

## Autotoxicity of peanut and identification of phytotoxic substances in rhizosphere soil

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### ABSTRACT

We evaluated the autotoxicity of peanut (*Arachis hypogaea* L.) leaf, stem and root extracts, and identified the phytotoxic substances accumulated in peanut rhizosphere soil during continuous cropping. We prepared aqueous extracts from peanut leaves, stems, and roots, and evaluated their effects on peanut germination and growth. Soil collected from areas in which peanut had been continuously cultivated for 1, 2, 3, or 4 years was analyzed by HPLC in 2009. Leaf extracts drastically inhibited the seed germination, followed by stem extracts and then root extracts. Root extracts severely inhibited the seedling growth, followed by stem extracts and then leaf extracts. We detected 4-types of phenolic acids (p-hydroxy benzoic acid, vanillic acid, coumaric acid, and coumarin) in peanut rhizosphere soil. After 4- years cropping, the vanillic acid and coumarin contents were 0.289 ug/g dry soil and 0.025 ug/g dry soil, respectively. The phenolic acids concentrations increased during the continuous cropping. After 4- year cropping, the vanillic acid concentration in soil was 12-times more than coumarin. The combined concentration of vanillic acid and coumarin increased during continuous cropping (from 0.067 to 0.314 µg/g dry soil over 4-years). This may be one reason underlying problems with continuous cropping. Thus peanut is a potentially autotoxic specie.

**Keywords:** Allelopathy, *Arachis hypogaea*, autotoxicity, continuous cropping, coumarin, phenolic acids, peanut, vanillic acid.

### INTRODUCTION

Peanut (*Arachis hypogaea* L.) is main vegetable oil crop in China. Hence its area is increasing, continuous cropping is becoming more common; however, peanut is unsuitable for continuous cropping system. In recent years, continuous cropping has caused severe yield losses in China's main peanut production areas. The greater the number of successive planting years, the greater was the yield loss. There are severe problems with continuous cropping (19, 32) and the production mechanisms in continuous cropping are also complex. The specific problems associated with continuous cropping differ among different crops, but the main problems are: (i) degradation of physico-chemical properties of soil, (ii) degradation of biological environmental in soil and (iii) autotoxicity, which is a special form of allelopathy.

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There is increasing evidence that allelopathy plays an important role in agricultural and ecological systems. Allelopathy is any direct or indirect harmful effect of one plant on another through the production of chemical compounds released into the environment (28). Many plants release allelochemicals by exudation, leaching, vaporization and decomposition of biomass. In the rhizosphere, allelochemicals may be toxic or stimulatory to the donor (releaser), to other plant species and to soil microorganisms (12, 21, 24, 30, 41). Autotoxicity is a special form of allelopathy that reduces the growth of the same species through the release of chemical substances into soil or nutrient solution. The effects of autotoxicity often are observed as reduction in crop yield and difficulty in re-establishing plants in the area due to low seed germination and poor seedling growth (31,43). Various toxic compounds that are potentially involved in autotoxicity are localized in the seed coat, fresh leaves, stems, crowns, dry hay, old roots, and soil residues (22). The following groups of compounds, (terpenoids and steroids, phenols, coumarins, flavonoids, tannins, alkaloids, and cyanogenic glycosides) and other compounds produce toxic effects (29). Phenolic acids have been intensively studied with regard to their phytotoxicity and have been suggested to cause autotoxicity problems in continuous cropping systems (7, 26). They are found in wide range of soils, and they are phytotoxic to various plants >1 mM concentrations (9, 13). Numerous studies have shown that phenolic acids are important autotoxic compounds causing problems in continuous cropping systems.

Recent research on autotoxicity has focused on rice, soybean, cucumber, and other crops grown around the world (6). There has also been recent research on autotoxicity of crops grown in China. Previous studies have shown that many crops are potentially autotoxic, but there is no information about whether peanut has autotoxic effects. This study aimed (i). to investigate the autotoxic effects of aqueous extracts of leaves, stems and roots of peanut plants, (ii). to isolate, identify, and quantify phenolic acids, which may be responsible for autotoxicity, from peanut rhizosphere soil. An in-depth understanding of the mechanisms of autotoxic interference in cropping systems will contribute to the development of new, environmentally safe strategies for sustainable agriculture.

