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Effects of Allelochemicals on Root Growth and Pod Yield in Response to Continuous Cropping Obstacle of Peanut

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ABSTRACT

Continuous cropping (CC) obstacle is a major threat in legume crops production; however, the underlying mechanisms concerning the roles allelochemicals play in CC obstacle are poorly understood. The current 2-year study was conducted to investigate the effects of different kinds and concentrations of allelochemicals, p-hydroxybenzoic acid (H), cinnamic acid (C), phthalic acid (P), and their mixtures (M) on peanut root growth and productivity in response to CC obstacle. Treatment with H, C, P, and M significantly decreased the plant height, dry weight of the leaves and stems, number of branches, and length of the lateral stem compared with control. Exogenous application of H, C, P, and M inhibited the peanut root growth as indicated by the decreased root morphological characters. The allelochemicals also induced the cell membrane oxidation even though the antioxidant enzymes activities were significantly increased in peanut roots. Meanwhile, treatment with H, C, P, and M reduced the contents of total soluble sugar and total soluble protein. Analysis of ATPase activity, nitrate reductase activity, and root system activity revealed that the inhibition effects of allelochemicals on peanut roots might be due to the decrease in activities of ATPase and NR, and the inhibition of root system. Consequently, allelochemicals significantly decreased the pod yield of peanut compared with control. Our results demonstrate that allelochemicals play a dominant role in CC obstacle-induced peanut growth inhibition and yield reduction through damaging the root antioxidant system, unbalancing the osmolytes accumulation, and decreasing the activities of root-related enzymes.

KEYWORDS

Peanut; continuous cropping obstacle; root growth; pod yield

1 Introduction

Legume crops belong to the Leguminosae or Fabaceae family and rank third in global crop production after cereal and oilseed crop species [1,2]. Legume crops are also key components of healthy diets and

productive crop rotations, which play important roles in global agricultural productivity and food security [3–6]. Continuous cropping (CC) is the concept that the same crop species or its relatives is grown on the same area of land over long-term (Long-term monoculture). CC obstacle is a global challenge that severely affects the growth and causes the degeneration of soil, induction of serious diseases, and decline in yields of legume crops even if the field management regime has been properly adopted [7–9]. CC obstacle, of which land degradation is considered a common type, is usually caused by overcultivation [10–12]. From the perspective of land, long-term CC could affect the microbial community structure and diversity in the soil which might lead to obstacle [13–15]. Consequently, CC obstacle could alter soil properties and ecological functions and ultimately prohibit the healthy growth and development of crop species especially legumes. However, the mechanisms regarding the side effects of CC obstacle in legume crops production are poorly understood.

In the past decades, the remediation effects on CC obstacle have been extensively investigated especially in legume crops [16–18]. Recently, Zeng et al. [15] reported that CC obstacle was ameliorated by intercropping patchouli with turmeric or ginger, probably through improving soil bacterial metabolism and the activities of soil enzymes. In another study, CC of strawberry led to three phases of changes in abiotic and biotic soil factors. Notably, the accumulation of phenolic acids have been demonstrated to be vital chemicals that significantly inhibited strawberry growth [10]. However, strategies in response to CC obstacle on legume crops especially in peanut production still remain elusive.

Allelochemicals are major components of root exudates in legume crops that have multiple biological functions. Most of the allelochemicals are secondary products released from plant root excretions or plant residues, such as phenolic acids, luteolin, and resveratrol, which severely accumulate in the soil of legumes CC systems [19–21]. Experiments indicated that allelochemicals were a group of promising compounds that could be taken as weed inhibitors due to their environmentally friendly properties [22–25]. As major components of allelochemicals induced by allelopathic effects, phenolic acids could influence the growth, productivity, and yield of the crops [26,27]. The underlying mechanisms concerning the allelopathic effects on crops including the inhibition of photosynthesis and enzyme action, the suppression of hormone activity, and the reduction of root growth and ion uptake rate [28–30]. To date, the mechanisms governing the roles allelochemicals play in CC obstacle of legume crops have not been determined in detail. In this regard, conducting research on the effects of allelochemicals especially phenolic acids on peanut growth and yield formation has important theoretical and practical significance for both scientific research and combating CC obstacle.

Therefore, the present study was carried out to interpret the causes of CC obstacle in relation to the allelochemicals (phenolic acids) in peanut growth and to clarify the physiological mechanisms governing this process. We hypothesize that allelochemicals might have negative effects on peanut growth, root physiology, and yield formation. Using different concentrations and kinds of allelochemicals, we investigated the changes in morphological characters, antioxidant systems, osmolytes accumulation, activities of NR and ATP, and nutrient content in peanut roots. The pod yield and yield components exposed to various allelochemicals were also compared with the control to test our hypothesis. This study revealed the underlying mechanism involved in allelochemicals on peanut root growth, thereby contributing to a better understanding of the involvement of allelochemicals in CC obstacle of legume crops.

2 Materials and Methods

2.1 Plant Materials and Growth Conditions

Peanut (*Arachis hypogaea* L.) cultivar Huayu 22 was used in this experiment. The 2-year pot-grown experiments were conducted at Yinmaquan experimental station, Jinan, China (36°43'15''N, 117°5'50''E) in 2016 and 2017. The seeds of uniform sizes were selected and soaked in a 2% (v/v) sodium hypochlorite for 15 min, and then thoroughly rinsed in sterile distilled water. The seeds were planted in

polystyrene pots (with 28 cm inner diameter, 42 cm depth, and small holes at the bottom, each pot contained six seeds) on 12 May 2016 and 14 May 2017. Each pot was filled with 18 kg of garden soil (obtained from 0 to 20 cm below the soil level) and basal synthetic fertilizers of 975 kg hm^{-2} (N:P$_2$O$_5$:K$_2$O = 1:1.5:1.5) before sowing. The main physical and chemical properties of the garden soil were as follows: pH, 7.02; bulk density, 1.21 g/cm^3; organic matter, 22.8 g/kg; nitrogen, 85.6 mg/kg; phosphorous, 21.4 mg/kg; and potassium, 81.3 mg/kg. The soil was initially free from uncontrolled allelochemicals as verified by the method of gas chromatography-mass spectrometry. After sowing, each pot was filled with 2 L of distilled water and covered with plastic film. All the pots were placed in a greenhouse with the following conditions: photoperiod of 14/10 h (light/dark), photosynthetic photon flux density (PPFD) of 1200 μmol m^{-2}s^{-1}, air temperature of 25/18°C (day/night), and air humidity of 60%. Each pot was watered with 500 mL distilled water every three days. On 01 June 2016 and 03 June 2017, the seedlings were thinned out with one strong seedling left in each pot. At four-leaf stage, pots with similar-looking seedlings were selected for the subsequent experiments.

