

ALLELOPATHIC EFFECT OF GINSENOSIDE RD ON GINSENG SEEDS AND SEEDLINGS

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Abstract. This study evaluated the physiological and ecological activities of (ginsenoside Rd, G-Rd) in ginseng seeds and seedlings. The effects of different concentrations (2, 1, 0.5, 0.25 mg L⁻¹) of G-Rd on the growth and development of ginseng seeds into seedlings were investigated. G-Rd had an allelopathic on the germination of ginseng seeds and increased the fresh weight of ginseng seedlings, both effects increasing with increasing concentrations. After G-Rd treatment, the antioxidant enzyme activities in young ginseng roots were lower than in the control group. Regarding antioxidant enzyme activities, the SOD, POD and CAT activities were higher than in the control group at low G-Rd treatment concentrations and significantly lower after treatment at high G-Rd concentrations ($P < 0.05$). The MDA content increased with increasing G-Rd treatment concentration. Regarding plant hormones, a low concentration of G-Rd could increase the GA content which decreased the ABA and IAA level, while high concentrations of G-Rd decreased the GA and IAA contents and increased the ABA content in the meantime. The allelopathic of different concentrations of G-Rd on ginseng seed germination, seedling growth and plant development were different. In short, G-Rd was proved to have a significant allelopathic activity.

Keywords: *ginseng, allelopathy, seed germination, antioxidant enzymes, hormones*

Introduction

The allelopathic effect, which refers to the release of metabolic secretions from plants into the environment and the inhibition of the growth of conspecifics, can play a key role in regulating plant biodiversity and productivity (Singh et al., 1999; Chou, 1999; Inderjit and Duke, 2003) with its allelopathy effects known more than 2000 years ago (Wu et al., 2008; Bi et al., 2010). In recent years, with the advances in science and technology and more sophisticated analytical instruments, the number of studies on allelopathic of ginseng has increased (Dorning and Cipollini, 2006; Yang et al., 2015). Allelopathic of ginseng have been systematically investigated in different disciplines, with great progress being made. However, a large body of research evidence suggests that failures in crop sowing and yield loss are associated with plant autotoxic substances (Singh et al., 1999). Hundreds of allelochemicals have been identified as possible autotoxic substances (Huang et al., 2013). The allelopathy mechanisms of some autotoxic substances have been found to include single or multiple effects of processes such as oxidative damage, phytohormone activity, DNA damage, photosynthesis, and mitochondrial function (Bais et al., 2003; Weir et al., 2004; Yang et al., 2011). However, only a few mechanisms of

the autotoxic substances have been identified in plants (Chi et al., 2013; Wu et al., 2015). During cultivation, chemosensitizing substances can interact with plants, soil microorganisms and soil physicochemical properties, inducing deterioration of soil physicochemical properties, microflora. This can lead to the formation of continuous crop disorders by inducing degradation of soil physicochemical properties, microbial flora disorders, reduced plant resistance, and the tendency of plant diseases to occur more frequently. In terms of release. In terms of the release pathway, allelopathic can be released into the soil through multiple pathways such as volatile leaching, root secretion, residue decomposition, etc., and through complex interaction with other environmental factors. In terms of release pathways, allelopathic can be released to the soil through volatile leaching, root secretion and residue decay, and can affect plant growth through complex interactions with other environmental factors. Therefore, to clarify the release pathways of ginseng allelochemicals the main release pathways and the mechanism of plant damage are of great significance to solve the problem of continuous crop barriers.

Ginseng (*Panax Ginseng* Mayer) is an important medicinal plant in China. Ginseng has a long history of cultivation in China, Korea and Japan (Coon and Ernst, 2002). Ginseng production areas are relatively concentrated due to the great economic benefits of ginseng cultivation and the limited areas of soil suitable for its cultivation. The scale of ginseng cultivation in harvested forests has also been decreasing in recent years so it has become increasingly important to produce high-quality ginseng. One important factor affecting the quality of ginseng is managing the various diseases that occur during its growth, with its continuous cultivation leading to a decreasing yield as well as an increase in diseases. Soil diseases affecting ginseng reduce seed germination, seedling growth, ginseng root quality, and can also cause severe disease infestation (Li et al., 2011). Soil diseases reduce ginseng production and cause major problems in plant recovery on old arable land. Autotoxic effects can be found in plants of the genera *Xanthium* (Kraus et al., 1995), *Jacopo* (Möhler et al., 2018), and *Cucumber* (Bu et al., 2016; Zhou et al., 2019).

Ginsenosides are characteristic medicinal components of the family *Ginsengaceae* and mostly accumulate in the peripheral skin tissue of ginseng roots. Ginsenosides are complex mixtures composed of several monomeric saponin components, with a varying proportion and solubility for each monomeric saponin. More than 40 ginsenosides have been identified and classified according to their saponin elements into the categories, protopanaxadiol saponins, protopanaxatriol saponins, and oleanolan saponins, which exhibit a variety of important physiological activities.

Ginsenosides are a group of secondary metabolites, which are triterpenoid saponins found almost exclusively in plants of the *Panax* species (family *Araliaceae*), such as *P. notoginseng*, *P. ginseng*, *P. quinquefolium*, *P. japonicas*, and *P. vietnamensis* (Court et al., 1996; Tran et al., 2001; Wei et al., 2007; Zhou et al., 2007; Christensen, 2009; Qi et al., 2011; Fujioka et al., 2016). Ginsenosides have been reported to be the autotoxins responsible for the failure of ginseng, American ginseng and *Panax ginseng* transplants (Nicol et al., 2002, 2003; Zhang and Cheng, 2006; Yang et al., 2015).

Secondary metabolites of perennial herbs, especially glycine (Guo et al., 2011), 3,4-dihydroxyacetophenone (Yang et al., 2015), and quinoic acid (Zhou et al., 2013), have been identified in diseased soils. Of these, G-Rd has been present in the root secretion or inter-root soil of ginseng (Nicol et al., 2003). Ginsenosides are the main bioactive compounds produced by ginseng plants (Kimura et al., 2006; Bernards et al., 2011). There are at least 20 different ginsenosides in ginseng, accounting for more than 6% of the dry weight of the plant. These compounds are released into the inter-root soil through root

secretions or into the environment through leaching and volatilization (Yang, 2006). The *Panax ginseng* autotoxin, Rd, exerts auto toxic effects on the seedling emergence, growth and root cell viability of *Panax ginseng* seedlings (Yang et al., 2015). G-Rd as an important bioactive substance in ginseng, but not much has been reported on its allelopathic on ginseng.