## MATERIALS AND METHOD

### I. Plant materials

Seeds from healthy, good quality pods of peanut (*A. hypogaea* L.) variety 'Huayu16' were used in this study. Plants were grown in plastic pots (32 × 25 × 15 cm) in an experimental glass greenhouse (27 ± 5°C, and 65 ± 10% relative humidity) at Shenyang Agricultural University. The soil was sandy loam and contained 12.2 g/kg organic matter, 0.9 g/kg total N, 0.5 g/kg total P, 10.9 g/kg total K, 61.9 mg/kg available N, 6.6 mg/kg available P, 3.9 mg/kg available K; and pH, 5.7. Each pot containing 10 kg soil was applied (0.5 g N, 1 g P<sub>2</sub>O<sub>5</sub>, 1 g K<sub>2</sub>O through fertilizers). Fresh plant materials were harvested at the blossom stage and immediately washed with tap water to remove any soil or other material. Plants leaves, stems, and roots were separated and stored at -20°C until analysis.

## II. Preparation of *A. hypogaea* aqueous extracts

Fresh leaves stems, and roots of *A. hypogaea* harvested at the blossom stage were cut into 1-2 cm long pieces. Each plant part was extracted separately. For extraction, 16 g plant material was extracted in 100 mL distilled water for 24 h on an orbital shaker at room temperature ( $25 \pm 1.0^\circ\text{C}$ ). Extracts were strained through two layers of cheesecloth to remove solid materials and then centrifuged at 5,000 g for 20 min. The supernatant was filtered through two layers of Whatman No. 1 filter paper. This extract was designated as the full-strength concentration (16 g/100 cc water), and was diluted with distilled water to obtain concentrations of 4, 8, and 16% (w/v) and kept in a refrigerator at  $4^\circ\text{C}$  until further testing. The pH of extracts was 6.8 to 7.4, (non-inhibitory to plant growth).

## III. Effect of aqueous extracts on germination of *A. hypogaea*

Twenty uniform *A. hypogaea* seeds were surface-sterilized in 5% sodium hypochlorite aqueous solution for 2 min, then washed 5-times with distilled water and dried between two paper towels. Then, the seeds were placed in separate Petri dishes lined with 15 cm Whatman No. 1 filter paper and 2 ml extract solution from each plant part or distilled water (as control) was added to each Petri dish. Each treatment consisted of three replicates. All Petri dishes were covered and kept in dark in incubator at  $25 \pm 1^\circ\text{C}$ . The number of germinated seeds was counted daily and 2 mL distilled water was added. Seeds were considered to be germinated when the emergent radical reached the half length of the seed. After 5-days, the germination potential (GP, in %) was determined. After 7-days, the germination percentage, germination index (GI) and root length (RL, in cm) were calculated.

$$\text{Germination (\%)} = n / N \times 100 \quad (\text{i})$$

$$\text{Germination potential (\%)} = m / N \times 100 \quad (\text{ii})$$

$$\text{Germination index} = \sum (Gt/Dt) \quad (\text{iii})$$

Where, N: Total number of seeds per replication, n: Number of germinated seeds after 7 days, m: Number of germinated seeds after 5 days, Dt : Number of days from the beginning of experiment and Gt : Number of germinated seeds on day Dt.

