii). Ginseng growth: Five plants were randomly selected from each treatment, the above ground and underground growth of ginseng plants was recorded at harvest.

(iv). Allelopathic response index: The allelopathic response index (RI) was calculated by Williamson's formula (17) :

$$RI = \frac{1-C}{T}(T \geq C) \text{ or } RI = \frac{T}{C-1}(T < C)$$

C: Control, T: Treatment, RI > 0: Stimulation, RI < 0: Inhibition.

Data processing

Xcalibur software (ThermoFisher Science) was used to process the UPLC-QQQ-MS data. The processed data were imported into SPSS software 13.0 for statistical analysis. Excel 2013, GraphPad Prism 7.0.4 were used for data recording, sorting and chart making.

RESULTS AND DISCUSSION

Ginsenoside content

The content of ginsenoside in different treatment groups was determined by UPLC-QQQ-MS. The peak area of the quantitative ion pair was integrated and the content of ginsenoside was calculated by the regression equation. The distribution of four ginsenosides in different soils is represented by a line in three growth periods, i.e. leaf developing stage, flower stage and harvest period (Fig. 1).

In the early stage of growth of ginseng, compared with HS group, the contents of saponin Rg_1, Rb_1, Re and Rd in soil of SEL, BS and RS groups were significantly increased, indicating that active saponins accumulated rapidly in different ways in the early growth stage; compared with CCS group, the four saponins in RS group were significantly increased, and Rg_1, Re and Rd contents in BS group were significantly increased, indicating that the secretion pathway had a great influence on the release of allelopathic substances, which might affect the plant growth. It showed a negative regulatory effect on plant growth.

In the late growth stage of ginseng plants, the accumulation of saponins in the soil of 5 groups was consistent. Compared with HS group, the content of four saponins in SEL, BS, RS and CCS groups increased gradually, and the differences between various treatment groups were significant. The content of Rg_1 in the CCS group was 21.48 μg/kg, Rb_1 was 27.19 μg/kg, Re content was 41.10 μg/kg, and Rd content was as high as 10.76 μg/kg. In the later stage of plant growth, the content of saponins in the release pathway of single allelopathy decreased and the content of saponins in the compound pathway increased. It indicated that with the growth of plants, the microorganisms environment and soil structure in soil changed constantly and there might be strong interactions between saponins.