The allelopathy of ginsenosides in soil has been studied, but not in soil after their release so they may play a role in ginseng soil diseases (Nicol et al., 2003; Yang et al., 2015).

In response to the serious threat from the self-toxic effects of ginseng plants, the present study aims to investigate the chemosensitizing substances of ginseng and their chemosensitizing potential. For this experiment, we selected ginseng plants at the early stage of growth and development after seed germination and investigated the effects of four concentrations of G-Rd on the activities of the antioxidant defense enzymes, superoxide dismutase (SOD), polyphenol oxidase (POD), and catalase (CAT). We also aim to investigate the effects of G-Rd on the growth hormones in the ginseng seed germination and root system, and explore the physiological adaptations of ginseng seeds and young roots to exogenous G-Rd. This will enable the exploration of the physiological and ecological mechanisms of ginsenosides and provide a theoretical basis to better understand the intrinsic causes of ginseng crop disorders.

Materials and methods

Materials

Ginseng (*Panax ginseng* C. A. Meyer) seeds were purchased from Wanliang Town, Fusong County, Jilin Province, China. All seeds were collected from uninfected healthy plants then stored at 4 °C until their experimental use.

Methods

Ginseng seed germination

The surfaces of the ginseng seeds were disinfected with carbendazim solution for 15 min, rinsed three times with distilled water then full-grained seeds were selected for studying autotoxicity. Three layers of gauze pads were placed in a 7 cm diameter Petri dish, and 20 ginseng seeds were placed in each Petri dish. Ten mL of G-Rd solution at different concentrations (0.25, 0.50, 1 and 2 mg L⁻¹) were added to the seeds in each dish with distilled water used as the control (CK). Each dish was incubated at 25 °C with 3 replicates in an artificial climate chamber under conditions of 17 h in the light (photoperiodic photon flux density, 40 µmol. m⁻²s⁻¹) followed by 7 h in the dark. The seeds were considered to have germinated when a 1-cm long radicle had emerged. The germination potential was recorded 5 d after G-Rd treatment and the germination rate after 10 d. The radicle length was measured with a ruler and the antioxidant enzyme activities and endogenous plant hormone contents were measured.

Ginseng seedling growth

The ginseng seeds were disinfected, and full-grained seeds selected as described above. Twenty ginseng seeds were placed on glass gauze in boxes at equal distances and watered once every two d. Ten mL of G-Rd solution at different concentrations (0.25, 0.50, 1 and 2 mg L⁻¹) were added after germination and emergence of the radicle, with

distilled water used as the control (CK). Each box was incubated at 25 °C with 3 replicates as described above. After 20 d, three ginseng seedlings were randomly selected from each box then their fresh weights recorded. The antioxidant enzyme activities and endogenous plant hormone contents were then measured in the leaves of seedlings.

Antioxidant enzyme activity

The CAT, SOD and POD activities and MDA (malondialdehyde) contents were determined using kits produced by Nanjing Jiancheng Co., Nanjing, China. CAT activity was defined as one unit of activity based on the amount of H₂O₂ decomposition of 1 µmol mg⁻¹ of tissue protein s⁻¹; SOD activity was defined as one unit of activity based on the amount of SOD corresponding to each g of plant tissue in the reaction system when SOD inhibition had reached 50%; POD activity was defined as the amount of enzyme catalyzing 1 µg of substrate min⁻¹ mg⁻¹ of histone at 37 °C; one unit of MDA content was defined as the amount of MDA catalyzing 1 nmol mg⁻¹ of histone.

Hormone content

The endogenous hormone content of the ginseng roots was determined by enzyme-linked immunosorbent assay, using a kit from Huangshi Yanke Biotechnology Co., Huangshi, China.

Antioxidant enzymes and hormones within one week of sample collection, other parameters on the day of sample collection.

Germination calculations

The seeds were incubated in the artificial climate chamber for 2 d where they started to germinate.

The germination potential (GP) was defined as follows:

$$\text{GP (\%)} = (\text{number of germinating seeds peaked on the day when the number of germinating / number of seeds for testing}) \times 100\% \text{ on day 5} \quad (\text{Eq.1})$$

The germination rate (GR) was defined as follows:

$$\text{GR (\%)} = (\text{number of grains of all normally germinated seeds in the specified days / number of seeds for testing}) \times 100\% \text{ on day 10} \quad (\text{Eq.2})$$

$$\text{Germination rate (\%)} = \text{number of germinated seeds/number of seeds sown} \times 100\% \quad (\text{Eq.3})$$

$$\text{Germination potential (\%)} = \text{number of germinated seeds at peak germination / number of seeds sown} \times 100\% \quad (\text{Eq.4})$$

Data analysis

One-way ANOVA using SPSS 10.0 statistical software (SPSS Inc., Chicago, IL, USA) followed by the LSD test was used to analyze the results to compare the significance of differences between mean values at the 0.05 level for the effects of different

concentrations of the allelopathy substance, G-Rd, on the POD, CAT and SOD enzyme activities and the levels of three growth hormones in the ginseng seeds and seedlings.

Results

Enzyme activities and phytohormones content in ginseng seed germination

Figure 1 shows that G-Rd at a concentration of 0.25 mg L⁻¹ did not significantly inhibit the ginseng seed germination rate compared with CK but at concentrations from 0.50 to 2 mg L⁻¹, it significantly inhibited the ginseng seed germination rate ($P < 0.05$). At the maximum G-Rd concentration of 2 mg L⁻¹, the germination rate was highest significance, thus exhibiting a strong allelopathy inhibition (Eq.2).