## IV. Effect of aqueous extracts on growth of *A. hypogaea* seedlings

Sandy soil cultures were used to examine the autotoxicity of *A. hypogaea* aqueous extracts to growth of peanut seedlings. To prepare the cultures, soil (170 g) was placed in a plastic cup (200 mL) and mixed thoroughly with 25 mL of each extract solution prepared from different parts of peanut plants or distilled water (control). Then, 20 uniformly germinated peanut seeds were selected and one germinated peanut seed was planted in each cup. Each treatment consisted of 3-replicates. Each cup was irrigated with 100 mL Hoagland's nutrient solution. Nutrients were maintained daily by adding 10-20 mL Hoagland's nutrient solution to avoid immobilization of nutrients. Distilled water was also added as necessary during the growth period. All treatments were kept in an air-conditioned room ( $25 \pm 1^\circ\text{C}$  and 16-h light/8-h dark photoperiod). At the seedling stage, we determined root length (RL in cm), shoot length (SL in cm), fresh weight (W in g), dry

weight (DW in g) and leaf area (LA in cm<sup>2</sup>). RL and SL were calculated from the base of the seed to the root tip and shoot tip, respectively, using a ruler ( $\pm 0.1$  cm). DW was determined by drying all components in an oven at 70°C for 48 h. Fresh and dry biomass was weighed using a digital balance ( $\pm 0.001$  g). LA was measured using a leaf area meter (CID CI-203).

#### V. Detection of phenolic acids in rhizosphere soil

**Plant materials:** These experiments were conducted from 2006 to 2009 at the Academy of Agricultural Sciences, Experiment Station, Zhanggutai, Liaoning Province. The soil type in this area is Aeolian soil, which is main soil type in agricultural production in northwest Liaoning. The field soil was of medium fertility (7.5 g/kg organic matter, 0.6 g/kg total N, 0.3 g/kg total P, 25.5 g/kg total K, 59.9 mg/kg available N, 1.98 mg/kg available P, 69.3 mg/kg available K). The test field had adequate irrigation and drainage. The experiments included four treatments: (i). 1-year cropping, (2009), (ii). 2-years cropping (2008-2009), (iii). 3-years cropping (2007-2009) and (iv). 4-years cropping (2006-2009). All treatments were randomly arranged and each treatment consisted of three replicates, making 12 test plots in total. Each plot was 8.88 m wide and 100 m long. The planting density in plots was  $1.18 \times 10^4$  plants/888m<sup>2</sup> (i.e. plant to plant spacing was 17 cm and row to row spacing 44 cm, respectively).

**Collection of soil samples:** Four soil samples were collected when plants reached the blossom stage in 2009. The soil samples were sieved (4-mm mesh sieve), air dried and stored at room temperature in the laboratory as per Dalton *et al.* (10). For each soil sample, 20 g soil was added to 20 mL distilled water and agitated for 2 h on a reciprocal shaker. The soil suspension was centrifuged at 8,000 g for 10 min and the supernatant was filtered through filter paper (Whatman No. 1) and then dried in a rotary evaporator at 51°C. The residue was dissolved in 2 mL ultra pure water. Each soil sample was processed in duplicate and the final extracts were analyzed by high-performance liquid chromatography (HPLC).

**HPLC analysis:** The concentration of autotoxic compounds in soil samples was determined using an HPLC system (Agilent 1100) equipped with a DAD detector. All separations were performed on a Shiseido ODS-C<sub>18</sub> column (250 mm  $\times$  2.1 mm, 5  $\mu$ m) with a constant flow rate of 1.0 mL/min. Eluted compounds were detected at 280 nm. The injection volume was 10  $\mu$ L and the column temperature was maintained at 35°C. HPLC separations were conducted using the following solutions: mobile phase A was methanol, and mobile phase B was 0.5% formic acid (methanol: 0.5% formic acid, 25:75). The concentration of each compound in the soil samples was determined by comparison of peak areas to those of external standards. The concentrations of compounds are shown as microgram per gram of dry soil.

Five phenolic acid standards were purchased from Acros Organics (<http://www.acros.com/>). These were p-hydroxy benzoic acid (506842), benzoic acid (AP-001N), vanillic acid (555794), coumarin (ALR-011N), and coumaric acid (C0391). Vanillin (high purity reagent) was purchased from Sinopharm Chemical Reagent Co. Ltd (<http://sinoreagent.cn.alibaba.com/>).