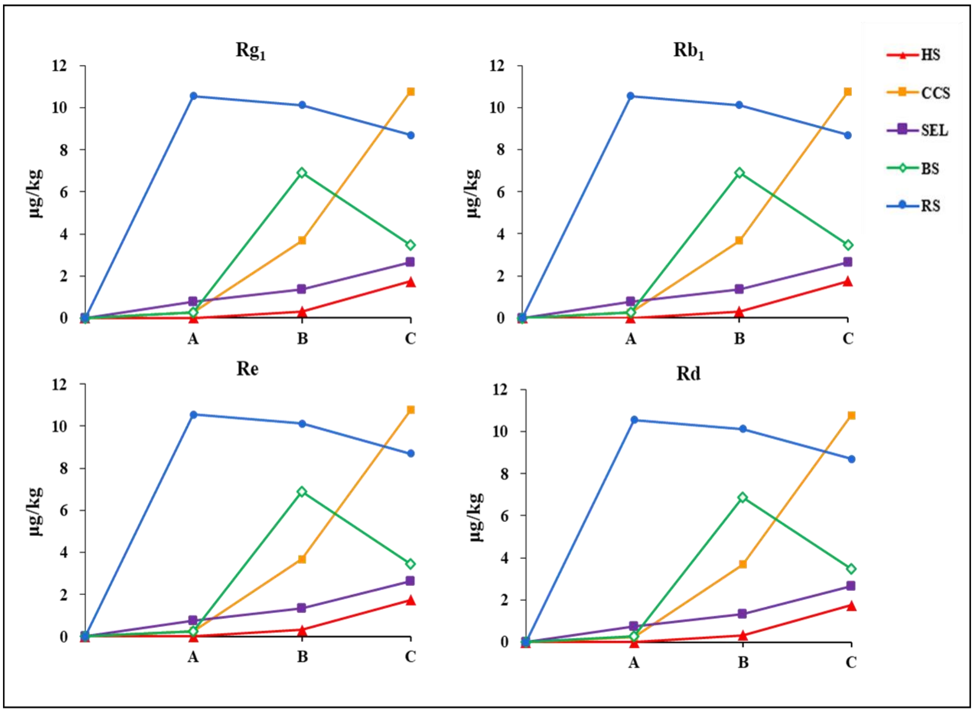

Figure 1. The content of Ginsenoside in different soil during the growth period of ginseng.
A: Leaf spreading stage, B: Flowering stage, C: Harvest stage.
HS: Healthy soil, SEL: Soil with stems and leaves extracts, BS: Soil + stems and leaves,
RS: Rhizosphere soil, CCS: Continuous cropping problem soil.

The accumulation analysis of four highly allelopathic active substances showed that Rg_1, Rb_1, Re and Rd all originated from the secretory pathway, and Rg_1 content was easy to accumulate in a single release pathway; Rb_1 content increased first and then decreased; the content of Re and Rd was very high in the early stage and slowly degraded in the later stage.

Soluble protein content in *Panax ginseng*

Soluble protein plays an important role in protecting the bio-membrane of the plant cell, it's also indispensable in plant stress resistance (21). The content of soluble protein in ginseng potted in different soil was analyzed (Fig. 2). The results showed that the soluble protein content of the RS treatment group was the lowest (1.65 g/L), while the soluble protein content of the SEL treatment group was the highest (2.45 g/L). There was no significant difference between CCS and HS treatment groups（$P > 0.05$, however, the levels of soluble protein in BS and SEL were significantly higher than HS ($P < 0.05$).

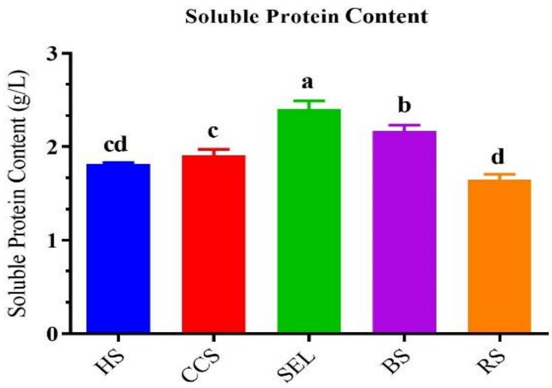

Figure 2. Effects of different kinds of soil on soluble protein content in *Panax ginseng*. Each value of extract represents means ±SE (standard errors) (n=5). Means with similar letters are not significantly different at $p < 0.05$.
HS: Healthy soil, SEL: Soil with stems and leaves extracts, BS: Soil + stems and leaves, RS: Rhizosphere soil, CCS: Continuous cropping problem soil.

Ginseng enzymes activities

The activities of antioxidant enzymes are closely related to the plant resistance to stress an important indicator for exploring the allelopathy of plants (6). The effects of different cultivated soils on CAT, POD, MDA, SOD of ginseng were studied. CAT is a monofunctional enzyme which contains heme tetrameric enzyme and mainly exists in the intracellular peroxisome (3,6,16). It has the function of scavenging H_2O_2 produced by photorespiration, which is decomposed into H_2O and O_2, and indirectly reduces the production of OH^{-1}. The results showed that CAT activity of CCS (1.11 U/mg protein) treatment group was the lowest and that of the SEL treatment group was the highest. There were significant differences between the HS treatment group and the CCS, SEL and BS treatment groups ($P < 0.05$) (Fig. 3). MDA activity can reflect the degree of lipid peroxidation, which indirectly reflects cell damage degree (15,16). Compared with the HS treatments, the activity of MDA in the SEL treatment group was the lowest (13.55 nmol/mg protein), the activity of MDA in (RS) treatment group was the highest ($P < 0.05$). POD activity can reflect the metabolic changes of plants in a certain period (12). There were significant differences in POD activity between different cultivation soils and the HS treatment group ($P < 0.05$). The activity of POD of RS treatment group was highest ($P < 0.05$), and POD activity of CCS, SEL and BS treatment groups were significantly lower than HS ($P < 0.05$). There was no significant effect on SOD activity in different cultivated soil ($P < 0.05$) (Fig. 3).

