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Tables: 3, Fig : -

Autotoxicity of phenolic compounds from the soil of American ginseng (*Panax quinquefolium* L.)

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ABSTRACT

The inhibitory activity of 9 phenolic compounds [*p*-hydroxybenzoic acid, vanillin, syringic acid, vanillic acid, coumaric acid, ferulic acid, cinnamic acid, salicylic acid, benzoic acid] was investigated *in vitro* on the radicle and shoot growth of American ginseng (*Panax quinquefolium* L.) seedlings. These compounds were detected in the soils of commercially cultivated American ginseng. Their test concentrations (0.1 mmol/L to 10.0 mmol/L) were selected based on their content in soils. All phenolic compounds inhibited the radicle and shoot growth of American ginseng in a dose-dependent response and their IC_{50} values were also calculated. Only 5-compounds caused < 50% inhibition in radicle growth of *American ginseng* and their autotoxicity followed the order: salicylic acid > cinnamic acid > coumaric acid > vanillic acid > syringic acid. However, *p*-hydroxybenzoic acid, vanillin, ferulic acid, benzoic acids even at the highest concentration (10.0 mmol/L) did not cause 50% inhibition in radicle elongation. Thus different phenolic compounds displayed variable phytoxicity to American ginseng (*Panax quinquefolium* L.) and there is a close relationship between the substitutions on the benzene ring of phenolic compounds and their phytotoxicity.

Key words: Autototoxicity, benzoic acid, bioassay, cinnamic acid, coumaric acid, ferulic acid, *p*-hydroxybenzoic acid, IC_{50}, *Panax quinquefolium* L., phenolic compounds, radicle, salicylic acid, seedling, shoot, syringic acid, vanillic acid, vanillin

INTRODUCTION

The role of allelopathy in agricultural and natural ecosystem has received increased attention in past few years (12,14). Much emphasis has also been placed on identifying the potential allelochemicals, hence, several categories of allelochemicals [phenolic acids, hydroxamic acids, other organic acids (10) and volatile substances (7)] in living and decomposing tissues of plant species have been identified. The phytotoxicity of phenolic compounds in different ecosystems has been studied in depth (3, 11).

American ginseng (*Panax quinquefolium* L.) is a herbaceous perennial plant (*Araliaceae* family) originated from eastern part of North America (2) and is an important traditional herbal medicine in China. The ginsenosides are mainly responsible for the

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pharmacological activities of American ginseng. In China, American ginseng (*Panax quinquefolius* L.) has been commercially cultivated since past few decades. The present supply of American ginseng is primarily from the plantations in the fields. Ginseng is sown in fall and emerges in the following spring. Its yield and quality is greatly reduced due to soil sickness, if replanted in the same soil successively (5,6), this problem occurs especially in horticultural crops (19,24). It is owing to accumulation of pathogenic pests, disorder in soil physical- chemical properties and autotoxicity (20). Nevertheless, the problem of re-establishment failure of American ginseng has not yet been solved, through supply of soil nutrients or decreasing soil-borne pathogens (1,18). Autotoxicity also occurs in rice (*Oryza sativa*), tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*), other Cucurbit crops, tea tree (*Camellia sinensis*), horsetail wood (*Casuarina equisetifolia*) and Chinese fir (*Cunninghamia lanceolata*) in South China (15). So autotoxicity may be the possible cause of re-seeding failure of American ginseng (25). He *et al* (2009) recently identified several phenolic acids and aliphatic compounds from the fibrous roots of American ginseng and these were found responsible for the inhibition of American ginseng seedlings growth (9).

Phenolic acids represent an important variety of plant secondary metabolites possessing different magnitudes of phytotoxicity on various receiver plants. Yu and Matsui (21,22) found that cucumber plant possess the allelopathic potential by exuding allelochemicals [benzoic and cinnamic acids (21, 22)]. Blum *et al* (3,4) reported that cinnamic acid derivatives were more inhibitory to cucumber seedlings than their corresponding benzoic acid derivatives (4,8). The relative inhibitory activity of eight phenolic acids to cucumber seedlings followed the order: ferulic acid and sinapic acid > *p*-coumaric acid, vanillic acid and syringic acid > caffeic acid, and *p*-hydroxybenzoic acid > protocatechuic acid (8). Among the salicylic acid, *p*-hydroxybenzoic, *m*-hydroxybenzoic acid and their *o*-methyl derivatives, the salicylic acid was most inhibitory to lettuce (*Lactuca sativa* L.) growth (13). In our preliminary experiments, many phenolic acids were found in ginseng soils and these compounds were inhibitory to the growth of American ginseng, however, the relative toxicity of several phenolic compounds on growth of American ginseng seedlings was not well known.

