Bangladesh J. Bot. **45**(4): 835-843, 2016 (September) Supplementary

IDENTIFICATION AND ALLELOCHEMICAL ECOLOGICAL TOXICITY OF GINSENG DECOMPOSITION PRODUCTS

YUJIA SONG* AND SHOUFA SONG[1]

Department of Environmental Engineering, Changchun University of Science and Technology, Changchun, P.R. China

Key words: Ginseng, Allelochemicals, GC-MS, Self-allelopathy, Plant physiology

Abstract

Results of quantitative identification of ginseng decomposition products' and their self-allelopathy indicated the presence of eight organic products. Biometric experiments were conducted on ginseng with various vegetative periods by analyzing the decomposition liquid of various concentrations. Results indicated that the peroxidase (POD) activity, superoxide dismutase (SOD) activity and chlorophyll content of the ginseng samples with various concentrations of decomposition liquid were all lower than identical parameters analyzed for the control group. The malondialdehyde (MDA) content was higher in studied samples than in the control group, indicating that the ginseng decomposition extract exhibited self-allelopathy towards the growth of ginseng. The effect of concentration on self-allelopathy was observed as generally increasing with increasing concentration.

Introduction

Panax ginseng C. A. Mey. is a perennial medicinal herb belonging to Araliaceae. The plant grows in northeast Asia, including China, North Korea, South Korea and Japan. In China it is particularly prevalent and its annual production accounts for approximately 70% of the total ginseng produced worldwide annually. However, ginseng cultivation faces serious obstacles, including continuous cropping (Jin *et al.* 2006). Generally, after three years of cultivation of ginseng in same fields, the seedling (root) rate falls below 25%, many researchers have studied the negative phenomena of continuous cropping obstacle from various aspects. It is generally reported that three factors may affect the process i.e., population change of the microorganism, imbalance of soil nutrients and allelopathy produced by the self secretion of ginseng plants (Zhang *et al.* 2008). Over the past two decades, research on the allelochemicals of ginseng has gradually risen to prominence as a new research aspect of continuous cropping. A number of research works were conducted to investigate root production to allelochemicals, which lead to the obstacles inherent to continuous cropping (Li *et al.* 2008a, Wang *et al.* 1994, Wang *et al.* 2005, Li *et al.* 2008a, b). Reported results indicate that perennial soil cultivation might have induced certain metabolic inhibitors, which can be extracted from water and citrate buffer. However, there was a shortage of research works addressing the presence of allelochemicals and the development of self-allelopathy of ginseng decomposition products. The present study has been aimed to identify the ginseng decomposition components and to investigate quantitatively the self-allelopathy of the plant.

Materials and Methods

One hundred grams of ginseng were cut into pieces approximately 0.5 cm in length, and placed into a plastic bottle. An equivalent of soil mass was added to the bottle, and deionized water was then added to reach a root to water ratio of 1 : 3 (W : W). The sample mixture was then

*Author for correspondence: <jlsongyujia@126.com>. [1]North-east Coal Industry Institute of Environmental Protection, Changchun, P.R. China.

cultivated in an incubator at 40°C with frequently stirring and addition of water. After 50 days, the ginseng decomposition liquid was obtained. It was combined with an even proportion of water, and then oscillated for one hour at 25°C at a frequency of 160 times per minute. After centrifugation at a rate of 6000 rpm, the remaining supernatant fluid represented the ginseng root decomposition liquid.

The decomposition liquid was poured into a processed and washed resin column. The velocity was controlled at 0.5 ml/min, and the effluent liquid was removed. The sample was eluted by methanol and an eluent velocity of 2.3 ml/min. The amount of eluent may be approximately 15 times the volume of resin. The methanol eluent (initial volume of eluent was 300 ml) was collected into the rotary evaporator, and concentrated to 10 ml at 40°C. Afterward, water separation of anhydrous sodium sulfate was conducted, and the sample was then transferred into a bottle and concentrated to 2 ml. The solution was then filtered over a 0.45 μm nylon membrane to conduct a GC/MS (AGILENT 6890N/ AGLILENT 5975) test.