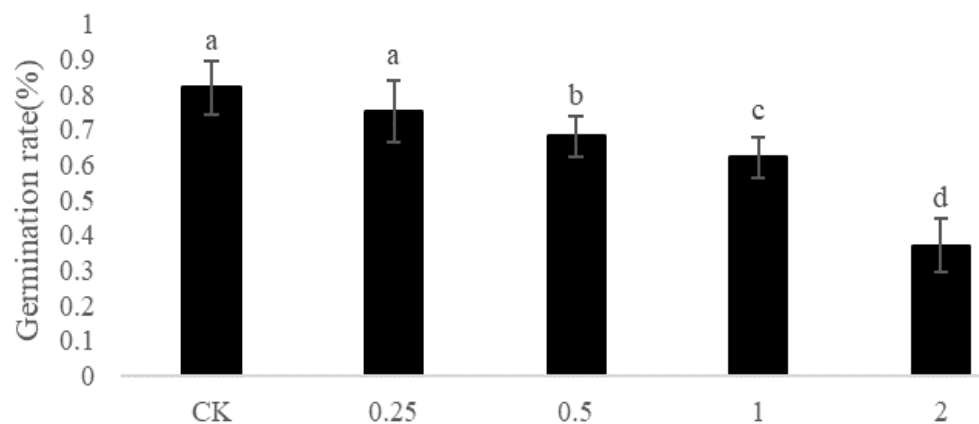


Figure 1. Effect of G-Rd concentration on the germination rate of *P. ginseng* seeds. Error bars present standard errors of three independent biological replicates. The same lower-case letter indicates a non-significant difference ($P > 0.05$), different lower-case letters indicate a significant difference ($P < 0.05$)

Effect of G-Rd concentration on the germination potential of ginseng seeds

Figure 2 shows that G-Rd at concentrations of 0.25 and 0.5 mg L⁻¹ showed some inhibition of the germination potential of ginseng seeds compared with CK, but did not reach a significant level but at concentrations of 1 and 2 mg L⁻¹, there was significant inhibition ($P < 0.05$), especially at 2 mg L⁻¹, where the germination potential was highest significance, thus showing a strong inhibitory effect (Eq.1).

Effect of G-Rd concentration on the growth of ginseng seed radicles

Figure 3 shows that, compared with CK, G-Rd significantly inhibited ginseng seed radicle growth at concentrations of 0.25 to 2 mg L⁻¹ ($P < 0.05$) and highest significance at concentrations of 2 mg L⁻¹, thus showing a strong inhibitory effect.

Effect of G-Rd concentration on enzyme activities and phytohormones content in germinated ginseng seeds

Figure 4 shows that the activities of CAT and POD in germinated ginseng seeds increased with G-RD at concentrations from 0.25 to 1 mg L⁻¹, but at the highest

concentration (2 mg L^{-1}), their activities decreased compared with CK. However, after G-Rd treatment, POD activity was concentration-dependent, with the activity increasing with increasing G-Rd concentration, with the maximum activity at 2 mg L^{-1} . At a G-Rd concentration of 1 mg L^{-1} , the activities of all the antioxidant enzymes, except SOD, were higher than those of CK. This indicated that G-Rd had a positive self-protective effect at low concentrations, but at higher concentrations (2 mg L^{-1}), it had a strong allelopathy effect which reduced antioxidant enzyme activity, reflecting a negative self-inactivating effect that reduced seed germination.

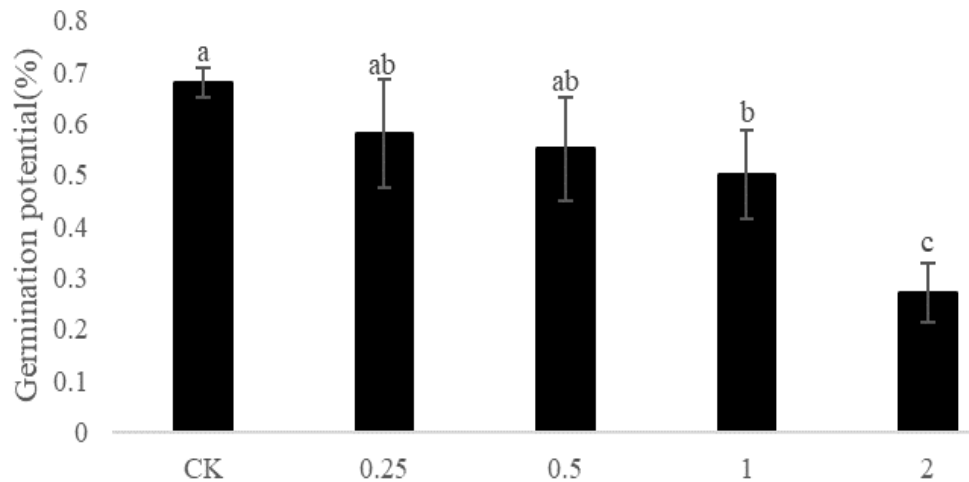


Figure 2. Effect of G-Rd concentration on the germination potential of *P. ginseng* seeds. Error bars present standard errors of three independent biological replicates. The same lower-case letter indicates a non-significant difference ($P > 0.05$), different lower-case letters indicate a significant difference ($P < 0.05$)

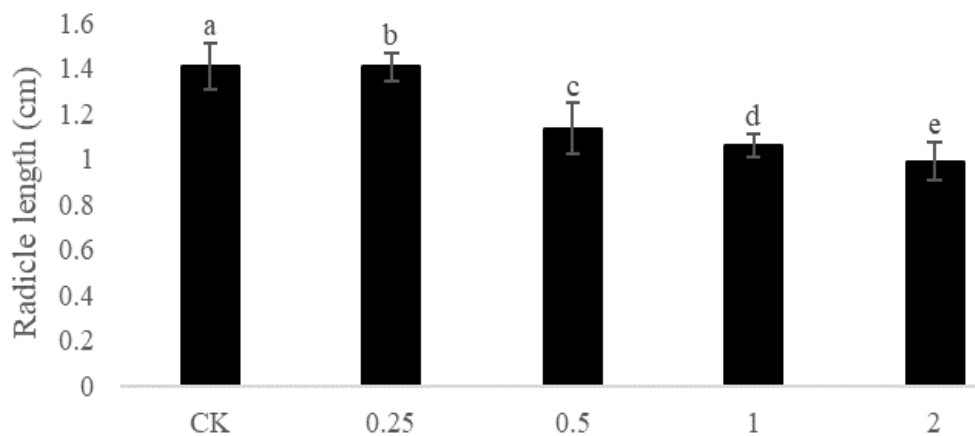


Figure 3. Effects of G-Rd concentration on the growth of radicles from *P. ginseng* seeds. Error bars present standard errors of three independent biological replicates. The same lower-case letter indicates a non-significant difference ($P > 0.05$), different lower-case letters indicate a significant difference ($P < 0.05$)