This study aimed to investigate the magnitude of inhibitory effects of 9 phenolic compounds [*p*-hydroxybenzoic acid, vanillin, syringic acid, vanillic acid, coumaric acid, ferulic acid, cinnamic acid, salicylic acid, benzoic acid] on the radicle and shoot growth of American ginseng, to understand the relationship between the structure and activity and the roles of various substitutions on the benzene ring in the phytotoxicity of phenolic compounds.

MATERIALS AND METHODS

The nine test phenolic compounds [*p*-hydroxybenzoic acid, vanillin, syringic acid, vanillic acid, coumaric acid, ferulic acid, cinnamic acid, salicylic acid benzoic acid] were purchased from Alfa Aesar company and their stock solutions [10.0, 5.0, 2.5, 1.0, 0.5, 0.1 mmol/L] were prepared in methanol.

Bioassays

American ginseng (*Panax quinquefolium* L.) seeds were provided by Beijing Tianhui Pharmaceutical Co., Ltd. Seeds were sterilized with distilled water: Clorox (95:5) for 10 min and rinsed 4 times with distilled water. Two ml of each compound was added to 9-cm dia Petri dishes containing two pieces of Whatman no. 1 filter paper. Controls received only methanol. The methanol was allowed to evaporate and 5 mL sterilized water was subsequently added to each Petri dish. Ten American ginseng seeds with radicles of 1 mm length were separately placed into each 9-cm Petri dish and then covered and sealed with Parafilm. Seeds were grown in dark at 25°C . All treatments were replicated five times. Radicle and shoot lengths were measured 7 day after treatment. Inhibitory rate was defined as (A-B/B)×100%, where, A is the mean net elongation of radicle of controls and B is the mean net elongation of radicle treated by phenolic compounds.

Data analysis: The data of radicle and shoot length of American ginseng seedlings were statistically analyzed in SPSS 13.0 program (SPSS Inc, Chicago, IL). One-way ANOVA and Dunnett and Duncan multiple comparison analysis were conducted to test the difference ($P<0.05$) in radicle and shoot length between the treated by individual phenolic compounds solution and untreated groups.

RESULTS AND DISCUSSION

All phenolic compounds inhibited the radicle and shoot growth of American ginseng seedlings compared to controls (Table 1, 2). Nine phenolic compounds possessed significant phytotoxicity to shoots at the concentration of 5mmol/L and 10mmol/L over the controls. Especially the seedlings' shoots were inhibited even with 0.1 mmol/l cinnamic acid and salicylic acid (Table 2). However, the inhibitory activity of same concentration of cinnamic acid and salicylic acid on the radicle of American ginseng was lower than on shoot, showing that shoot growth was more sensitive to these two phenolics. Although phytotoxicity of phenolic compounds to shoot growth of American ginseng increased with the increasing applied concentrations, but did not follow linear trend. However, the inhibitory impact of nine compounds on radicle growth was best described by a linear toxicity regression equation ($P<0.05$). Even 10.0 mmol/L (highest concentration) of *p*-hydroxybenzoic acid, vanillin, ferulic acid, benzoic acid did not cause 50% inhibition in American ginseng root elongation. So IC_{50} values of other five compounds ranged from 3.71 (salicylic acid) to 12.93 (syringic acid) (Table 3). Thus various phenolic compounds had different magnitude of toxicity to radicle elongation of American ginseng. Salicylic acid proved most inhibitory. The toxicity of five phenolic compounds against American ginseng radicle growth was in order: salicylic acid > cinnamic acid > coumaric acid > vanillic acid > syringic acid. These results demonstrated that different substitutions in various phenolic compounds on their benzene ring, affects their toxic activity.