The GC working conditions were as follows: Initial temperature = 40°C, increased at a rate of 5°C per minute up to 100°C; increased at a rate of 15°C per minute up to 280°C; post-test temperature = 0°C; post-test time = 0.00 min; run time = 33.00 minutes by temperature-program; the injection method was split injection near the front introduction port; the introduction temperature = 280°C; pressure = 7.05 psi; split ratio = 50 : 1; split rate = 99.8 ml/min, the carrier gas was high-purity heliu; the column type was Agilent 19091Z - 433; HP-1 methyl siloxane flow rate = 1.0 ml/min. The MS utilized electronic ionization; its bombardment voltage = 70 eV, scan range = 20-700 amu, the quadrupole temperature = 150°C; the entire sample can be scanned within 0.2 seconds; the ionization source temperature = 230°C; the mass spectrometry database was NIST; and each sample was subjected to two replications.

The entire experiment was conducted indoors. The seedlings which exhibited consistent growth were transplanted to cultivation pots of 15 cm × 15 cm, which were filled with 2 kg soil. Two ginseng plants were planted in each pot, with three replications of each. The pots were placed into the lighting culture chamber. The average daytime temperature was 25°C, the average night temperature was 20°C, relative humidity was 65, the culture chamber was illuminated for 12 hrs each day at a light intensity of 4000 lux.

The ginseng decomposition liquid was collected once (group I), three times (group II) and five times (group III), and the samples were then used to water the potted ginseng plants. The samples in the control group were watered simultaneously, and testing was conducted to evaluate peroxidase and superoxide dismutase activity of the root, as well as to analyze the chlorophyll and malondialdehyde content of plant leaves in the germination-frondesce stage, flowering season and initial fruiting period.

Spectrophotometry was employed to determine the peroxidase activity of the samples (Zhang *et al*. 2004). The superoxide dismutase activity of the sample plants was evaluated by pyrogallol autoxidation (Zhang *et al.* 2004). Chlorophyll content was measured by spectrophotometry, and the malondialdehyde content was evaluated according to the thiobarbituric acid method (Zhang *et al.* 2004).

In order to ensure the precision of sampling technology throughout the experiment, the standard quality control procedure and repeated sampling were employed. In order to ensure analytical precision, the repeated experiment was set. The standard curve was determined before each test, to serve as the quality control of experimental measurements.

Results and Discussion

This study innovatively identified the composition of ginseng decomposition liquid quantitatively; previous research into allelochemicals had simply described theoretically the relevant organic matter that may be produced in the decomposition residue, which had been identified as a likely source of allelochemicals in plants (Zhan *et al.* 2004). This study serves as the first quantitative identification of the composition of ginseng decomposition material. The decomposition liquid was prepared with ginseng root, and its composition was identified accordingly. In summation, eight substances were reliably identified (Fig. 1).