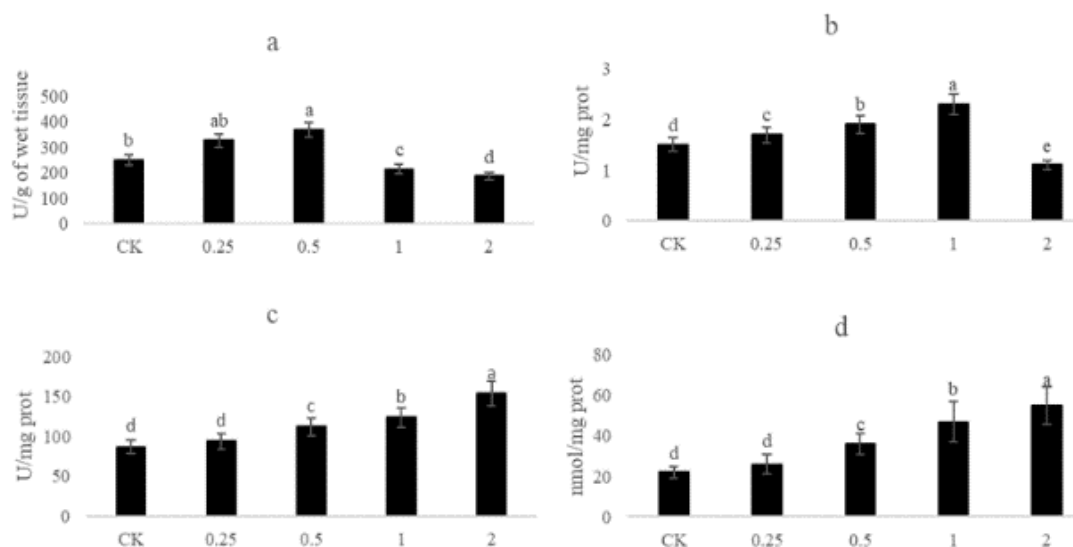


Figure 4. Effects of G-Rd concentration on the activity of antioxidant enzymes in germinated ginseng seeds: (a) SOD; (b) CAT; (c) POD; and (d) MDA. Error bars present standard errors of three independent biological replicates. The same lower-case letter indicates a non-significant difference ($P > 0.05$), different lower-case letters indicate a significant difference ($P < 0.05$)

Figure 5 shows that G-Rd at a concentration of 0.25 mg L⁻¹ had significantly inhibitory effects on the IAA contents in germinated ginseng seeds, and insignificant effect on their ABA and GA content. G-Rd at a concentration of 0.5 mg L⁻¹ significantly enhanced the contents of IAA and GA ($P < 0.05$) but not that of ABA. At 1 mg L⁻¹, G-Rd significantly enhanced the contents of ABA, IAA, and GA ($P < 0.05$). However, at 2 mg L⁻¹, G-Rd significantly inhibited the contents of IAA ($P < 0.05$) and GA ($P < 0.05$), with a significant enhancing effect on ABA content ($P < 0.05$).

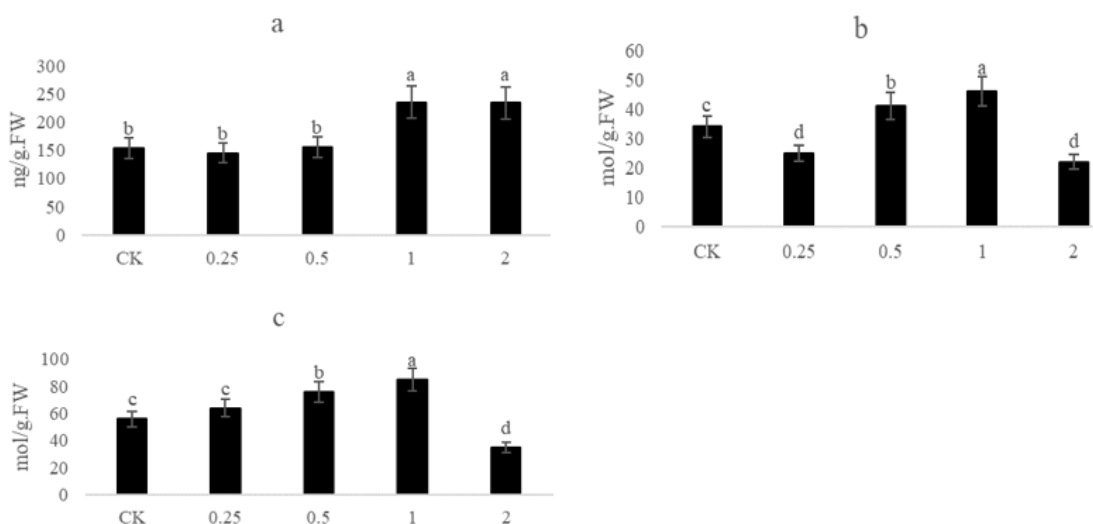


Figure 5. Effects of G-Rd concentration on the contents of phytohormones in germinated ginseng seeds: (a) ABA; (b) IAA; and (c) GA. Error bars present standard errors of three independent biological replicates. The same lower-case letter indicates a non-significant difference ($P > 0.05$), different lower-case letters indicate a significant difference ($P < 0.05$)

Effects of G-Rd concentration on the early growth of ginseng seedlings

Figure 6 shows that G-Rd at concentrations of 0.25 and 0.5 mg L⁻¹ had a beneficial and significant effect on increasing the fresh weight of ginseng seedlings. However, G-Rd concentrations of 1 and 2 mg L⁻¹ significantly reduced the fresh weight of ginseng seedlings ($P < 0.05$), especially at the maximum concentration of 2 mg L⁻¹, where the fresh weight was highest significance ($P < 0.05$), thus showing a strong allelopathy inhibitory effect.