Ginseng growth

The growth of ginseng in different treatments was observed during harvesting time (Fig. 4). The pictures showed that except for the HS treatment group, the others were all involved in soil sickness, of which the rhizosphere soil RS treatment group was the most serious. Meanwhile, the radical length, radical width, the number of lateral roots and fibrous roots and seedling height of the ginseng plant were measured, the allelopathic effect index (RI) was calculated as well (Table 2) (Fig. 5). The results showed that the radical length of CCS, BS and RS groups was significantly inhibited, and RI of these groups were (-0.36), (-0.41) and (-0.47) respectively. The radical length, radical width, number of transverse roots and fibrous roots and seedling height of HS were significantly different between RS group and control group (HS). The factors of radical width, lateral and fibrous roots amount and seedling height in the CCS, SEL, BS and RS group were inhibited, while RS group caused the most serious inhibition with allelopathic effect index (total) -2.71.

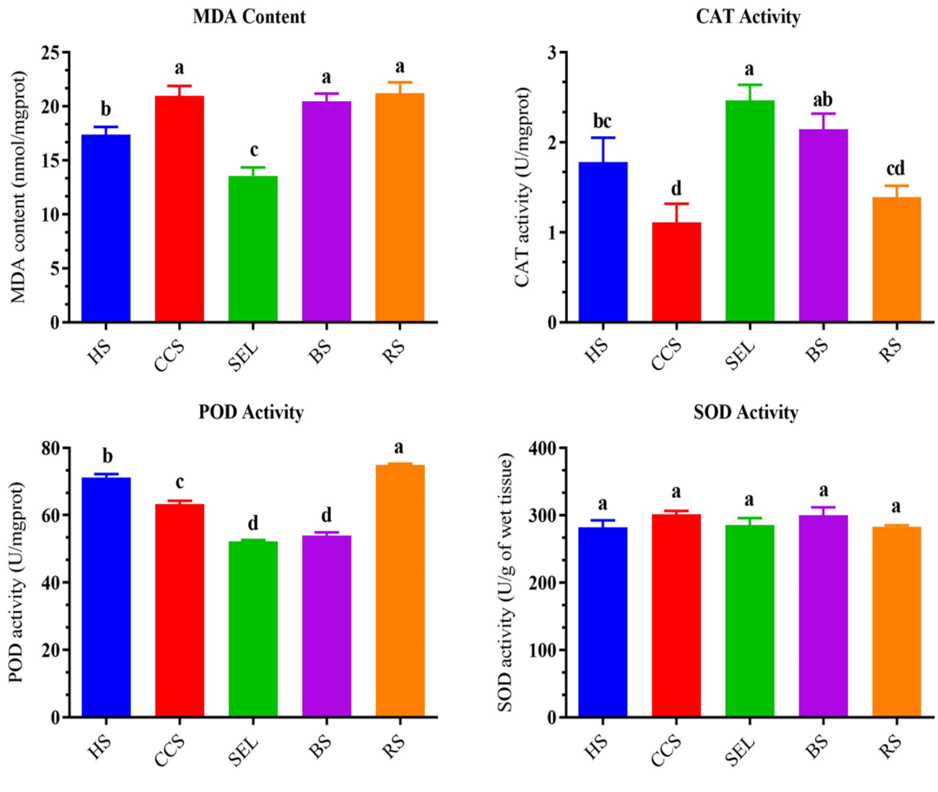

Figure 3. The effects of different soils on CAT, MDA, POD and SOD activity. Each value of extract represents means ±SE (standard errors) (n=5). Means with similar letters are not significantly different at $p < 0.05$.
HS: Healthy soil, CCS: Continuous cropping problem soil, SEL: Soil with stems and leaves extracts, BS: Soil + stems and leaves, RS: Rhizosphere soil