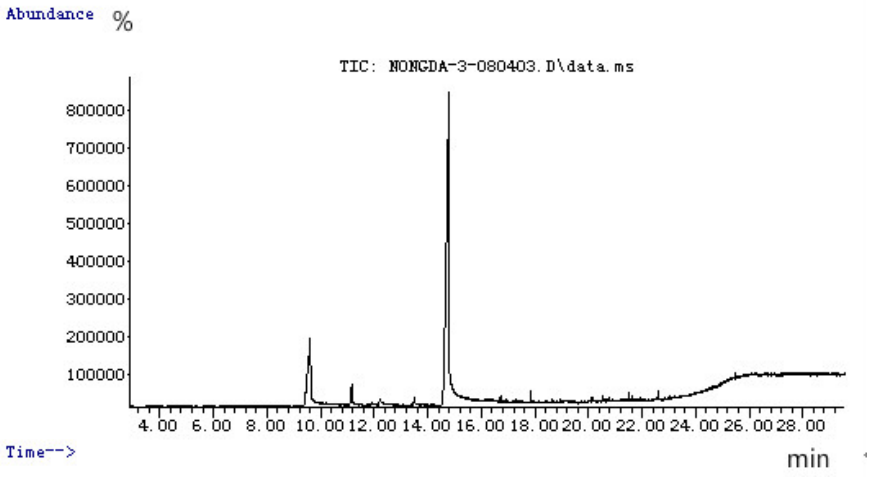

Fig. 1. The total ion chromatogram of ginseng decomposing products.

Table 1 shows the eight identified substances; of which organic acids account for five while aliphatic hydrocarbons account for three. The organic acids comprised not only the majority of the species, but account for 98.9% of the total content. Among the eight identified substances, four were typical allelochemicals (2-ethyl-hexyl acid, octylic acid, tetradecane and tridecane) indicating that there are a variety of allelochemicals in ginseng decomposition products. This also indicated that not only living ginseng is capable of releasing allelochemicals into the outside environment, but the decomposition residue can also release allelochemicals into the soil. As indicated by the single-matter content of samples, octylic acid accounted for 74.6% of the total content. This indicated that octylic acid is one of the primary allelochemical components produced by ginseng. Additionally, hexanoic acid, n-heptane acid, and 2-methyl-hexyl acid had not been identified by previous allelochemical research; however, according to the allelochemical classification theory as provided by Rice *et al.* (1986), all three substances can be regarded as allelochemicals.

As shown in Fig. 2, every processing group exhibiting inhibited peroxidase (POD) activity in ginseng as compared to the control group. In the germination-frondesce stage, the POD activities of processing groups were less affected than in the flowering season and initial fruiting period, which reached 72.2, 80.1 and 82.7%, respectively, as compared to the control group. During the flowering season and initial fruiting period, the POD activity of processing group I was equal to 40.8 and 45.0%, respectively; the POD activity of processing group II reached 41.5 and 42.8%, respectively, as compared to the control group; and the POD activity of processing group III

corresponded to 46.9 and 52.2%, respectively, as compared to the control group. The POD activity exhibited by all processing groups were much lower than that of the control group, although processing group I demonstrated the greatest degree of impact among the three studied processing groups.

Table 1. List of organic ingredients of ginseng decomposing products.

Number	Retention time (min)	Material name	Percentage
1	9.52	Hexanoic acid	18.4
2	11.162	2-methyl-hexanoic acid	2.9
3	12.21	Heptanoic acid	2.0
4	13.49	2-ethyl-hexanoic acid	1.1
5	14.73	Octanoic acid	74.6
6	16.72	Tridecane	0.3
7	17.85	Tetradecane	0.4
8	21.50	2, 6, 11-trimethyl-dodecane	0.4

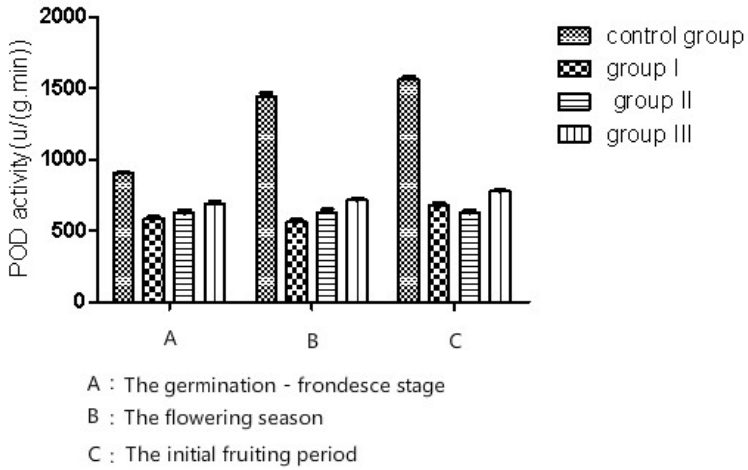

Fig. 2. The inhibition of ginseng decomposing products on the activity of POD.