The nine test phenolic compounds [cinnamic, benzoic and coumalic acid derivatives. Salicylic acid, vanillic acid, syringic acid] were categorized into benzoic acid derivatives. The inhibitory effects of salicylic acid was strongest (IC_{50}=3.71). One of the reasons might be that the *ortho*-hydroxyl substitution was attributable to the higher phytotoxicity of salicylic acid. This result can be supported by other reports. Iqbal *et al.*

Table 1. Effects of nine phenolic compounds from American ginseng soil on its own seedlings radicle growth

Conc. (mmol/L)	*p*-Hydroxy-benzoic acid	Vanillic acid	Syringic acid	Vanillin	Coumaric acid	Ferulic acid	Salicylic acid	Benzoic acid	Cinnamic acid
0	1.12a	1.12a	1.12a	1.12a	1.12a	1.12a	1.12a	1.12a	0.82a
0.1	0.95ab	1.01ab	1.07ab	1.02ab	0.95b	1.04a	1.04ab	0.96b	0.75ab
0.5	0.89bc	0.95bc	0.98bc	0.96ab	0.91b	0.85bc	0.98ab	0.95b	0.63b
1.0	0.89bc	0.88c	0.91cd	0.95ab	0.77c	0.89bc	0.88b	0.89bc	0.60bc
2.5	0.73cd	0.65d	0.84e	0.86bc	0.65c	0.90b	0.67c	0.78cd	0.47cd
5.0	0.68d	0.61de	0.67e	0.71cd	0.52d	0.73c	0.49cd	0.75d	0.40de
10.0	0.64d	0.49f	0.50f	0.57cd	0.52d	0.57d	0.30d	0.54e	0.30f

Values in the same column followed by the same letter are not statistically different at P=0.05 by Duncan's test

Table 2. Effects of nine phenolic compounds from American ginseng soil on its own seedlings shoot growth (cm)

Conc. (mmol/L).	*p*-Hydroxy-benzoic acid	Vanillic acid	Syringic acid	Vanillin	Coumaric acid	Ferulic acid	Salicylic acid	Benzoic acid	Cinnamic acid
0	2.73±0.16	2.73±0.16	2.73±0.16	2.73±0.16	2.73±0.16	2.73±0.16	2.73±0.16	2.73±0.16	2.73±0.16
0.1	1.99 ±0.71	2.39±0.09	2.50±0.20	2.31±0.18	2.32±0.21	2.21±0.56	2.00±0.33*	2.45±0.27	0.00±0.00*
0.5	2.69±0.37	2.54±0.40	2.39±0.37	2.22±0.34	2.26±0.66	2.42±0.42	2.31±0.29	2.47±0.31	0.00±0.00*
1	2.04±0.61	2.55±0.52	2.21±0.21	2.17±0.39	1.15±0.78*	2.10±0.25	2.04±0.51*	2.72±0.18	0.00±0.00*
2.5	2.02±1.16	1.70±0.41*	2.17±0.35	2.30± 0.33	1.47±0.98*	1.96±0.57	0.71±0.68*	2.12±0.36*	0.00±0.00*
5	0.95±0.89*	1.08±0.27*	0.85±0.40*	2.09±0.32*	0.29±0.35*	1.59±0.97*	0.36±0.39*	1.69±0.53*	0.00±0.00*
10	0.32±0.51*	1.09±0.62*	0.91±0.58*	1.22±0.32*	0.00±0.00*	0.83±0.43*	0.00±0.00*	0.53±0.25*	0.00±0.00*

The data represent mean ± SD (n=5). * indicates that values in the same column are statistically different at P=0.05 by Dunnett's test

Table 3. Toxicity regression equation, r^2 value and IC_{50} value for the radicle length (cm) of American ginseng seedlings treated with nine phenolic compounds

Phenolic compounds	Toxicity regression equation	r^2	IC_{50} (mmol/L)
p-Hydroxybenzoic acid	y = 0.1514x + 0.2686	0.9055	---
Vanillic acid	y = 0.2476x + 0.2841	0.9183	7.45
Syringic acid	y = 0.2448x + 0.2279	0.8951	12.93
Vanillin	y = 0.1944x + 0.2198	0.8375	---
Coumaric acid	y = 0.2221x + 0.3277	0.9236	5.97
Ferulic acid	y = 0.1757x + 0.2356	0.7808	---
Salicylic acid	y = 0.3407x + 0.306	0.8971	3.71
Benzoic acid	y = 0.1755x + 0.252	0.8078	---
Cinnamic acid	y = 0.2738x + 0.3234	0.9714	4.42