2.2 Experimental Design

The phenolic acid allelochemicals treatments were exposed to the seedlings on 13 June 2016 and 15 June 2017 (four-leaf stage). In order to simulate the actual CC obstacle in peanut production, the concentrations of allelochemicals used in this study were based on our preliminary experiments that were identical to those in the root exudates of peanut when continuous cropping for 5 successive years [31]. Each pot was well watered with 2 L of distilled water (CK), 360 μg L^{-1} of p-hydroxybenzoic acid (H1), 720 μg L^{-1} of p-hydroxybenzoic acid (H2), 360 μg L^{-1} of cinnamic acid (C1), 720 μg L^{-1} of cinnamic acid (C2), 360 μg L^{-1} of phthalic acid (P1), 720 μg L^{-1} of phthalic acid (P2), 360 μg L^{-1} of the mixture of H1, C1, P1 (4:10:7, w:w:w) (M1), and 720 μg L^{-1} of the mixture of H2, C2, P2 (4:10:7, w:w:w) (M2). The measurements and samples were taken at 75 d after emergence unless otherwise stated. In total, 9 treatments were composed with three replicates, and each replicate consisted of 12 plants.

2.3 Agronomic Characteristics Analysis

The height of the main stem was firstly determined from the base to the top of the main stem of the peanut plant. Meanwhile, the number of branches and the length of the lateral stems were also investigated. Then the samples were separated into stems and leaves, oven-heated at 105°C for 30 min to deactivate enzymes, and dried to constant weight at 75°C before the dry weights were recorded [32].

2.4 Antioxidant Enzyme Activity and MDA Content Assay

In order to assay the activities of antioxidant enzymes, the root samples (0.5 g) were immediately ground with 5 mL 25 mM HEPES-NaOH buffer (pH 7.8) using pre-chilled mortar and pestle. The main components of the buffer were as follows: 20% (v/v) glycerol, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ascorbic acid (AsA), 1 mM dithiothreitol (DTT), 5 mM MgCl$_2$, and 1 mM reducing glutathione (GSH). The homogenates were centrifuged at 12,000 g and 4°C for 20 min using a centrifuge (5810R, Eppendorf, Germany), then the supernatants were collected for the determination of the enzymatic activity. All of the parameters were determined using a spectrophotometer (UV-2401, Shimadzu, Japan). According to Bradford [33], the protein content was determined prior to the determination of antioxidant enzymes. Superoxide dismutase (SOD) activity was determined by the measurement of the reduction of nitro-blue tetrazolium at 560 nm according to Stewart et al. [34]. Peroxidase (POD) activity was assayed by taken guaiacol oxidation as a substrate at 470 nm as modified by the method of Cakmak et al. [35]. Catalase (CAT) activity was measured as a decline at 240 nm based on the method of Patra et al. [36]. The lipid peroxidation was determined according to the thiobarbituric acid (TCA) reaction through determination of the malondialdehyde (MDA) content as mentioned by Hodges et al. [37]. MDA content was calculated at 532 nm by subtracting the absorbance at 600 nm for non-MDA compounds.

2.5 Root Morphology and Activity of Root System Assay

The root morphological characters were analyzed as modified by the method of Jiang et al. [38], the fresh roots were carefully washed twice with distilled water, and then scanned with a dual lens scanning system (Epson V700, Nagano-ken, Japan). The root images were analyzed with WinRHIZO software (Regent Instruments, Canada). The data of the total root length, average root diameter and root surface area were collected. Then the root samples were over-dried at 80°C for 2 d to get the root dry weight data.

The activity of root system was evaluated at 45, 75, and 105 d after emergence as previously described by Ruf et al. [39] and Begum et al. [40]. The fresh root samples were taken immediately and determined using the triphenyl tetrazolium chloride (TTC) method and the root activity was expressed as the deoxidization ability of TTC with the formula: TTC reducing intensity ($\mu g\ TTC\ g^{-1}\ h^{-1}$) = TTC reduction mass ($\mu$g)/Root weight (g) × time (h).

2.6 NR and ATPase Activity Assay

The nitrate reductase (NR) activity was assayed at 45, 75, and 105 d after emergence with some modifications as originally described by Scheible et al. [41]. Briefly, frozen leaf tissues (0.3 g FW) were ground in 100 mM phosphate buffered solution (pH 7.5). The determination was started by mixing one part of extract with five parts of reaction buffer (0.25 mM NADH, 5 mM KNO_3) and incubated at 25°C for 30 min. The reaction was then stopped by adding 0.1 M zinc acetate. After reaction for 15 min, the tubes were centrifuged at 13,000 g for 10 min. The nitrite produced was then measured by adding 1 mL of 1% (w/v) sulfanilamide in 3 M HCl plus 1 mL of 0.02% (v/v) N-(1-naphthyl)-ethylenediamine in distilled water using spectrophotometry analysis at 520 nm.

The ATPase activity was determined at 45, 75, and 105 d after emergence as previously described [42,43] with some modifications. This method was to determine the Pi released by the microsomal ATPase during ATP hydrolysis after incubation in the assay medium (50 mM BTP-Cl (pH 6.5), 100 mM KCl, 5 mM BTP-ATP, and 5 mM $MgSO_4$) at 30°C for 30 min. Then the reaction solution was determined using a spectrophotometer at 820 nm.