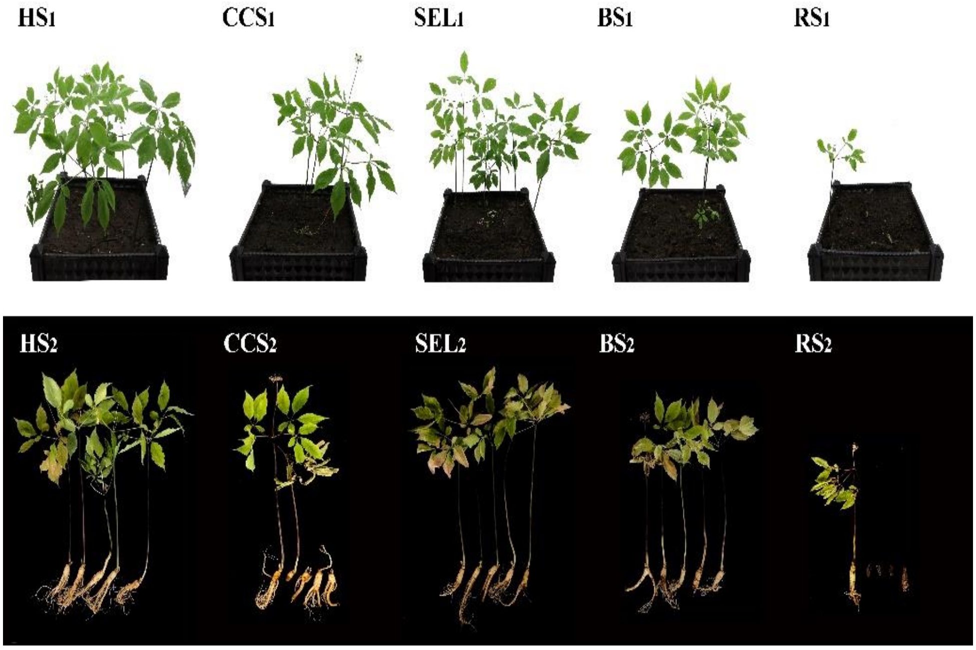

Figure 4. The growth performance of ginseng in different soils.
HS: Healthy soil, CCS: Continuous cropping problem soil, SEL: Soil with stems and leaves extracts, BS: Soil + stems and leaves, RS: Rhizosphere soil

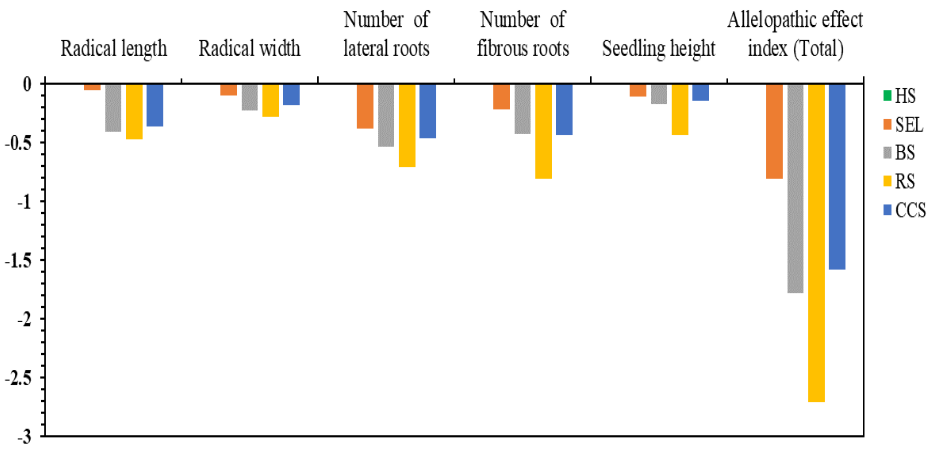

Figure 5. Allelopathic index (RI) of Radical length, Radical width, The number of lateral roots, The number of fibrous roots, seedling height and Allelopathic effect index (Total) of *Panax ginseng*
HS: Healthy soil, CCS: Continuous cropping problem soil, SEL: Soil with stems and leaves extracts, BS: Soil + stems and leaves, RS: Rhizosphere soil