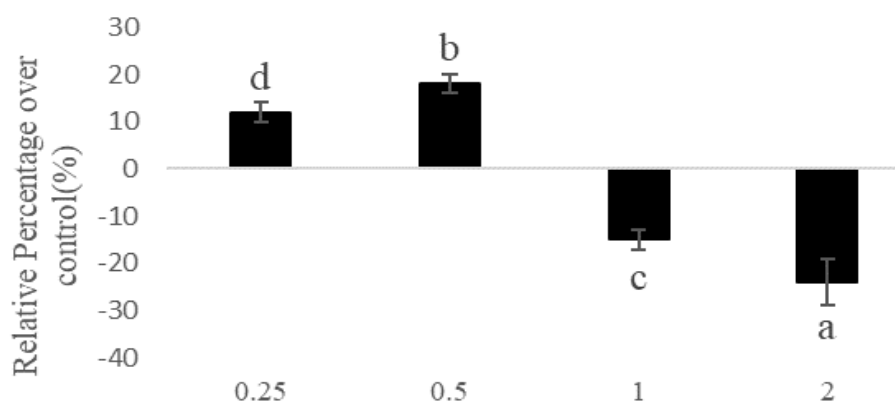


Figure 6. Effects of G-Rd concentration on the growth of *P. ginseng* seedlings. Error bars present standard errors of three independent biological replicates. The same lower-case letter indicates a non-significant difference ($P > 0.05$), different lower-case letters indicate a significant difference ($P < 0.05$)

Effect of G-Rd concentration on the enzyme activity in the leaves of *P. ginseng* seedlings

Figure 7 shows that the activities of SOD, CAT, and POD increased in the leaves of ginseng seedlings at G-Rd concentrations from 0.25 to 1 mg L⁻¹, but decreased significantly when the concentration reached 2 mg L⁻¹. However, the effect on POD activity was concentration-dependent, with the activity increasing with increasing G-Rd concentration, with the maximum activity at a concentration of 2 mg L⁻¹. At a G-Rd concentration of 1 mg L⁻¹, the activities of all the antioxidant enzymes were higher than that of CK, indicating that ginseng has a positive self-protective effect at low concentrations. There were also significant differences between treatment groups. However, the strong allelopathy effect of G-Rd on ginseng at high concentrations reduced the antioxidant enzyme activities, reflecting the self-deactivation effect of the plant and thus the reduced seed germination.

Effect of G-Rd concentration on the hormone content in the leaves of *P. ginseng* seedlings

Figure 8 shows that G-Rd at 0.25 mg L⁻¹ had a significant inhibitory effect on the contents of ABA and IAA ($P < 0.05$) in the leaves of ginseng seedlings but a significant increase in their GA content. At concentrations of 0.5 and 1 mg L⁻¹, G-Rd significantly enhanced the ABA, IAA, and GA contents ($P < 0.05$). There were also significant differences between treatment groups. G-Rd at 2 mg L⁻¹ significantly inhibited the contents of IAA and GA and significantly enhanced the contents of ABA ($P < 0.05$).

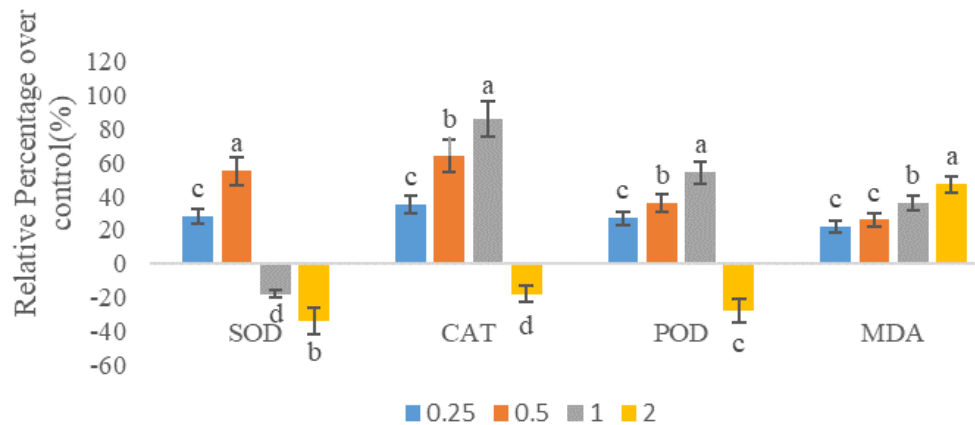


Figure 7. Effects of G-Rd concentration on the activity of antioxidant enzymes in the leaves of *P. ginseng* seedlings. Error bars present standard errors of three independent biological replicates. The same lower-case letter indicates a non-significant difference ($P > 0.05$), different lower-case letters indicate a significant difference ($P < 0.05$)

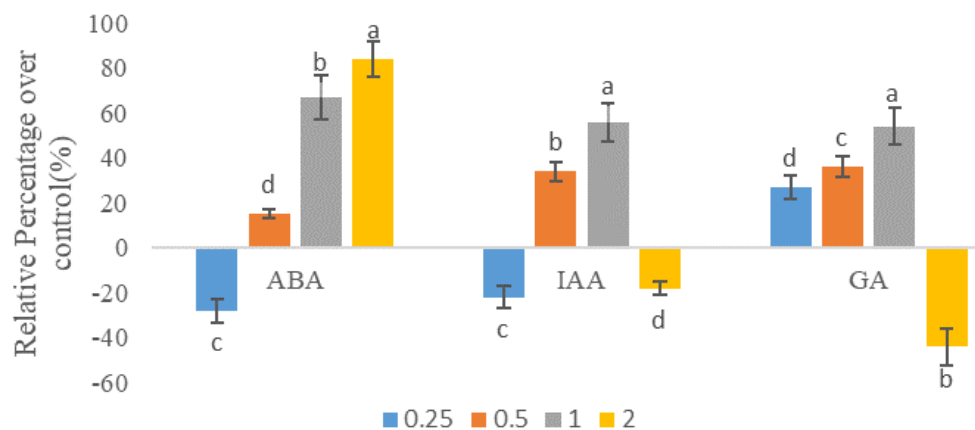


Figure 8. Effects of G-Rd concentration on the content of endogenous plant hormones in the leaves of *P. ginseng* seedlings. Error bars present standard errors of three independent biological replicates. The same lower-case letter indicates a non-significant difference ($P > 0.05$), different lower-case letters indicate a significant difference ($P < 0.05$)

Discussion

Ginseng crop disorder is a common problem in cultivation and production. Studies have shown that there are many causes of continuous crop disorders. The weakening of the main physical and chemical properties of the soil, the increase of pathogenic microorganisms and soil-borne diseases are all important causes, but none of them has. However, none of them has revealed the fundamental mechanism of the generation and formation of the continuous crop disorder. Numerous studies have shown that chemosensitizing substances have an important influence in ginseng crop disorders. The allelopathic of root secretions, which is an important cause of crop disorder, has become a hot topic of research on plant crop disorder in recent years (Chen et al., 2018). The allelopathic of root secretions, which is an important cause of crop disorder, has become a hot topic of plant crop disorder research in recent years (Whitehead et al., 2018). The

present study has reported the effects of exogenous G-Rd and seedling ginsenoside on ginseng seed germination, ginseng seedling root length growth and the fresh weight of young roots, as well as its effects on the activities of various antioxidant defense enzymes such as SOD, POD and CAT and the contents of growth hormones in the seedlings. The study has also explored the physiological adaptations of ginseng seeds and seedlings in response to exogenous G-Rd to gain a better understanding of the physiological and ecological mechanisms of ginsenoside. These mechanisms were investigated to provide a theoretical basis for revealing the intrinsic causes of ginseng crop disorders.