According to the above analysis, every processing group of ginseng samples demonstrated weakened oxygen clearing capabilities. The O_2- and H_2O_2 free radicals and other reactive oxygen species may be accumulated extensively, thereby inducing lipid peroxidation of unsaturated fatty acids in the membrane. This phemonenon is not conducive to ginseng growth, and may result in declining yield and quality of the plant (Yang *et al.* 2011).

As shown in Fig. 3, superoxide dismutase (SOD) activity increased accordingly from the germination-frondesce stage, to the the flowering season and through the initial fruiting period. During the germination-frondesce stage (Fig. 3), SOD activity serves as the most effective inhibitor, which is least effecting in the initial fruiting period. The inhibition effectiveness from high to low is observed as follows: germination- frondesce stage > flowering season > initial fruiting period.

Different treatment options may lead to different SOD inhibition during the different periods of ginseng growth. The ginseng decomposition liquid was collected once (group I), three times (group II) and five times (group III), processing groups I are greatly impacted by SOD activity during the germination-frondesce stage; inhibition levels reached 43.2 and 38.6%, respectively, as compared to the control group. The SOD inhibition level of processing group II was 17.3% as compared to the control group, while the SOD inhibition level of processing group I was 26.4%, as compared to the control group. The SOD inhibition levels of processing groups I and II were 26.9, 17.2 and 23.3%, respectively, as obtained by the above analysis. Inhibition effectiveness from high to low occurs according to the following categories : I > III > II.

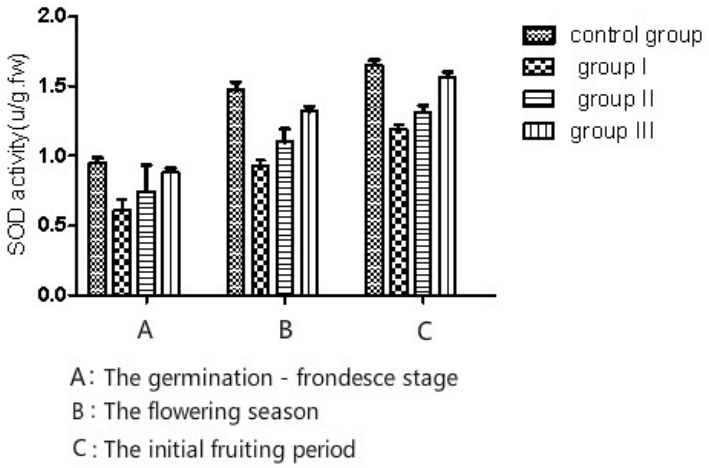

Fig. 3. The inhibition of ginseng decomposing products on the activity of SOD.

Ginseng root secretion extracts demonstrated various SOD inhibition effectiveness, which is in accordance with previous reports which indicated that root secretions degrade membrane function (Roshchina and Roshchina 1993). This indicated that allelochemicals in root secretions inhibited SOD activity of the plants, thus resulting in the acceleration of reactive oxygen *in vivo*, initiating lipid peroxidation and thereby undermining the structure of the membrane (Zhang and Lin 2009).

As shown in Figs 4 and 5, the chlorophyll content in ginseng increases greatly according to the following categorization, as compared to levels observed in the control group: germination-frondesce stage→ flowering season → initial fruiting period. Processing groups I , II and III promoted various degrees of chlorophyll inhibition in ginseng. During the germination-frondesce stage, the chlorophyll A inhibition rate of the processing liquid reached 33.00, 50.7 and 22.5% in groups I, II and III, respectively (Fig. 4); chlorophyll B inhibition reached 60.6, 73.4 and 18.9% in groups I, II and III, respectively (Fig. 5).