2.7 Determination of the Contents of the Total Soluble Sugars and Total Protein

For the assay of the contents of the total soluble sugars and total protein, the third fully expanded leaves on the main stem grown in the same positions were firstly oven-dried for 15 min at 105°C and then 85°C for 3 d. Then the samples were ground in a high-speed ball mill (MM400; Retsch, Haan, Germany) and mixed thoroughly. The root instances (0.1 g) were extracted twice with 80% (v/v) ethanol at 80°C for 10 min and centrifuged at 3,000 × g for 30 min. The supernatant was then collected (repeated three times) in a 30 ml glass tube and the contents of soluble sugars and free amino acids were measured consequently. The total soluble sugar content was assayed at 620 nm using anthrone method according to the method of Buysse et al. [44]. The total protein content was determined following the trichloroacetic acid (TCA) propanone sediment method as described by Si et al. [45].

2.8 Determination of the Nutrient Uptake

Dried materials from harvests were weighed and ground in a high-speed ball mill (MM400; Retsch, Haan, Germany) and sifted through a 0.5 mm screen for the analysis of total nitrogen, total phosphorus, and total potassium. After slaking with nitric-perchloric acid (5:3), the total nitrogen concentration was analyzed by the micro-Kjeldahl method according to Aljazairi et al. [46]. After acid digestion, the concentration of phosphorus was quantified by colorimetry following the method of Chi et al. [47]. The potassium concentration was determined by using an atomic adsorption spectrophotometer based on the protocol of Guo et al. [4]. Concentrations of all nutrients were expressed on a dry weight percentage basis.

2.9 Pod Yield and Yield Components

The peanut pods were manually harvested upon maturity on 26 September 2016 and 27 September 2017. The pod yield and yield components of peanut including pods number per plant, 100-seed weight and shelling rate were measured for all plants in the sampling area after sun-drying for 15 d.

2.10 Statistical Analysis

The experimental design was a completely randomized block design with three replicates. Statistical analysis of the bioassays was performed using the SPSS statistical package, and differences between the treatment means were compared by Tukey's test at a level of $P < 0.05$.

3 Results

3.1 Effect of Allelochemical Treatment on Peanut Agronomic Characteristics

To examine the effect of allelochemicals on peanut growth, we determined the agronomic characteristics of peanut seedlings. As shown in Table 1, both the low (360 µg L^{-1}) and high (720 µg L^{-1}) concentration of p-hydroxybenzoic acid (H), cinnamic acid (C), phthalic acid (P), and their mixtures (M) induced a substantial decrease in the height of the main stem, the number of branches, and the length of the lateral stem in peanut seedlings. Meanwhile, allelochemical treatment also significantly decreased the dry weight of the stems and leaves in peanut seedlings (Table 1). Notably, high allelochemical treatment such as H2, C2, P2, and M2 induced higher reduction of these agronomic characteristics as compared with low allelochemical treatment. Particularly, plants treated with M2 displayed severe reduction in height of the main stem (38.4% in 2016 and 38.5% in 2017), number of branches (33.3% in 2016 and 35.3% in 2017), length of the lateral stem (36.3% in 2016 and 34.4% in 2017), dry weight of the stems (37.4% in 2016 and 40.3% in 2017), and dry weight of the leaves (42.5% in 2016 and 42.9% in 2017) when compared with those in control.

Table 1: Effects of allelochemical treatment on peanut agronomic characteristics

Year	Treatment	Height of the main stem (cm)	Number of branches	Length of the lateral stem (cm)	Dry weight of the stems (g)	Dry weight of the leaves (g)
	CK	34.9a	15a	36.6a	12.23a	11.06a
	H1	28.9c	12bc	31.4c	9.26c	8.36c
	H2	26.9d	10c	27.8d	8.23e	7.01ef
	C1	29.2c	12bc	31.9c	9.37c	8.45c
2016	C2	27.2cd	10c	28.5d	8.29e	7.08ef
	P1	31.3b	13b	34.8b	9.98b	8.91b
	P2	27.7cd	10c	29.7c	8.43de	7.14e
	M1	25.9d	12bc	27.1d	8.76d	7.86d
	M2	21.5e	10c	23.3e	7.66f	6.36f
	CK	38.2a	17a	39.2a	13.44a	12.41a
	H1	31.6c	14ab	34.5b	10.19c	9.28c
	H2	29.4e	12bc	30.6d	9.05e	7.81e
	C1	32.0c	13abc	35.1b	10.29c	9.21c

(Continued)

Table 1 (continued)

Year	Treatment	Height of the main stem (cm)	Number of branches	Length of the lateral stem (cm)	Dry weight of the stems (g)	Dry weight of the leaves (g)
2017	C2	30.1d	11bc	31.4cd	9.09e	7.87e
	P1	34.2b	13abc	38.4a	11.01b	9.76b
	P2	30.7d	10c	32.5c	9.30de	7.92e
	M1	28.7e	13abc	29.8d	9.61d	8.54d
	M2	23.5f	11bc	25.7e	8.02f	7.08f

Note: Means denoted by different letters within the same column indicate significant differences according to Tukey's test ($P < 0.05$).

3.2 Effect of Allelochemical Treatment on Peanut Root Morphological Characters

To characterize the role of exogenous allelochemicals on peanut root growth characters, we first concentrated on the phenotypic changes that occurred in the peanut seedlings. In line with the effect of allelochemicals on agronomic characteristics, seedlings treated with H, C, P, and M showed significantly reduced total root length, root surface area, and root dry weight compared with the water-treated control (Table 2). It appears that the high concentration of allelochemicals (H2, C2, and P2) had stronger effect than the low concentration treatments on the reduction of these characters. Additionally, the mixture of these allelochemicals (M1 and M2) showed strongest inhibition of peanut root growth. By contrast, exogenous application of allelochemicals significantly increased the average root diameter especially in M1 and M2 treatments as evident by the increment of 20.9% and 30.2% in 2016 and 20.8% and 29.2% in 2017, respectively compared with CK.