A series of solutions containing six kinds of phenolic acid standards at appropriate concentrations in distilled water were prepared for the construction of calibration curves. The standard curves were constructed by plotting peak area versus concentration of the solutes and the limit of detection of different standards were concluded (Table 1). The recovery was measured to determine the accuracy of the proposed assay, the six kinds of phenolic acid standards' recoveries were: p-hydroxy benzoic acid (90.4%), Benzoic acid (89.6%), Vanillic acid (90.1%), Coumarin (98.4%), Coumaric acid (89.1%), Vanillin (91.3%). HPLC chromatogram includes 6-kinds of phenolic acids (Figure 1).

Table 1. Calibration curves of the standards chosen under HPLC condition

Standards	Calibration curve	Regression coefficient	Method Detection limitation ( $\mu\text{g}$ )
p-hydroxy benzoic acid	$Y = 829.83X - 0.743$	0.996	0.01
Benzoic acid	$Y = 272.73X + 0.252$	0.999	0.01
Vanillic acid	$Y = 504.084X - 2.165$	0.995	0.025
Coumarin	$Y = 2305.532X - 6.89$	0.998	0.005
Coumaric acid	$Y = 829.83X - 0.743$	0.999	0.01
Vanillin	$Y = 1680.965X - 5.90$	0.998	0.005

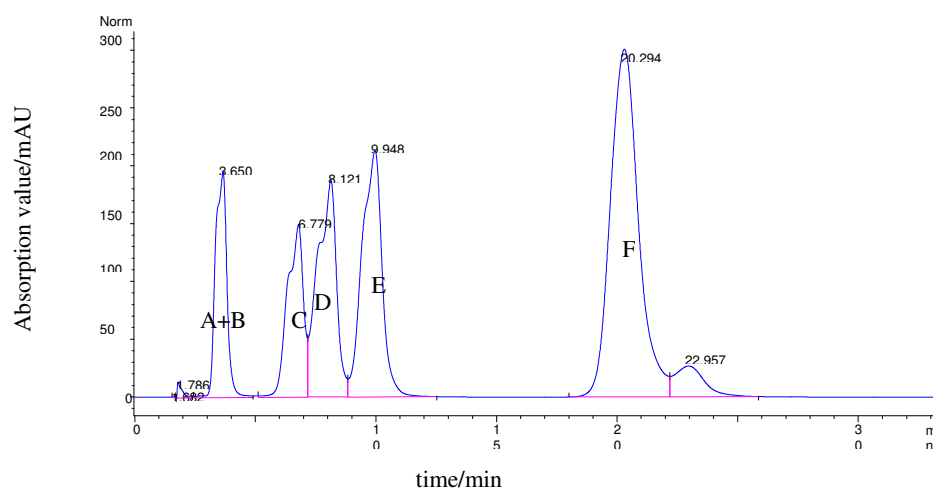


Figure 1. Chromatogram of 6 phenolic acids. A: Benzoic acid B: Coumaric acid C: p-hydroxy benzoic acid D: Vanillic acid E: Vanillin F: Coumarin

### Statistical analyses

The response index (RI) referred to in this paper was that put forward by Williamson (38). This index is a measure of the autotoxic effects of aqueous extracts from different plant parts of peanut plants.

$$\text{RI} = 1 - C/T \quad \text{when } T \geq C$$

$$\text{RI} = T/C - 1 \quad \text{when } T < C$$

Where C: Control value and T: Treatment value. When  $RI > 0$ , Extract has promoting effect; when  $RI < 0$ , Extract has inhibitory effect. The control RI is 0. All data were subjected to one-way ANOVA and means were compared with least significant difference (LSD) and Duncan's multiple range test at the 5% level. All statistical analyses were conducted using SPSS (version 11.5).