During the flowering season, the reduction of chlorophylls A and B content appeared to be near in groups II and III.

During the initial fruiting period, chlorophyll A content reduced up to 24.2, 90.6 and 9.5% in groups I, II and III, respectively, as compared to the control, and chlorophyll B inhibition 22.2, 3.5 and 5.5% in groups I, II and III, as compared to the control.

Chlorophyll A is involved in the collection and conversion of solar energy, while chlorophyll B is involved only in the collection of solar energy. Thus, chlorophyll is integral past of the photosynthetic process of plants. The above analysis indicated that extracts of the studied concentrations inhibit chlorophyll synthesis, thus reducing the energy collection and conversion capacity of the plant and decreasing leaf photosynthesis. This in turn affects the synthesis of dry matter, and reduces ginseng productivity.

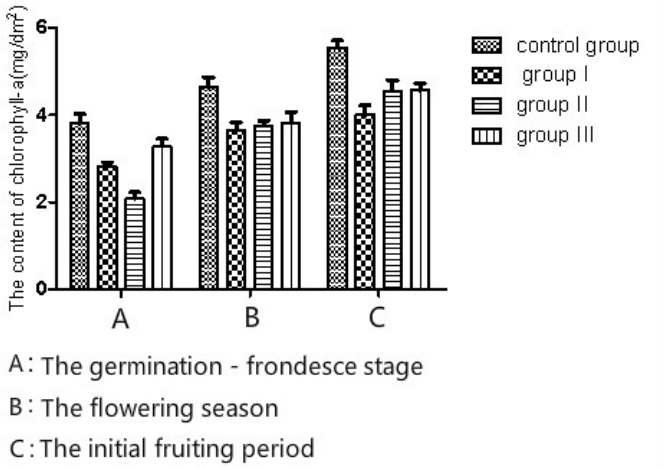

Fig. 4. The effect of ginseng decomposing products on the content of chlorophyll A.

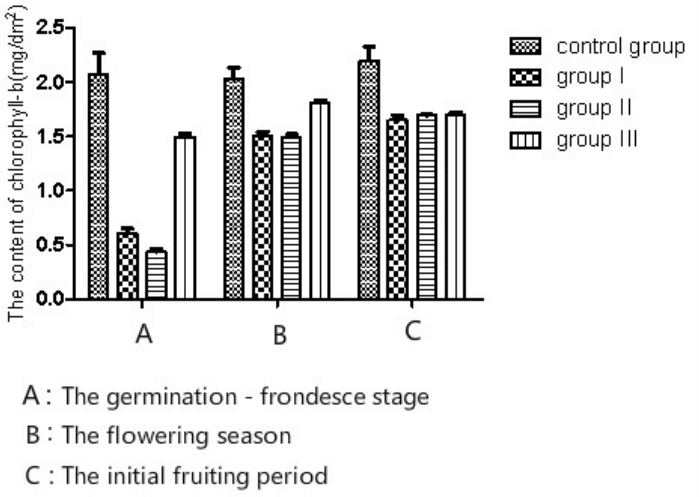

Fig. 5. The effect of ginseng decomposing products on the content of chlorophyll B.

As shown in Fig. 6, during the germination-frondesce stage, the flowering season and the initial fruiting period, we observed that the malondialdehyde (MDA) content of ginseng plants appeared to increase in accordance with increasing MDA content.

According to the above trends, the soil in all processing groups induced high MDA content in ginseng as compared to the control group. The activated oxygen in ginseng simultaneously improved, interrupting the optimal balance between the production and removal of free radicals in the activated oxygen, which may accumulate and damage the membrane. The extent of damage observed in the processing group appeared to occur according to the following classification: I >III > II>control group.

The production and reaction mechanisms of allelochemicals are very complex, and their production methods are many and varied. Production of allelochemical substances likely impacts every stage and physiological process of plant growth (Kong and Hu 2001, Rice 1984, Catherine *et al.* 2003).