Table 2: Effects of allelochemical treatment on peanut root morphological characters

Year	Treatment	Total root length (cm)	Average root diameter (mm)	Root surface area (mm^2)	Root dry weight (g)
	CK	16103.6a	0.43d	7180.7a	4.25a
	H1	11129.3c	0.49c	4987.2c	2.96c
	H2	10211.7ef	0.54ab	3869.6ef	2.29e
	C1	11281.3bc	0.49c	5096.2bc	3.08c
2016	C2	10148.2def	0.53b	4031.6def	2.33e
	P1	13391.5b	0.48cd	5117.8bc	3.13b
	P2	11691.1de	0.51bc	4110.9de	2.41e
	M1	10580.3d	0.52bc	4896.7d	2.68d
	M2	9739.4f	0.56a	3678.2f	2.05f
	CK	17894.4a	0.48d	7908.9a	4.65a
	H1	12328.7cde	0.56abc	5484.4c	3.29c
	H2	12001.8de	0.61a	4241.8g	3.18e
	C1	12500.2cd	0.54bcd	5612.2b	3.31c
2017	C2	11149.1fg	0.60ab	4443.9f	2.61e
	P1	14690.9b	0.52cd	5618.6b	3.54b
	P2	12869.5c	0.58abc	4540.0e	2.70e
	M1	11598.5ef	0.58abc	5386.4d	2.92d
	M2	10670.6g	0.62a	4054.0h	2.28f

Note: Means denoted by different letters within the same column indicate significant differences according to Tukey's test ($P < 0.05$).

3.3 Effect of Allelochemical Treatment on Activities of Antioxidant Enzymes and Oxidative Damage of Peanut Root

To investigate the role of allelochemical in the response of antioxidant system in peanut roots, we assessed the activities of SOD, POD, and CAT. The exogenous application of allelochemicals significantly induced the activities of SOD (Figs. 1A and 1E), POD (Figs. 1B and 1F), and CAT (Figs. 1C and 1G) in both 2016 and 2017. Interestingly, different concentrations of allelochemicals induced further increases in the activities of these enzymes at various degrees. Allelochemical treatment at 720 μg L^{-1} (H2, C2, P2, and M2) concentration showed the better effect in this regard than those treatment of 360 μg L^{-1} (H1, C1, P1, and M1) compared with CK. Likewise, the increment of activities of SOD, POD, and CAT in M2 was significantly higher than other treatments. In line with the data of antioxidant enzymes, exogenous application of allelochemicals induced lipid peroxidation in peanut roots, as evidenced by the significantly increased malondialdehyde (MDA) content in both 2016 and 2017 (Figs. 1D and 1H). The higher concentration of allelochemicals exhibited higher MDA content than the lower concentration treatments. M1 and M2 caused the greatest aggravation of membrane peroxidation resulting in the induction of MDA content by 53.6/76.1% in 2016 (Figs. 1D) and 75.5/93.3% in 2017 (Figs. 1H), respectively compared with CK in peanut roots.

3.4 Effect of Allelochemical Treatment on Activities of NR, ATPase, and Root System of Peanut Root
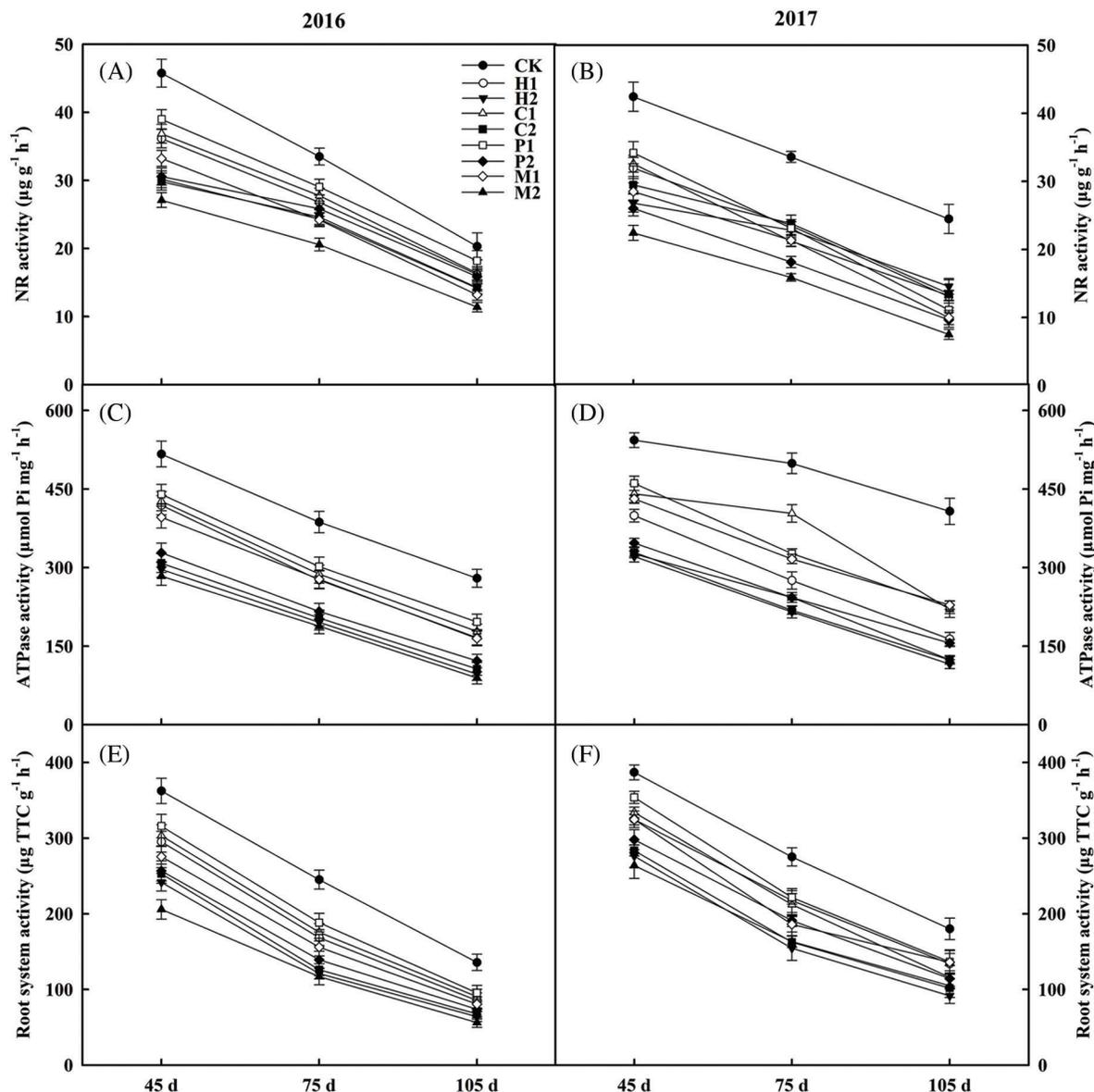
To understand the effects of allelochemical treatment on the enzymes of NR, ATPase, and root system, the time course changes of NR, ATPase, and root system were determined under allelochemical treatment. Exogenous application of H, C, and P significantly decreased NR activity at 45 d after emergence in both 2016 and 2017 (Figs. 2A and 2B). Then the activities of NR were gradually decreased in all the treatments at 75 and 105 d after emergence. Particularly, the NR activity in M2 was 40.8% in 2016 and 47.1% in 2017, respectively lower than the untreated seedlings of CK at 45 d after emergence and remained at the lowest value during the remaining period of the experiments in both 2016 and 2017. Similarly, the activities of ATPase (Figs. 2C and 2D) and root system (Figs. 2E and 2F) showed similar changes to NR activity where application of allelochemicals significantly reduced the activities of ATPase and root system at 45 d after emergence and decreased at 75 and 105 d after emergence. Again, M2 treatment exhibited the lowest activities of ATPase and root system during the whole period of the experiment in both years.