Under normal conditions, the scavenging of reactive oxygen species (ROS) and free radicals in plants is in dynamic balance but once this balance is disrupted, free radicals and ROS accumulate, allowing unsaturated fatty acids in the lipid bilayer of the membrane to be easily broken down by oxidation and cause membrane damage. MDA is the main product of cell membrane lipid peroxidation. Treating ginseng seedlings with ginsenosides increased the MDA content, indicating that the original balance of ROS production and scavenging in the leaves of young ginseng roots treated with exogenous ginsenosides had been disrupted, resulting in irreversible peroxidative damage to the membrane lipids. POD, SOD and CAT are three important cytoprotective enzymes. Their activities in ginseng roots increased significantly after treatment with different concentrations of ginsenosides, gradually increasing the activities of each enzyme with increasing concentration. This indicated that oxidative stress had led to an increase in the activities of antioxidant enzymes in the ginseng root cells. However, this increase did not correspond to the production of ROS, thus leading to membrane peroxidation in the ginseng root cells. The SOD activity did not differ significantly between the control and treatments with G-Rd at low to medium concentrations and was lower than the control after medium to high concentration treatment. The POD activity increased significantly after treatment with medium to high concentrations of G-Rd but decreased significantly after treatment at a high concentration. The levels of CAT activity after G-Rd treatment were all lower than that of CK. When plants are subjected to high levels of adverse stress, the increase in protective enzyme activity is not sufficient to resist it. This means that some structures of the plant cells are damaged and metabolic and physiological functions are disrupted. The activity of the protective enzyme (SOD) will then decrease, along with that of CAT. The scavenging capacity then decreases, leading to a large surplus of ROS, thus increasing the level of membrane peroxidation to cause the damage suffered by plants under adversity or the plant's resistance to adversity. The variation in the resistance of plants under adversity is often related to the level of SOD activity *in vivo*. Under adversity, the normal oxygen metabolism of plants is disturbed, increasing the rate of ROS production while, at the same time, disrupting the SOD-dominated cytoprotective system. Under the action of these two factors, the chain reaction of membrane lipid peroxidation in the plants accelerates, thereby increasing the accumulation of the harmful products of peroxidation. This then leads to the destruction of the cell membrane system, damage to the macromolecules essential for life, and finally, to plant death. The results of the present study agree with those on other antioxidant enzymes under allelopathy chemical stress (Coelho et al., 2017). It was found that plants produce a large number of antioxidant enzymes to scavenge reactive oxygen species and mitigate chemosensory damage to cells under chemosensory stress. Superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) constitute the major antioxidant enzyme systems in plants (Mir et al., 2018). Medium to low concentrations of ginsenosides isolated from ginseng have been found to significantly stimulate the activities of SOD, POD, and CAT in the

roots of American ginseng (Zhang et al., 2011). Similarly, the plant growth retardant and triazole fungicide, paclobutrazol, increased the contents of antioxidant enzymes in wheat leaves (Kraus et al., 1995) and glycine betaine in wheat leaves (Ali et al., 2015). These are self-protective mechanisms in plants which respond to biotic and abiotic stresses. However, in the present study, the activity of antioxidant enzymes decreased at a G-Rd concentration of 2 mg L^{-1} , a phenomenon also observed by Yang (2006).

The significant increase in ABA content may have indicated the existence of additive and synergistic effects from different allelopathy effects, which together regulate ABA content, accelerate the inhibition of the elongation of shoot and root organs, affect differentiation, accelerate the abscission of plant organs, and induce the occurrence of old ginseng diseases. The significant decrease in IAA content indicates the existence of strong additive and synergistic effects between different allelopathy effects. The decrease in IAA content indicated the presence of a strong additive and synergistic effect between different allelopathy effects, which is consistent with the trend in the ABA results. The changes in its content may have caused some *in vivo* division and physiologically inhibited growth metabolism in the root tip, stem tip, young leaves and other tissues of the ginseng seedlings. The allelopathy effects of different concentrations of the endogenous hormone, GA, in ginseng may exhibit a phenomenon of low promotion and high inhibition, or because of the interaction between hormones, GA may combine with other hormone indicators to adapt to environmental changes leading to hormone homeostasis. Allelochemicals have been shown to increase the phytohormone content of crops and weeds (Ren et al., 2018; Yuan and Yang, 2018). Wei et al. found that plant growth in *Arabidopsis* was stunted when gibberellin content was inhibited by chemosensitive substances (Wei et al., 2018). Physiological studies have also shown that IAA and GA affect cell expansion and balance plant growth (Hui et al., 2018; Li et al., 2018; Zhang et al., 2018). In the present study, the root length and fresh weight were both related to the IAA content and the GA content was influenced by G-Rd, similar to results on abiotic and biotic stress treatments in other plants, such as snap bean and rice (Saleh et al., 2015; Shu et al., 2018; Shen et al., 2019). The present results suggest that endogenous hormones may interact in the ginseng seedlings in response and adaptation to G-Rd stress. Therefore, the regulatory mechanisms of endogenous hormones on the growth of ginseng seedlings under G-Rd stress need to be investigated further.

Conclusion

Ginsenoside Rd may be one possible factor causing disorders in ginseng crops. G-Rd was able to inhibit ginseng seed germination, radicle elongation and reduce fresh weight at high concentrations (0.5 to 2 mg L^{-1}) and promote them at a low concentration (0.25 mg L^{-1}). Different concentrations of G-Rd exhibited different effects on the antioxidant enzyme activities and phytohormone contents of ginseng seedlings. Low concentrations (0.25 and 0.50 mg L^{-1}) of G-Rd could increase antioxidant enzyme activity and had a positive effect on maintaining the balance between ROS production and scavenging. High concentrations of G-Rd (1 to 2 mg L^{-1}) induced excessive ROS production and inhibited the growth of the ginseng seedlings. The present study has confirmed that G-Rd is one of the chemosensitizing factors affecting ginseng crops. This has enriched the theoretical background for the study of ginseng production and revealed more of the intrinsic and essential causes of ginseng crop failure.