## RESULTS

### Seed germination

All aqueous extracts from different plant parts of *A. hypogaea* L. affected the peanut seed germination (Table 2). At all concentrations, the aqueous extracts from different plant parts reduced the germination (%), germination potential, germination index, and root length, compared with water control. Thus, the extracts inhibited the peanut seed germination. At the lowest extract concentration (4%), root, stem, and leaf extracts reduced germination by 14, 10, and 3%, respectively. At the highest extract concentration (16%), root, stem, and leaf extracts reduced germination by 33, 14, and 60%, respectively. At the lowest extract concentration (4%), root, stem, and leaf extracts reduced germination potential by 0, 66, and 66%, respectively. At the highest extract concentration of 16%, root, stem and leaf extracts reduced germination potential by 20, 94, and 100% respectively. The germination index and root length results showed similar trends. These results confirm that the inhibitory effects of peanut plant extracts on seed germination were concentration-dependent. The autotoxic effects of aqueous extracts from different plant parts of peanut plants are shown in Fig. 2. The inhibitory effect was greater with increasing concentration of the aqueous extracts. The autotoxic effects of aqueous extracts from different parts of peanut plants followed the order: leaf > stem > root. That is, leaf extracts were most inhibitory to seed germination than other extracts.

Table 2. Effects of aqueous extracts from different parts of *A. hypogaea* L. on seed germination indices (Petri plate)

Aqueous extract Conc (%)	Germination (%)	Germination Potential (%)	Germination Index	Root Length (cm)
Control	0.70±0.29a	0.35±0.18a	2.95±1.10a	3.57±1.14a
<b>Root extracts</b>				
4	0.60abc	0.35±0.09a	2.67±0.98ab	2.11±0.34b
8	0.60abc	0.30±0.07a	2.63±0.95ab	2.08±0.34b
16	0.47±0.12c	0.28±0.08a	2.09±0.83bc	1.83±0.21bc
<b>Stem extracts</b>				
4	0.63±0.12ab	0.12±0.05b	2.14±0.84bc	1.97±0.13bc
8	0.67±0.06a	0.08±0.01b	2.18±0.85bc	1.86±0.13bc
16	0.60±0.09abc	0.02±0.01b	1.84±0.67c	1.54±0.04bc
<b>Leaf extracts</b>				
4	0.68±0.13a	0.12±0.02b	2.30±0.77abc	1.69±0.19bc
8	0.62±0.14ab	0.08±0.01b	2.04±0.71bc	1.55±0.12bc
16	0.28±0.13d	0b	0.86±0.29d	1.31±0.26d

Note: Different letters indicate significant differences among treatments at the 5% level.

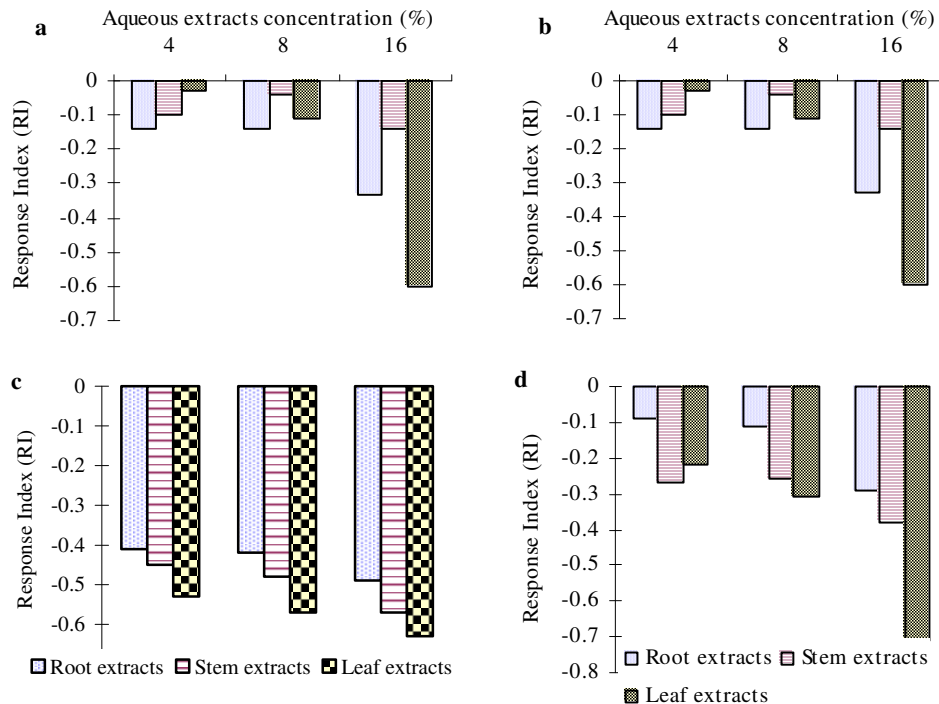


Figure 2. Autotoxic effects of aqueous extracts from different parts of *A. hypogaea* L. on seed germination indices. a: Germination; b: Germination potential; c: Germination index; d: Root length.