3.5 Effect of Allelochemical Treatment on Osmolytes Accumulation and Nutrient Content of Peanut Root

To determine the mechanism of the side effects of allelochemicals on peanut root, we analyzed the osmolytes accumulation involved in stress response. Treatment with H, C, P, and M significantly reduced the accumulation of osmolytes as evidenced by the decreased contents of total soluble sugar and total soluble protein in both 2016 and 2017 (Fig. 3). It is noteworthy that the magnitude of reduction for total soluble sugar and protein in low concentration of allelochemicals (H1, C2, P1, and M1) was augmented by high concentration of allelochemicals (H2, C2, P2, and M2). Allelochemical treatment led to evident reductions of total soluble sugar by 51.9, 50.8, 42.9, and 54.1% in 2016 (Fig. 3A) and 52.4, 51.4, 40.1, and 45.8% in 2017 (Fig. 3C), respectively in H2, C2, P2, and M2 seedlings compared with CK. Moreover, the allelochemical-induced reductions of total protein content were 49.1, 46.1, 49.8, and 50.2% in 2016 (Fig. 3B) and 50.8, 48.1 51.1 and 51.9% in 2017 (Fig. 3D), respectively in H2, C2, P2, and M2 seedlings compared with CK.
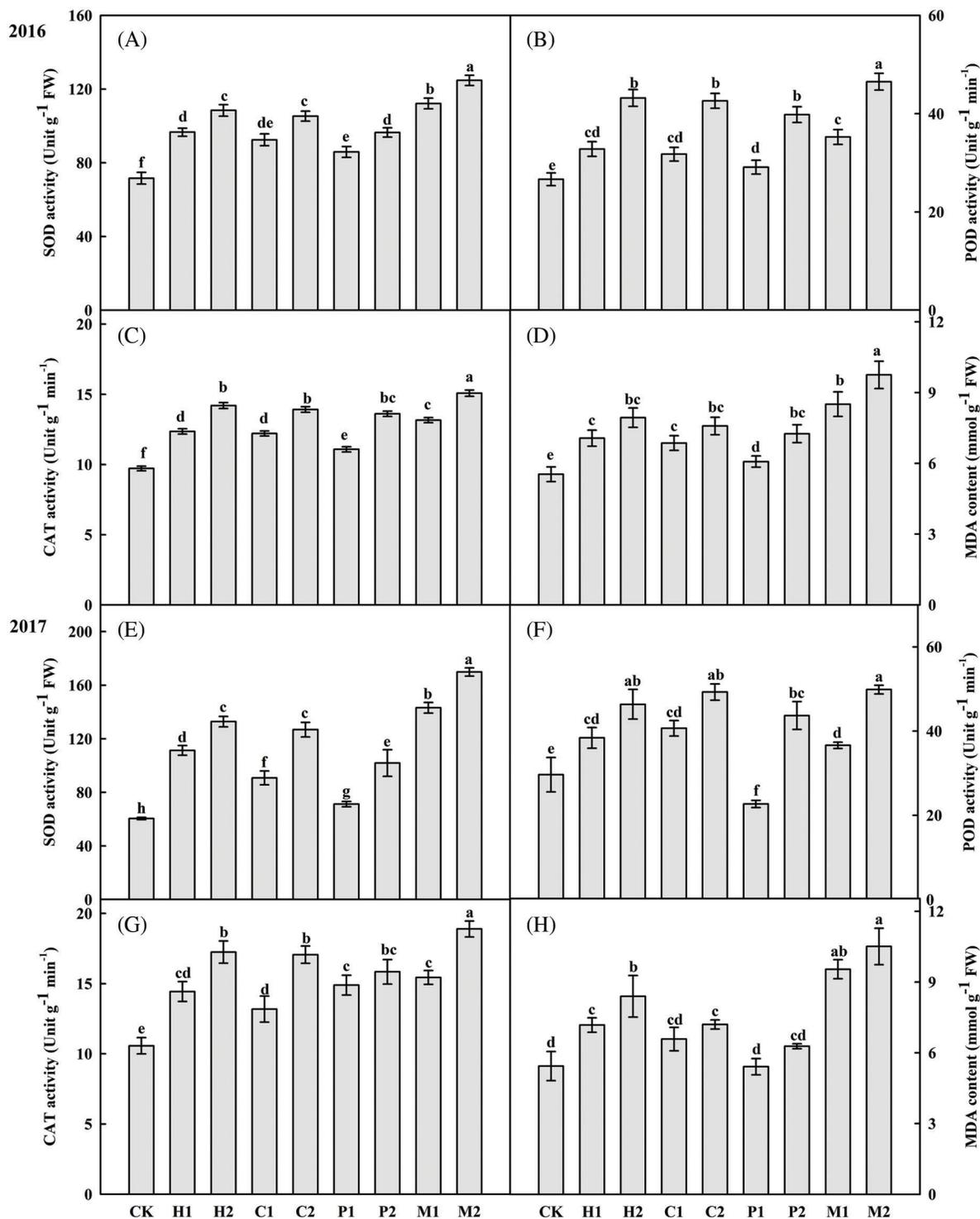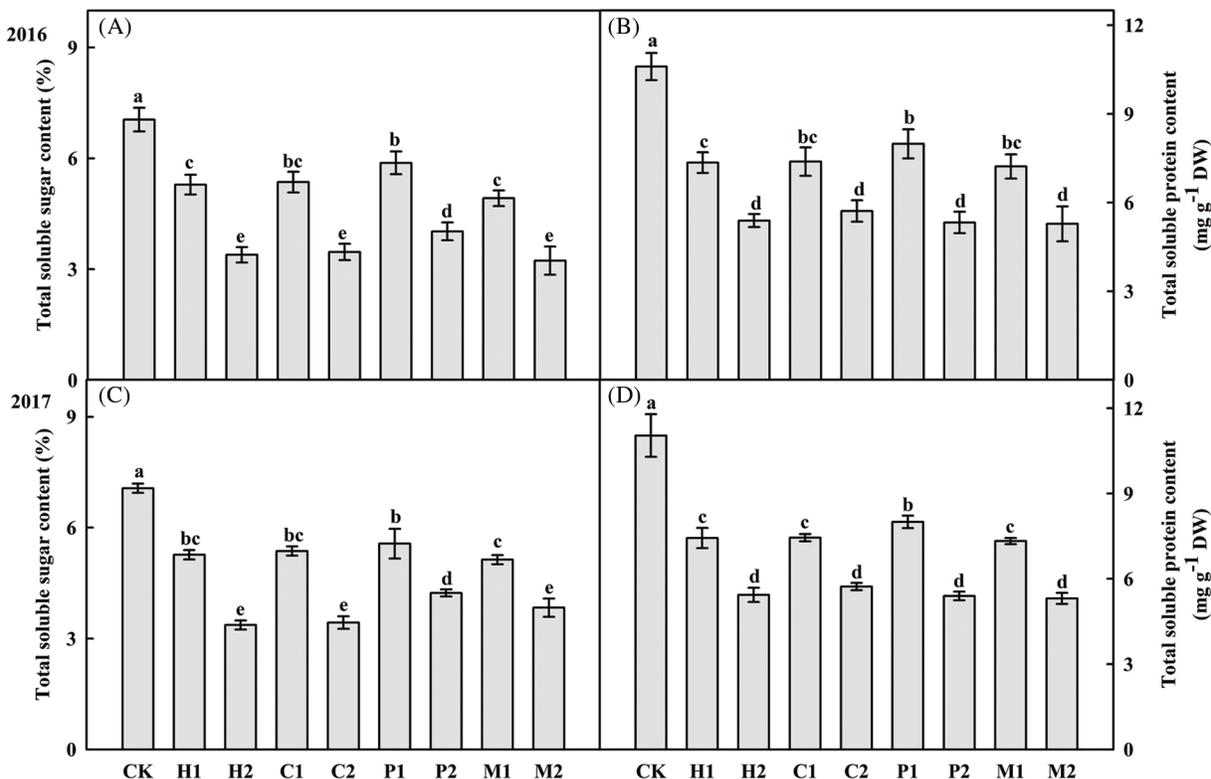
Figure 1: Effects of allelochemical treatment on activities of the antioxidant enzymes and lipid peroxidation in peanut roots. Peanut seedlings at the four-leaf stage were treated with allelochemicals. Activities of SOD (A, E), POD (B, F), CAT (C, G), and content of MDA (D, H) in peanut roots were determined at 75 d after emergence. Data are the means (±SD) of three biological replicates. Different letters indicate significant differences according to Tukey's test ($P < 0.05$)