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REFERENCES

- [1] Ali, S., Chaudhary, A., Rizwan, M., Anwar, H. T., Adrees, M., Farid, M., Irshad, M. K., Hayat, T., Anjum, S. A. (2015): Alleviation of chromium toxicity by glycinebetaine is related to elevated antioxidant enzymes and suppressed chromium uptake and oxidative stress in wheat (*Triticum aestivum* L.). – *Environ Sci Pollution Research* 22: 10669-10678.
- [2] Bais, H. P., Vepachedu, R., Gilroy, S., Callaway, R. M., Vivanco, J. M. (2003): Allelopathy and exotic plant invasion: from molecules and genes to species interactions. – *Science* 301: 1377-1380.
- [3] Bernards, M. A., Ivanov, D. A., Neculai, M. A., Nicol, R. W. (2011): Ginsenosides: Phytoanticipins or host recognition factors? – In: Gang, D. R. (eds.) *The Biological Activity of Phytochemicals*. Springer, New York, pp. 13-32.
- [4] Bi, X., Yang, J., Gao, W. (2010): Autotoxicity of phenolic compounds from the soil of American ginseng (*Panax quinquefolium* L.). – *Allelopathy J* 25: 115-122.
- [5] Bu, R., Xie, J., Yu, J., Liao, W., Xiao, X., Lv, J., Wang, C., Ye, J., Calderón-Urrea, A. (2016): Autotoxicity in cucumber (*Cucumis sativus* L.) seedlings is alleviated by silicon through an increase in the activity of antioxidant enzymes and by mitigating lipid peroxidation. – *J Plant Biol* 59: 247-259.
- [6] Chen, W., Teng, Y., Li, Z., Liu, W., Ren, W., Luo, Y., Christie, P. (2018): Mechanisms by which organic fertilizer and effective microbes mitigate peanut continuous cropping yield constraints in a red soil of south China. – *Applied Soil Ecology* 128: 23-34.
- [7] Chi, W. C., Chen, Y. A., Hsiung, Y. C., Fu, S. F., Chou, C. H., Trinh, N. N., Chen, Y. C., Huang, H. J. (2013): Autotoxicity mechanism of *Oryza sativa*: transcriptome response in rice roots exposed to ferulic acid. – *BMC Genomics* 14: 351.
- [8] Chou, C. (1999): Roles of allelopathy in plant biodiversity and sustainable agriculture. – *Crit. Rev. Plant Sci.* 18: 609-636.
- [9] Christensen, L. P. (2009): Ginsenosides: chemistry, biosynthesis, analysis, and potential health effects. – *Adv. Food Nutr. Res.* 55: 1-99.
- [10] Coelho, É. M. P., Barbosa, M. C., Mito, M. S., Mantovanelli, G. C., Oliveira, R. S., Ishii-Iwamoto, E. L. (2017): The activity of the antioxidant defense system of the weed species *Senna obtusifolia* L. and its resistance to allelochemical stress. – *J Chemical Ecol* 43: 725-738.
- [11] Coon, J. T., Ernst, E. (2002): *Panax ginseng*. – *Drug Safety* 25: 323-344.
- [12] Court, W. A., Hendel, J. G., Elmi, J. (1996): Reversed-phase high performance liquid chromatography determination of ginsenosides of *Panax quinquefolium*. – *J. Chromatogr. A* 755: 11-17.
- [13] Dorning, M., Cipollini, D. (2006): Leaf and root extracts of the invasive shrub, *Lonicera maackii*, inhibit seed germination of three herbs with no autotoxic effects. – *Plant Ecol* 184: 287-296.
- [14] Fujioka, N., Kohda, H., Yamasaki, K., Kasai, R., Shoyama, Y., Nishioka, I. (1989): Dammarane and oleanane saponins from callus tissue of *Panax japonicus*. – *Phytochemistry* 28: 1855-1858.
- [15] Guo, Z. Y., Kong, C. H., Wang, J. G., Wang, Y. F. (2011): Rhizosphere isoflavones (Daidzein and genistein) levels and their relation to the microbial community structure of mono-cropped soybean soil in field and controlled conditions. – *Soil Biol Biochemistry* 43: 2257-2264.

- [16] Huang, L. F., Song, L. X., Xia, X. J., Mao, W. H., Shi, K., Zhou, Y. H., Yu, J. Q. (2013): Plant-soil feedbacks and soil sickness: from mechanisms to application in agriculture. – J. Chem. Ecol. 39: 232-242.
- [17] Hui, W., Wang, Y., Yan, S., Shi, J., Huang, W., Zayed, M. Z., Peng, C., Chen, X., Wu, G. (2018): Simultaneous analysis of endogenous plant growth substances during floral sex differentiation in *Jatropha curcas* L. using HPLC-ESI-MS/MS. – Scientia Horticulturae 241: 209-217.
- [18] Inderjit, Duke, S. O. (2003): Ecophysiological aspects of allelopathy. – Planta 217: 529-539.
- [19] Kimura, Y., Sumiyoshi, M., Kawahira, K., Sakanaka, M. (2006): Effects of ginseng saponins isolated from Red Ginseng roots on burn wound healing in mice. – British J Pharmacology 148: 860-870.
- [20] Kraus, T. E., McKersie, B. D., Fletcher, R. A. (1995): Paclobutrazol-induced tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity. – J Plant Physiology 145: 570-576.
- [21] Li, Y., Huang, X., Ding, W. (2011): Autotoxicity of *Panax ginseng* rhizosphere and non-rhizosphere soil extracts on early seedlings growth and identification of chemicals. – Allelopathy J 28: 95-102.
- [22] Li, X., Wang, L., Wang, S., Yang, Q., Zhou, Q., Huang, X. (2018): A preliminary analysis of the effects of bisphenol A on the plant root growth via changes in endogenous plant hormones. – Ecotoxicology Environ Safety 150: 152-158.
- [23] Mir, M. A., John, R., Alyemeni, M. N., Alam, P., Ahmad, P. (2018): Jasmonic acid ameliorates alkaline stress by improving growth performance, ascorbate glutathione cycle and glyoxylase system in maize seedlings. – Scientific Reports 8: 2831.
- [24] Möhler, H., Diekötter, T., Herrmann, J. D., Donath, T. W. (2018): Allelopathic vs. autotoxic potential of a grassland weed-evidence from a seed germination experiment. – Plant Ecol & Diversity 11: 539-549.
- [25] Nicol, R. W., Traquair, J. A., Bernards, M. A. (2002): Ginsenosides as host resistance factors in American ginseng (*Panax quinquefolius*). – Can. J. Bot. 80: 557-562.
- [26] Nicol, R. W., Yousef, L., Traquair, J. A., Bernards, M. A. (2003): Ginsenosides stimulate the growth of soilborne pathogens of American ginseng. – Phytochemistry 64: 257-264.
- [27] Qi, L. W., Wang, C. Z., Yuan, C. S. (2011): Ginsenosides from American ginseng: chemical and pharmacological diversity. – Phytochemistry 72: 689-699.
- [28] Ren, K., Hayat, S., Qi, X., Liu, T., Cheng, Z. (2018): The garlic allelochemical DADS influences cucumber root growth involved in regulating hormone levels and modulating cell cycling. – J Plant Physiology 230: 51-60.
- [29] Saleh, A. M., Madany, M. M., González, L. (2015): The effects of coumarin application on early growth and some physiological parameters in Faba bean (*Vicia faba* L.). – J Plant Growth Regulation 34: 233-241.
- [30] Shen, S., Liu, Y., Wang, F., Yao, G., Xie, L., Xu, B. (2019): Graphene oxide regulates root development and influences IAA concentration in rice. – Journal of Plant Growth Regulation 38: 241-248.
- [31] Shu, K., Zhou, W., Chen, F., Luo, X., Yang, W. (2018): Absciscic acid and gibberellins antagonistically mediate plant development and abiotic stress responses. – Front in Plant Sci 9: 416.
- [32] Singh, H. P., Batish, D. R., Kohli, R. K. (1999): Autotoxicity: concept, organisms, and ecological significance. – Crit. Rev. Plant Sci. 18: 757-772.
- [33] Tran, Q. L., Adnyana, I. K., Tezuka, Y., Nagaoka, T., Tran, Q. K., Kadota, S. (2001): Triterpene saponins from Vietnamese ginseng (*Panax vietnamensis*) and their hepatocytoprotective activity. – J. Nat. Prod. 64: 456-461.
- [34] Wei, S., Wang, Y., Li, J., Zhang, H., Ding, L. (2007): Investigation of ginsenosides in different parts and ages of *Panax ginseng*. – Food Chem. 102: 664-668.