### Seedling growth

The aqueous extracts from different parts of peanut plants inhibited the seedling growth (Table 3). At all concentrations, the aqueous extracts from different plant parts decreased the shoot length, root length, dry weight, and leaf area compared to water control. Therefore, the extracts had an inhibitory effect on the seedling growth, the higher concentrations of extracts were more inhibitory (Figure 3). However, there were no significant differences ( $P < 0.05$ ) on shoot length and leaf area of seedlings treated with leaf extracts. At the lowest extract concentration (4%), root, stem, and leaf extracts reduced root length by 26, 22, and 7%, respectively. At the highest extract concentration (16%), these extracts reduced root length by 29, 27, and 22%, respectively. Similar to root length, all extracts at all concentrations significantly decreased the seedlings dry weight, and the effect was concentration-dependent. At the lowest extract concentration (4%), root and stem extracts reduced shoot length by 39 and 29%, respectively. At the highest extract concentration (16%), root and stem extracts reduced shoot length by 44 and 32%, respectively. The shoot length of seedlings was not affected by the leaf extracts. Similar to shoot length, increasing concentrations of root and stem extracts significantly decreased

Table 3. Effects of aqueous extracts from different parts of peanut on seedling growth (Pot culture)

Aqueous extract Conc (%)	Stem Length (cm)	Root Length (cm)	Dry Weight (g/Plant)	Leaf Area (cm <sup>2</sup> )
Control	17.63±2.04a	20.07±4.00a	0.97±0.13a	30.31±6.71a
<b>Root extracts</b>				
4	10.70±0.91bc	14.78±4.58bc	0.84±0.19abc	12.45±3.87b
8	10.11±2.12c	14.21±2.49c	0.78±0.19bc	11.86±4.74b
16	9.92±1.21c	14.32±5.32c	0.71±0.06bc	11.06±3.85b
<b>Stem extracts</b>				
4	12.59±1.61b	15.67±4.26bc	0.90±0.21ab	16.20±3.76b
8	11.87±3.04bc	15.31±2.11bc	0.88±0.30ab	15.96±3.59b
16	12.03±1.50bc	14.72±2.90bc	0.80±0.28abc	13.90±3.80b
<b>Leaf extracts</b>				
4	17.26±2.44a	18.58±3.62ab	0.96±0.17a	28.68±6.91a
8	16.73±1.93a	17.49±4.31abc	0.85±0.08abc	27.28±4.97a
16	16.10±2.98a	15.60±5.08bc	0.84±0.08abc	26.37±9.20a

Note: Different letters indicate significant differences among treatments at the 5% level.