Figure 2: Time course changes of activities of NR (A, B), ATPase (C, D), and root system (E, F) post allelochemical treatment in peanut roots. Peanut seedlings at the four-leaf stage were treated with allelochemicals. Activities of NR, ATPase, and root system in peanut roots were determined at 45, 75, and 105 d after emergence. Data are the means (±SD) of three biological replicates

Application of allelochemicals significantly inhibited the contents of nitrogen (N), phosphorus (P), and potassium (K) in peanut roots at 75 d after emergence (Fig. 4). The highest decline in N content was observed for M2 (28.7%), followed by M1 (23.6%), H2 (22.5%), and C2 (21.6%) compared with CK in 2016 (Fig. 4A). In 2017, the highest decline in N content was observed for M2 (22.8%), followed by C2 (22.6%), M1 (22.4%), and H2 (22.3%) compared with CK (Fig. 4B). In line with N content, similar results were obtained in the contents of P and K where the highest decline was observed in M2 by 33.9% (Fig. 4C) and 33.8% (Fig. 4E) in 2016 and 35.9% (Fig. 4D) and 34.0% in 2017 (Fig. 4F) of P content and K content, respectively compared with CK. Notably, the dosage effect of allelochemicals were also

detected where application of 720 µg L^{-1} allelochemicals exhibited more reduction in the contents of N, P, and K than those of 360 µg L^{-1} compared with untreated control seedlings in both 2016 and 2017.
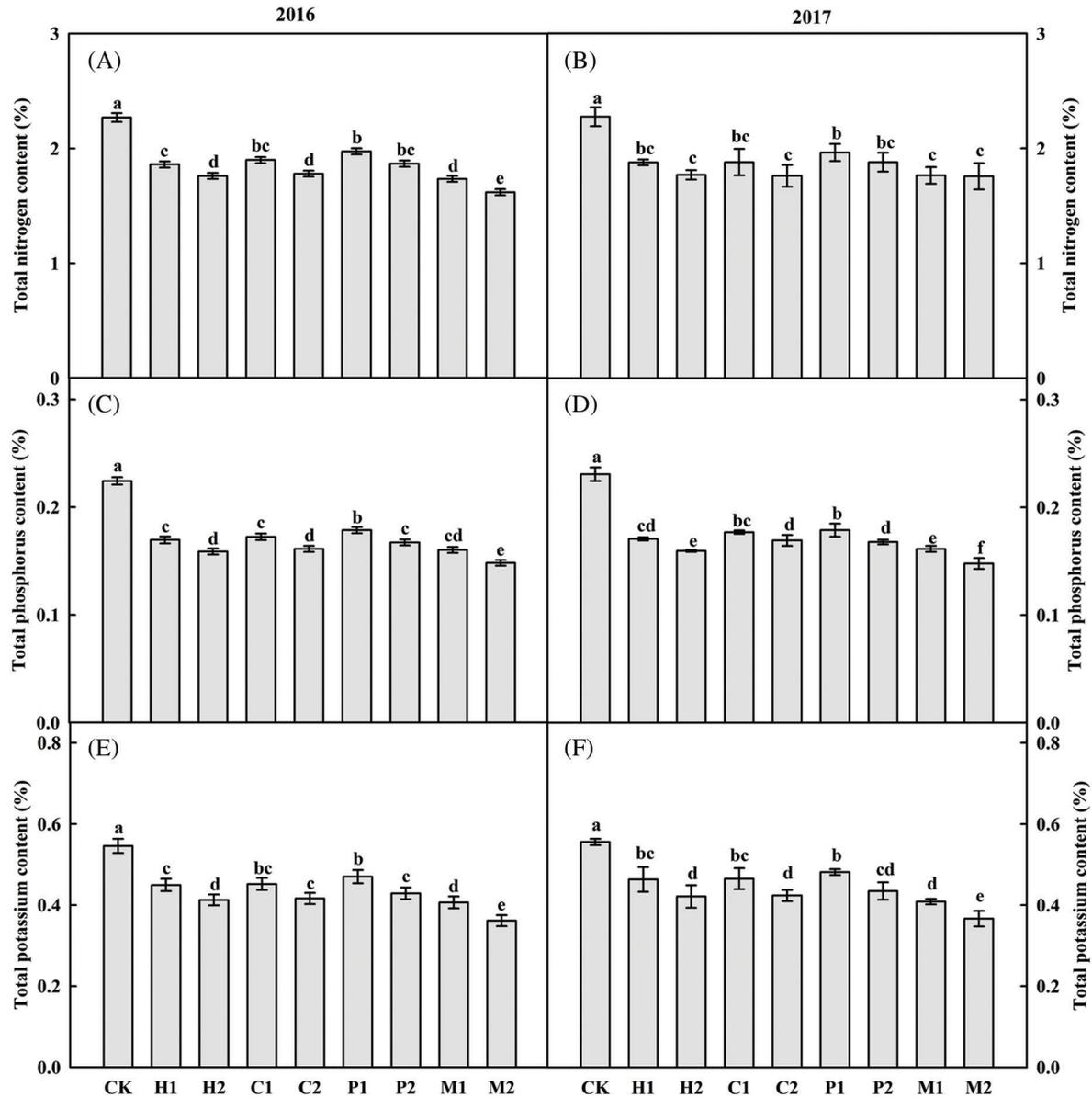

Figure 3: Effects of allelochemical treatment on osmolytes accumulation in peanut roots. Peanut seedlings at the four-leaf stage were treated with allelochemicals. Contents of total soluble sugar (A, C) and total soluble protein (B, D) in peanut roots were determined at 75 d after emergence. Data are the means (±SD) of three biological replicates. Different letters indicate significant differences according to Tukey's test ($P < 0.05$)

3.6 Effect of Allelochemical Treatment on Pod Yield and Yield Components of Peanut

The dry fruit weight per pot was significantly decreased by the 360 µg L^{-1} of allelochemical treatment in H1, C1, and P1. The reduction of dry fruit weight per pot was aggravated when the concentration was higher in H2, C2, and P2. Again, the reduction was more pronounced in M1 and M2 as evidenced by a decline of 42.4% and 49.6% in 2016, and 43.0% and 49.7% in 2017, respectively compared with CK (Table 3). Similar results were obtained in the yield components data where