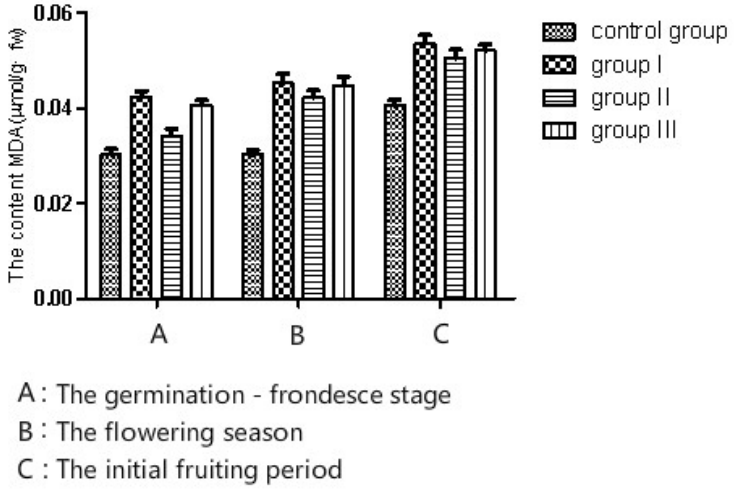

Fig. 6. The effect of ginseng decomposing products on the content of MDA.

Previous researches utilized living plants for analysis, to determine how they produce and release allelochemicals during plant growth. However, research investigating plant production of allelochemicals still remains in the theoretical and preliminary stages (Lijeroth *et al.* 1990, Liu 1998). This study conducted quantitative research of ginseng decomposition products, varifying that some plants continue to release allelochemicals into the surrounding environment after their natural decomposition, and thus impact the future growth of their own species or other creatures, which is one of the primary causes of continuous cropping problems.

Allelochemicals can affect almost every aspect of plant physiology and biochemistry; the majority of allelochemicals affect the cell membrane, energy generation and energy useage, while some allelochemicals only affect specific enzymes and thus interfere with the senior metabolic and growth regulation systems of plants (Wang and Cao 1993, Zhang and Gao 2000, Ji and Xu 1995, Zhu and Wang 1999, Hu *et al.* 2001, Yu and Matsui 1999, Chen *et al.* 2015, Portales-Reyes *et al.* 2015). The current experiment proved that allelochemicals produced by decomposing ginseng negatively affect its own physiological indicators and create obstacles to continuous cropping which results in root rot, disease, low yields and other phenomena related to ginseng cultivation.

Currently, the recognized allelochemicals include organic acids, phenolic acids and their derivatives, flavonoids, terpenoids, alkaloids and 14 additional chemical categories. The current

identified eight substances from ginseng decomposition products, all of which are included in the 14 species, which is in accordance with previous research that identified concentration effects of allelochemicals on self-allelopathy. Therefore, future studies on the ecological toxicity of ginseng allelochemicals are necessary to surmounting continuous cropping obstacles.

Acknowledgements

This project was supported by the National Natural Science Foundation of China (40871005) and funded by the Scientific Research Foundation of the Changchun University of Science and Technology. This project was also supported by the "Simulation and regulation of nitrogen and phosphorus transfer in small drainage basins on the periphery of urban areas" research program, which is funded by the Thirteenth Five-Year Scientific Research Program of the Jilin Province Department of Education.

References

Catherine LK, Rengefors GOF and Edna G 2003. Allelopathyn in phytoplankton-biochemical, ecological and evolutionary aspects. J. Phyclo. **42**: 406-419.

Chen SY, Chi WC, Trinh NN, Cheng KT, Chen YA, Lin TC and Chiang TY 2015. Alleviation of allelochemical juglone-induced phytotoxicity in tobacco plants by proline. J. Plant Interact. **10**: 167-172.

Hu YG, Zeng ZH, and Cheng X 2001. The progress and prospect of alfalfa autotoutoxicity. Grassland and Turf. **4**: 9-11.