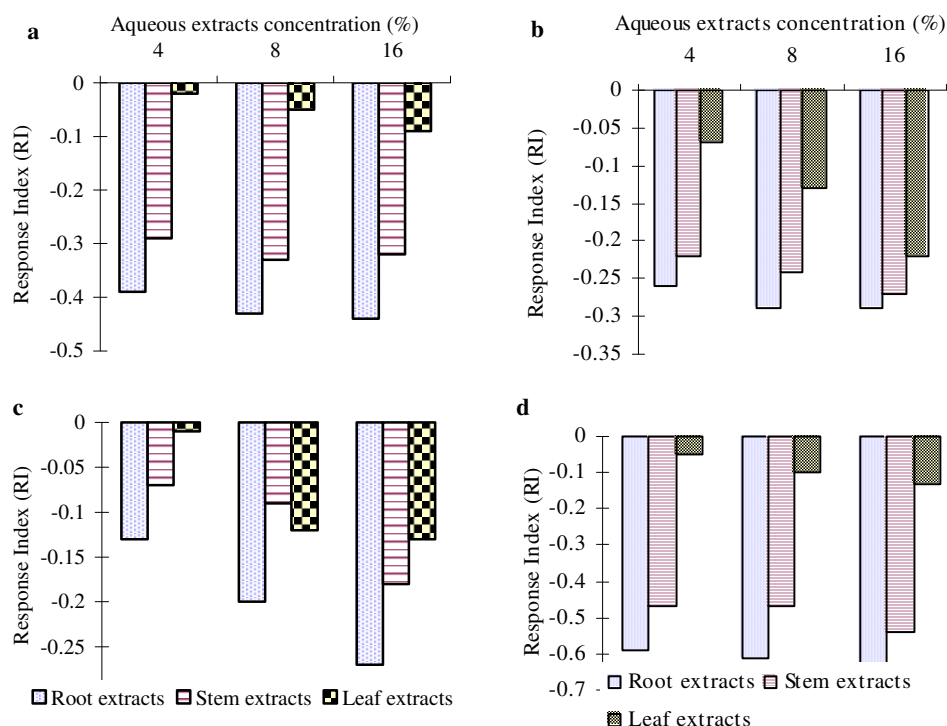


Figure 3. Autotoxic effects of aqueous extracts from different parts of peanut on seedling growth. a: Shoot length; b: Root length; c: Dry weight; d: Leaf area.

seedling leaf area, but the leaf area of seedlings were less affected by leaf extracts. These results confirmed that aqueous extracts from peanut plant parts showed concentration-dependent inhibitory effects on peanut seedling growth. The data indicated that autotoxic effects of aqueous extracts from different plant parts of *A. hypogaea* L. followed the order: root > stem > leaf. This differed from the result obtained for seed germination; however, seedling growth was slightly inhibited by leaf extracts.

#### Phenolic acids found in peanut rhizosphere soil

The main phenolic acids found in peanut rhizosphere soil in different cropping years were: p-hydroxy benzoic acid, vanillic acid, coumaric acid, and coumarin. Before peanut sowings, the phenolic acids concentration was near to zero i.e. < detection limits. Vanillic acid and coumarin were present in higher concentrations than the other two phenolic acids, and their concentrations increased during 4-years of continuous cropping (Table 4). The vanillic acid concentration was 0.059 µg/g dry soil after 1- year cropping and became 4.9-times greater (0.289 µg/g dry soil) after 4- years cropping. The coumarin concentration was 0.008 µg/g dry soil after 1-year cropping and increased to 3.12-times higher (0.025 µg/g dry soil) after 4- years cropping. The vanillic acid concentration in soil after 4-years cropping was greater than coumarin, e.g. the vanillic acid concentration was 11.56-times higher than coumarin. The total concentration of these two phenolic acids after 4- years cropping was 0.314 µg/g dry soil, which was 4-Times higher than their combined concentration (0.067 µg/g dry soil) after 1- year cropping.

Table 4. Types and concentrations of phenolic acids in peanut rhizosphere soil after 4-years continuous cropping

Cropping years	Vanillic acid (µg/g dry soil)	Coumarin (µg/g dry soil)	Total (µg/g dry soil)
1-Year cropping (2009)	0.056±0.002	0.008	0.067±0.003
2-Years cropping (2008-2009)	0.132±0.010	0.019±0.001	0.151±0.011
3-Years cropping (2007-2009)	0.187±0.050	0.021±0.001	0.208±0.051
4-Years cropping (2006-2009)	0.289±0.060	0.025±0.001	0.314±0.061

## DISCUSSION

Owing to its importance in agriculture, autotoxicity has been studied in many crops (1, 2, 15, 23, 33, 39, 42). In this study, the aqueous extracts from peanut plants had inhibitory effects on seed germination and seedling growth, and the degree of inhibition increased with increasing extract concentration. Thus under controlled laboratory conditions, *A. hypogaea* shows allelopathic potential against itself. Thus, it is a potentially autotoxic species.