allelochemical treatment significantly decreased the pod number per plant, 100-pod weight, and full fruit rate while significantly increased the pod number per kilogram. Notably, the dosage effect still exists that high concentration of allelochemicals (H2, C2, and P2) showed more impact on the yield components than the low concentration treatments (H1, C1, and P1). Moreover, application of the mixture of allelochemicals (M1 and M2) have been shown to be the most severe treatments that impacted by allelochemicals where the pod number per plant was reduced by 46.1% and 50.7% in 2016, and 47.7% and 50.6% in 2017.The 100-pod weight was reduced by 10.3% and 11.4% in 2016, and 10.6% and 12.0% in 2017. The full fruit rate was reduced by 31.8% and 40.8% in 2016 and 31.5% and 41.7% in 2017 in M1 and M2, respectively compared with

CK. However, the pod number per kilogram was induced by 38.7% and 53.7% in 2016 and 41.0% and 52.5% in 2017 in M1 and M2, respectively compared with CK.

Figure 4: Effects of allelochemical treatment on the nutrient content in peanut roots. Peanut seedlings at the four-leaf stage were treated with allelochemicals. Contents of total nitrogen (A, B), total phosphorus (C, D), and total potassium (E, F) in peanut roots were determined at 75 d after emergence. Data are the means (±SD) of three biological replicates. Different letters indicate significant differences according to Tukey's test ($P < 0.05$)

Table 3: Effects of allelochemical treatment on peanut yield and yield components

Year	Treatment	Dried fruit weight per pot	Pod No. per plant	100-pod weight (g)	Pod No. per kilogram	Full fruit rate (%)
	CK	168.0a	21.9a	269.1a	856e	68.6a
	H1	112.6c	14.4c	249.3b	936d	53.3bc
	H2	86.0ef	11.2ef	245.6bc	1132b	42.2de
	C1	121.1bc	14.9bc	253.4b	967d	54.1bc
2016	C2	90.1def	11.7def	246.4bc	1166b	43.8de
	P1	123.7b	15.3b	255.6b	1068c	58.8b
	P2	94.6de	12.1de	251.1b	1223b	45.2d
	M1	96.7d	11.8d	241.3c	1187b	46.8d
	M2	84.7f	10.8f	238.3c	1316a	40.6f
	CK	184.4a	24.3a	297.7a	824f	75.6a
	H1	124.1c	15.8d	274.9c	911e	58.9c
	H2	95.2f	12.4fg	270.4d	1109c	47.0e
	C1	132.6b	16.5c	280.3b	931e	59.8c
2017	C2	100.0e	12.8f	271.5d	1151b	48.0e
	P1	135.5b	17.4b	281.4b	1052d	64.2b
	P2	104.6d	13.9e	276.8c	1168b	50.1d
	M1	105.1d	12.7fg	266.2e	1162b	51.8d
	M2	92.7f	12.0g	262.1f	1257a	44.1f

Note: Means denoted by different letters within the same column indicate significant differences according to Tukey's test ($P < 0.05$).