- [35] Weir, T. L., Park, S. W., Vivanco, J. M. (2004): Biochemical and physiological mechanisms mediated by allelochemicals. – *Curr. Opin. Plant Biol.* 7: 472-479.
- [36] Whitehead, J., Wittemann, M., Cronberg, N. (2018): Allelopathy in bryophytes - a review. – *Lindbergia* 1: 01097.
- [37] Wu, L., Zhao, Y., Guan, Y., Pang, S. (2008): A review on studies of the reason and control methods of succession cropping obstacle of *Panax ginseng* CA Mey. – *Special Wild Economic Animal Plant Research* 2: 68-72.
- [38] Wu, B., Long, Q., Gao, Y., Wang, Z., Shao, T., Liu, Y., Li, Y., Ding, W. (2015): Comprehensive characterization of a time-course transcriptional response induced by autotoxins in *Panax ginseng*, using RNA-Seq. – *BMC Genomics* 16: 1010.
- [39] Xiong, W., Ye, T. T., Yao, X., Liu, X., Ma, S., Chen, X., Chen, M. L., Feng, Y. Q., Wu, Y. (2018): The dioxygenase GIM_2 functions in seed germination by altering gibberellin production in *Arabidopsis*. – *Journal of Integrative Plant Biology* 60(04): 276-291.
- [40] Yang, G. (2006): Physiological effects of allelochemicals from leachates of *Ageratina adenophora* (Spreng.) on rice seedlings. – *Allelopathy J* 18: 237-246.
- [41] Yang, C., Liu, S., Zhou, S., Wu, H., Yu, J., Xia, C. (2011): Allelochemical ethyl 2-methyl acetoacetate (EMA) induces oxidative damage and antioxidant responses in *Phaeodactylum tricornutum*. – *Pestic. Biochem. Physiol.* 100: 93-103.
- [42] Yang, M., Zhang, X., Xu, Y., Mei, X., Jiang, B., Liao, J., Yin, Z., Zheng, J., Zhao, Z., Fan, L. (2015): Autotoxic ginsenosides in the rhizosphere contribute to the replant failure of *Panax notoginseng*. – *PLoS One* 10: e0118555.
- [43] Yuan, X. K., Yang, Z. Q. (2018): The effects of endogenous hormones on plant morphology and fruit quality of tomato under difference between day and night temperature. – *Horticultural Sci* 45: 131-138.
- [44] Zhang, H., Cheng, Y. (2006): Solid-phase extraction and liquid chromatography-electrospray mass spectrometric analysis of saponins in a Chinese patent medicine of formulated *Salvia miltiorrhiza* and *Panax notoginseng*. – *J. Pharmaceut. Biomed.* 40: 429-432.
- [45] Zhang, A. H., Lei, F. J., Fang, S. W., Jia, M. H., Zhang, L.X. (2011): Effects of ginsenosides on the growth and activity of antioxidant enzymes in American ginseng seedlings. – *J Medicinal Plants Research* 5: 3217-3223.
- [46] Zhang, W., Lu, L. Y., Hu, L. Y., Cao, W., Sun, K., Sun, Q. B., Siddikee, M. A., Shi, R. H., Dai, C. C. (2018): Evidences for the involvement of auxin, ethylene and ROS signaling during allelochemical benzoic acid-mediated primary root inhibition of *Arabidopsis*. – *Plant and Cell Physiology* 59: 1889-1904.
- [47] Zhou, J. M., Cui, X. M., Zeng, J., Zhu, L., Zhao, A. (2007): Investigation on research progress and activity utilization of ginsenosides from different parts of *Panax notoginseng*. – *J. Chinese Med Mater.* 30: 1615-1618.
- [48] Zhou, B., Kong, C. H., Li, Y. H., Wang, P., Xu, X. H. (2013): Crabgrass (*Digitaria sanguinalis*) allelochemicals that interfere with crop growth and the soil microbial community. – *J Agric Food Chem* 61: 5310-5317.
- [49] Zhou, X., Wang, J., Jin, X., Li, D., Shi, Y., Wu, F. (2019): Effects of selected cucumber root exudates components on soil *Trichoderma* spp. communities. – *Allelopathy J* 47: 257-266.