Ji ZC, and Xu WZ 1995. The change of soil environment with reducing yield of continue cropping in soybean. Soybean Sci. **14**: 321-329.

Jin H, Yu SL and Cao ZQ 2006. Improvement of long-cultivated land of ginseng and farmland for continuous cultivation of ginseng and quinguefolium. Guid. J. tradit. Chin. Med. Pharm. **8**: 84-86.

Kong CH and Hu F 2001. Allelopathy and its application. Beijing, China Agriculture Press. pp. 56-63.

Li Y, Huang XF and Ding W 2008a. Allelopathic effects of soil extracts on the growth of ginseng seeds and its chemical composition. Ecol. Envi. **17**: 1173-1178.

Li Y, Zhu DL and Huang XF 2008b. Inhibition of different soil extracts on growth of ginseng seeds. Inf. Tradit. Chin. Med. **39**: 1070 -1074.

Lijeroth E Mathiasson l and Lundborg T 1990. Assimilate translocatlon to the rhizosphere of two wheat lines and subsequent utilization by rhizosphere microorganism at two soil nitrogen concentrations. Plant Soil **22**: 1015-1022.

Liu SP 1998. Root exudates. Ecological Agricultural Research **6**: 34-36.

Portales-Reyes C, Van Doornik T, Schultheis EH and Suwa T 2015. A novel impact of a novel weapon: allelochemicals in *Alliaria petiolata* disrupt the legume-rhizobia mutualism. Biol. Invasions **17**(9): 2779-2791.

Rice EL 1984. Allelopathy (Second edition). Beijing, Academic Press. pp. 13-19.

Rice EL, Putnam AR and Tang CS 1986. The Science of Allelopathy. New York, John Wiley. pp. 33-67.

Roshchina VV and Roshchina VD 1993. The excretory function of higher plant. New York, Spinger-Verlag. pp. 70-83.

Wang JD, Fu XQ and Li TJ 1994. Effect and properties of growth inhibitors of ginseng seeds and their isolation and identification. J. Jilin U. **9**: 94-95.

Wang JL and Cao ZH 1993. Nutrition environment of plant rhizosphere in relation to sustainable agriculture. Plant Physiol. Communi. **29**: 329-336.

Wang YP, Zhao YJ and Shao D 2005. A preliminary study of American ginseng root exudates. Acta. Chin. Med. Pharm. **30**: 229-231.

Yang HG, Yang CF, Chen HJ, Yan SL and Chen DG 2011. Response of physiology of phalaenopsis cultivars with different heat tolerance to high temperature stress at seeding stage. Chin. Agric. Sci. Bull. **27**: 144-150.

Yu JQ and Matsui Y 1999. Autointoxication of root exudates in *Pisum sativus*. Acta Horticulturae Sinica **26**: 175-179.

Zhan XM, Han XR and Yang JF 2004. The effect of succession cropping and soybean stubble on soybean root exudates. Chin. J. Soil Sci. **10**: 632-633.

Zhang LX, Chen CB and Wang YP 2008. Study on discontinuous cultivating of *Panax ginseng* and its workable solution. J. Jilin Agri. U. **30**: 481-485.

Zhang SX and Gao ZQ 2000. Continuous cropping obstacle and rhizospheric microecology. Chin. J. Appl. Ecol. **11**: 152-156.

Zhang ZA, Zhang MS and Wei RH 2004. Plant Physiology guide. Beijing, China Agricultural Science and Technology Press. pp. 101-107.

Zhu B and Wang WZ 1999. Problem of Chinese fir continuous planting, its causes and solutions. J. Centr. South Forest. **19**: 76-78.

Zhang CY and Lin WX 2009. Continuous cropping obstacle and allelopathic autotoxicity of medicinal plants. Chin. J. Eco-Agroc. **17**: 189-196.

(Manuscript received on 14 April, 2016; revised on 20 September, 2016)