Table 2. The effects of different soils on the ginseng growth

Treated soil Group	Radical Length (cm)	Radical Width (cm)	Number of lateral roots	Number of fibrous roots	Seedling Height (cm)
HS	6.91±1.27a	1.95±0.05a	6±1a	22±2a	45.64±1.19a
CCS	4.44±0.94b	1.60±0.07bc	3±0bc	12±3b	39.15±1.43b
SEL	6.66±0.44a	1.75±0.23ab	4±0b	17±5ab	40.78±2.02b
BS	3.73±0.42bc	1.50±0.19bc	3±1c	13±3b	37.67±1.37b
RS	3.37±0.48bc	1.40±0.07c	2±0d	4±2c	25.79±1.15c

Different normal letters indicate significant difference at 0.05 level.
HS: Healthy soil, CCS: Continuous cropping problem soil, SEL: Soil with stems and leaves extracts, BS: Soil + stems and leaves, RS: Rhizosphere soil

Allelopathy can significantly affect the soluble protein content, antioxidant enzyme activity, growth and quality of plant (1,14,18). Soluble protein plays a role in protecting the bio-membrane of the plant cell, it's also indispensable in plant stress resistance. Reactive oxygen species are produced in plant aerobic metabolism, which is an important signal molecule involved in the regulation of physiological and abiotic stress responses in plants. The high concentration of Reactive oxygen species ROS can inhibit plant growth and even lead to death (6). To maintain their normal physiological metabolism, plants tend to scavenge reactive oxygen species by antioxidant enzyme system. High concentration of allelochemicals can damage the cell structure of plants, while promoting the degradation of plant litter and organic compounds, which ended up in plant growth inhibition (4,11,20). Different secretion pathways of ginseng allelochemicals were simulated to determine the above factors and differences were found in soluble protein content, antioxidant enzyme activity and growth of ginseng plants cultivated in different kinds of soil, water extracts from stems and leaves SEL.

CONCLUSIONS

All treatments of soil significantly increased the content of soluble protease. Compared with healthy soil (HS), rhizosphere soil (RS) reduced the CAT activity but increased the POD activity. Ginseng growth was different in 5-kinds of soils. The experimental soils: HS, RS, BS and SEL had negative allelopathic effects and the allelopathic effects of RS soil were lowest (-2.71).

The accumulation analysis of four highly allelopathic active substances showed that Rg1, Rb1, Re and Rd all originated from the secretory pathway, but Rg1 content was easily accumulated in single release pathway; Rb1 content increased first and then decreased; the content of Re and Rd was very high in the early stage and decreased in later stage.

ACKNOWLEDGEMENTS

This research was financially supported by the National Natural Science Foundation of China (82073969), the Natural Science Foundation of Jilin province, China (No.20190201297JC), the Major Science and Technology Project of Jilin Province, China (No.20200504003YY), National Key Research and Development Program of China (No.2017YFC1702101).