4 Discussion

This study elucidated some unique functions of allelochemicals in response to CC obstacle in peanut, an important oil seed crop worldwide. We noticed that plant growth inhibition could be induced by both high (720 μg L^{-1}) and low (360 μg L^{-1}) concentrations of allelochemicals (Table 1). Notably, exogenous application of allelochemicals H, C, P, and their mixture M significantly decreased the plant growth of both aboveground part and underground part as evidenced by the reduced plant height, number of branches, dry weight of stems and leaves, and root morphological characters (Tables 1 and 2). Meanwhile, allelochemicals also aggravated the membrane peroxidation even though the activities of antioxidant enzymes were induced (Fig. 1). Besides, allelochemicals restricted the uptake of root nutrition of N, P, K, and the accumulation of osmolytes such as soluble sugar and protein (Figs. 3 and 4). Ultimately, the application of allelochemicals reduced the pod yield of peanut plants (Table 3). We therefore propose that allelochemicals play a crucial role in CC obstacle induced peanut growth inhibition and yield reduction. The findings of the present study could provide novel information about the long-term monoculture from the perspective of allelochemicals.

Phenolic acids such as p-hydroxybenzoic acid, cinnamic acid, and phthalic acid used in this study have been reported to have a commercial value for human health due to their antiaging, antitumor, and antiinflammatory properties [48–52]. As major components of allelochemicals, phenolic acids always induce changes in soil microbial properties and enzymatic activity [53] and act as signaling molecules in plant-microorganism interactions [54], however, their functions in CC obstacle were less defined. As

shown in Table 1, exogenous application of phenolic acids showed negative effects on peanut growth especially the underground part of the plants. In peanut production, CC obstacle has been considered as the major limitation of plant growth and yield formation. These results hinting a possibility that application of phenolic acids resembles that of CC obstacle, namely, CC obstacle induced allelochemicals especially phenolic acids play a dominant role in the inhibition of peanut root growth.

The induction of the activities of antioxidant enzymes such as SOD, POD, and CAT are demonstrated to be important for scavenging reactive oxygen species (ROS) [55–57]. In the present study, exposure of peanut seedlings to both low and high concentrations of allelochemicals induced substantial increases in activities of SOD, POD, and CAT (Fig. 1). However, such increase in activities of antioxidant enzymes was unable to ameliorate oxidative stress possibly due to insufficient ROS scavenging ability in parallel with allelochemical-induced ROS generation as characterized by the allelochemical-induced the membrane peroxidation (MDA accumulation) in peanut roots (Figs. 1D and 1H). We therefore speculate that the induction of antioxidant systems by allelochemicals may indicate an adaptive response by peanut as allelochemicals are well-recognized as signal molecules in response to plant environmental stress [58–60], which warrant further investigation. These observations confirmed that the antioxidant systems stimulated by exogenous allelochemicals were insufficient to confer CC obstacle induced peanut root inhibition.

The metabolisms of mineral nutrients such as N, P, and K, as well as osmolytes such as soluble sugar and soluble protein reveal the plant growing potential and root activity. Moreover, the composition of mineral nutrients and osmolytes may function as indicators for the assessment of the plant physiological parameters [61–63]. It is well accepted that the synthesis of soluble sugar and soluble protein could be partially attributed to the activities of root NR and ATPase for their functions in assimilation and transformation of N in plant roots [64–67]. NR catalyses the transfer of two electrons from NAD(P)H to nitrate to produce nitrite [68] while ATPase plays a key role in the maintenance of optimum photosynthetic rate and ensuring effective energy flow for crop growth [69]. In this paper, the reductions of NR activity and ATPase activity (Figs. 2A–2D) were observed under allelochemical treatments, which were consistent with the changes of total soluble sugar and protein content (Fig. 3), indicating that NR and ATPase play crucial roles in the uptake of mineral nutrients by peanut roots under CC obstacle. In line with the osmolytes data, the performed analysis exhibited a dramatic decline in dry weight of shoots and roots under allelochemical treatments (Table 1), suggesting that application of allelochemicals reduced the capacity of synthesis and transportation of carbohydrates through decreasing the content of total soluble sugar in peanut plants. The data of dried fruit weight per pot further pointing out that allelochemicals showed negative effects on peanut pod yield formation (Table 3). Consequently, the inhibited root physiological traits weakened the absorption of nitrogen and induced early senescence, which ultimately decreased the pod yield of peanut. A similar set of observations was made in the yield components where pod No. per plant, 100-pod weight, and full fruit rate were significantly reduced by the application of allelochemicals (Table 3). In line with the previous studies, the yield and yield components decreases for these treatments were possibly due to aggravating of CC obstacle as a result of positioned rotation [47,70].

In summary, our study has concentrated on addressing the allelochemical-induced peanut growth response and feedback to the pod yield under CC obstacle. Application of allelochemicals manifested remarkable inhibitions to peanut plants in terms of seedling growth, antioxidant system, osmolytes accumulation, and root activity. Moreover, the decreased nutrition absorption by roots might contribute to the peanut yield reduction (Fig. 5). The findings of this study provide new clues that could help to develop new strategies to combat CC obstacle in legume crops production through reducing the accumulation of allelochemicals. Further researches should be taken under field conditions to obtain practical conclusions in support of the involvement of allelochemicals in CC obstacle of legume crops.
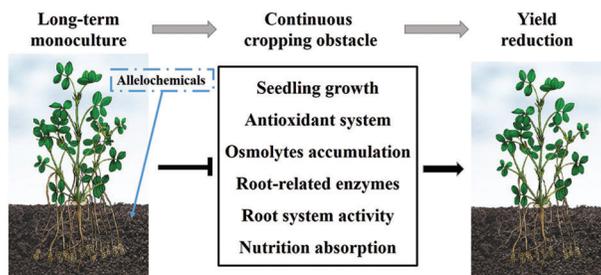
Figure 5: The working model illustrating the mechanisms of allelochemicals in reducing peanut yield

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