The aqueous extracts of different peanut plant parts showed different degrees of phytotoxicity. These results confirm previous reports that autotoxic effects of aqueous extracts differ depending on the plant part they are derived from (4,8). Such differences might be related to greater quantities of allelopathic compounds in certain tissues, imparting a higher level of autotoxicity. The release of phytotoxic compounds could also be affected by tissue type. Different plant tissues including leaves, stems, roots, and seeds

show allelopathic potential (25, 37); however, this potential varies among different tissues (16). Leaves are the most consistent source of chemicals involved in phytotoxicity, while roots contain fewer and less potent toxins (3, 11, 27). In contrast, Oueslati I (23) concluded that barley (*Hordeum vulgare* L.) stem extracts were more inhibitory to radicle growth of test plants than leaf or root extracts. However, our results showed that leaf and stem extracts most strongly inhibited the seed germination, while root extracts were most inhibitory to seedling growth. Further research is required to investigate the reasons for this difference. One explanation may be that proposed by Kobayashi (18), who argued that differences in sensitivity to phytotoxic substances among various plant species under laboratory conditions depends on the physiological and biochemical characteristics of each species. The results of this study showed that peanut plant extracts have autotoxic effects, this may be one of the reasons for the problems associated with continuous cropping of peanut.

Previous studies showed that some cropping soil extracts showed phytotoxic effects. There have been a number of reports on the accumulation of phenolic acids in cropping soil (14, 17, 34, 40). Whether continuous cropping can significantly alter the types and concentrations of phenolic acids in soil is unclear. Therefore, research on phenolic acids in soil is one way to explore the mechanisms underlying the problems in continuous cropping. We analyzed peanut rhizosphere soil collected over 4-years of continuous cropping, focusing on the following phenolic acids: p-hydroxy benzoic acid, vanillic acid, coumaric acid and coumarin. The concentrations of vanillic acid and coumarin were higher than those of other compounds and their concentrations increased over the 4 cropping years. In contrast, there were low concentrations of p-hydroxy benzoic acid and coumaric acid in the soil, and their concentrations did not change markedly over time. It is worth noting that the content of vanillic acid and coumarin increased with greater number of cropping years. This implies that substances from peanut residues may accumulate over time and become more toxic in the cropping soil. We found only two main kinds of phenolic acids in peanut rhizosphere soil, and they were not present at high concentrations. However, various types of phenolic acids are abundant in the rhizosphere soil of other crops. Therefore, although we identified two main phenolic acids, there are likely to be many other types of allelochemicals in peanut rhizosphere soil, and further research is required to identify and quantify them. It is difficult to analyze allelochemicals in root exudates without disrupting the roots. In addition, allelochemicals exuded from roots are difficult to analyze as they are present in very small quantities. Several studies have analyzed active allelochemicals in root exudates in controlled laboratory conditions; however, such systems cannot mimic the complexities of field conditions (35,36). It is preferable to analyze allelochemicals using systems in which plants are grown in soil, since allelopathy occurs in soil. Even if some chemicals are present at high concentrations in root exudates, this does not mean that they are able to exert allelopathic effects in soil under natural conditions (20).

Plants can release allelochemicals into the environment by exudation, leaching, vaporization and decomposition and so there are many sources of allelochemicals in the soil. It is difficult to identify the allelochemicals in the soil, because they are easily absorbed and/or transformed into different forms via the activity of microorganisms. This can alter their allelopathic properties. The structure and the physicochemical properties of soil are significantly correlated with its ability to retain and absorb allelochemicals. Soil

pH can also indirectly affect the production and degradation of allelochemicals. In addition to the two factors above, soil microbial decomposition also plays an important role in the activity of allelochemicals. Some phenolic acids are decomposed or consumed by microorganisms, and therefore, the phenolic acids cannot accumulate to sufficient levels to exert phytotoxic effects. In the present study, our results showed that there were low concentrations of phenolic acids in rhizosphere soil of peanut. There may be several reasons for these low concentrations of phenolic acids. Therefore, further research is underway to further analyze the soil to determine whether other phenolic acids are present, and whether they affect the growth of peanut plants and/or the microbial community in the soil.

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