COMPLIANCE WITH ETHICAL STANDARDS

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

REFERENCES

1. Abenavoli, M.R., Cacco, G., Sorgona, A., Marabottini, R., Paolacci, A.R., Ciaffi, M. and Badiani, M. (2006). The inhibitory effects of coumarin on the germination of durum wheat (*Triticum turgidum* ssp durum, cv. Simeto) seeds. *Journal of Chemical Ecology* **32**: 489-506.
2. Bi, X.B., Yang, J.X. and Gao, W.W. (2010). Autotoxicity of phenolic compounds from the soil of American ginseng (*Panax quinquefolium* L.). *Allelopathy Journal* **25**: 115-121.
3. Blokhina, O., Virolainen, E. and Fagerstedt, K.V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Annals of Botany* **91**: 179-194.
4. Cesarano, G., Zotti, M., Antignani, V., Marra, R., Scala, F. and Bonanomi, G. (2017). Soil sickness and negative plant-soil feedback: A re-appraisal of hypotheses. *Journal of Plant Pathology* **99**: 545-570.
5. Drost, D.C., Doll, J.D. and Moody, K. (1980). Effect of live plant leaching and different residue sources on the allelopathy of *Cyperus esculentus* L. *Philippine Journal of Weed Science* **9**: 229-233.
6. Gill, S.S. and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* **48**: 909-930.
7. Hwang, E., Park, S.Y., Yin, C.S., Kim, H.T., Kim, Y.M. and Yi, T.H. (2017). Anti-ageing effects of the mixture of *Panax ginseng* and *Crataegus pinnatifida* in human dermal fibroblasts and healthy human skin. *Journal of Ginseng Research* **41**: 69-77.
8. Kang, A., Hao, H., Zheng, X., Liang, Y., Xie, Y., Xie, T., Dai, C., Zhao, Q., Xie, W.L. and Al., E. (2011). Peripheral anti-inflammatory effects explain the ginsenosides paradox between poor brain distribution and anti-depression efficacy. *Journal of Neuroinflammation* **8**:100-100.
9. Lei, F., Zhang, A., Xu, Y. and Zhang, L. (2010). Allelopathic effects of ginsenosides on *in-vitro* growth and antioxidant enzymes activity of ginseng callus. *Allelopathy Journal* **26**:13-22
10. Li, Q., Zhang, L.X., Guan, T.Z., Xu, Y.H. and Chen, C B. (2020b). Allelopathic effects of ginsenoside Rgl on seed germination and seedling growth of *Panax ginseng*. *Allelopathy Journal* **49**: 229-242.
11. Li, Q., Zhang, L., Xu, Y. and Chen, C. (2020a). Soil Sickness of *Panax gingseng* : Current status and future perspectives. *Allelopathy Journal* **50**: 23-33.
12. Meloni, D.A., Oliva, M.A., Martinez, C.A. and Cambraia, J. (2003). Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environmental and Experimental Botany* **49**: 69-76.
13. Park, S.Y. (2010). Potential therapeutic agents against Alzheimer's disease from natural sources. *Archives of Pharmacal Research* **33**: 1589.
14. Qian, H., Xu, X., Chen, W., Jiang, H., Jin, Y., Liu, W. and Fu, Z. (2009). Allelochemical stress causes oxidative damage and inhibition of photosynthesis in *Chlorella vulgaris*. *Chemosphere* **75**: 368-375.
15. Shah, K., Kumar, R.G., Verma, S. and Dubey, R.S. (2001). Effects of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Science* **161**: 1135-1144.
16. Velikova, V., Yordanov, I. and Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants - Protective role of exogenous polyamines. *Plant Science* **151**: 59-66.

17. Weidenhamer, J.D., Mohney, B.K., Shihada, N. and Rupasinghe, M. (2014). Spatial and temporal dynamics of root exudation: How important is heterogeneity in allelopathic interactions? *Journal of Chemical Ecology* **40**: 940-952.
18. Wu, Z., Deng, P., Wu, X., Luo, S. and Gao, Y. (2007). Allelopathic effects of the submerged macrophyte *Potamogeton malaianus* on *Scenedesmus obliquus*. *Hydrobiologia* **592**: 465-474.
19. Yang, M., Zhang, X., Xu, Y., Mei, X., Jiang, B., Liao, J., Yin, Z., Zheng, J., Zhao, Z., Fan, L., He, X., Zhu, Y. and Zhu, S. (2015). Autotoxic ginsenosides in the rhizosphere contribute to the replant failure of *Panax notoginseng*. *PLoS One* **10**: e0118555-0118571.
20. Ye, C., Fang, H.Y., Liu, H.J., Yang, M. and Zhu, S.S. (2019). Current status of soil sickness research on *Panax notoginseng* in Yunnan, China. *Allelopathy Journal* **47**: 1-14.
21. Zhang, H., Dong, J., Zhao, X., Zhang, Y., Ren, J., Xing, L., Jiang, C., Wang, X., Wang, J., Zhao, S. and Yu, H. (2019). Research progress in membrane lipid metabolism and molecular mechanism in Peanut cold tolerance. *Frontiers in Plant Science* **10**: 838-850.

PUBLISHER NOTE