Note: x denotes the concentration (lg10). y denotes the inhibitory rate of American ginseng radicle growth. r^2 denotes the correlation coefficient. --- denotes the corresponding compound does not cause 50% inhibition at the highest concentration (10mmol/L)

(13) found that the growth inhibitory activity of salicylic acid on the root growth of *Lactuca sativa* L. was highest through investigating the phytotoxic activity of monohydroxybenzoic acids and their *o*-methyl derivatives. Yu and Matsui (23) also found that *o*-hydroxybenzoic acid showed strongest activity on iron uptake by cucumber seedlings among three groups of carboxylic acid, such as benzonic acid, *o*-hydroxybenzoic acid (salicylic acid), *p*-hydroxybenzoic acid, vanilic acid, cinnamic acid, ferulic acid. The magnitude of toxicity of syringic acid (IC_{50}=12.93) to American ginseng growth was lower than vanillic acid (IC_{50}=7.45). One of reasons for this difference was that the numbers of methoxyl groups played a role in influencing the activity of compounds. The phytotoxicity decreased with an increase in number of methoxyl groups on the benzene ring for vanillic acid. It was concluded that the substitition of methoxyl group on the benzene ring (vanilic and syringic acids) decreased the inhibitory activity on the radicle growth of American ginseng, indicating that the hydrophobicity (or lipophilicity) of the phenolic acids plays an important role in the inhibitory activity.

Cinnamic acid (IC_{50}=4.42) belonging to cinnamic acid derivatives also expressed stronger phytotoxic activity than vanillic acid (IC_{50}=7.45), syringic acid (IC_{50}=12.93). Although Blum *et al* (3,4) had reported that the cinnamic acid derivatives were more inhibitory to cucumber seedlings than the benzoic acid derivatives (5,8), cinnamic acid possessing higher phytotoxicity to American ginseng than vanillic acid, syringic acid was reported first time. This result was also consistent with other reports. Lyu *et al* (16,17) reported that vanillic acid, *p*-hydroxycinnamic acid and ferulic acids were the potential allelochemicals inhibitory to uptake of $H_2PO_4^-$ by cucumber seedlings. Yu and Matsui (23) also demonstrated that cinnamic acid inhibited the ion uptake by cucumber seedlings more strongly than these phenolic acids and that the activity of phenolic acid was partly modified by substitutions such as hydroxyl and methoxyl groups.

Coumaric acid had strong inhibitory activity which listed the third place in the toxicity range of all five compounds (IC_{50}=5.97). Moreover, coumaric acid constituted a large proportion in the phenolic compounds detected from the soils in the experiments (9). However, whether coumaric acid was the main allelochemical of American ginseng still needed further investigation.

All test nine phenolic compounds were found in the commercially cultivated American ginseng soils (9). However, *p*-hydroxybenzoic acid, vanillin, ferulic acid and benzoic acids even at maximum concentration (10.0 mmol/L) did not cause 50% inhibition in American ginseng shoot elongation. These laboratory results might indicate that *p*-hydroxybenzoic acid, vanillin, ferulic acid, benzoic acid were not mainly responsible for the re-establishment problem of American ginseng. The inhibitory activity of vanillic acid was stronger than vanillin, which might demonstrate that different redox status could cause the difference in phytoxicity to American ginseng seedlings.

CONCLUSIONS

These results may help to better understand the relationship between structure and activity of nine test phenolic compounds. The differences in structure of compounds could lead to the various change in their activity. The number, position and kind of substitutions such as hydroxyl group and methoxyl group on the benzene ring could contribute to the change in phytotoxicity of compounds to American ginseng seedlings. Hydrophilicity or hydrophobicity of allelochemicals, for example phenolic compounds tested here, is responsible for the relative phytotoxic activity of allelochemicals. This study showed that elucidation of the role of the carboxyl, hydroxyl and methoxyl groups in the inhibitory activity on radicle growth of American ginseng will contribute in evaluating the activity of other phenolic analogs ubiquitous in nature.

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