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Autotoxic effects of *Angelica sinensis* (Oliv.) Diels

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ABSTRACT

Laboratory and pot experiments were done to determine the autotoxicity potential of aqueous extracts (rhizomes, shoots, soil extracts) and rhizome exudates of *Angelica sinensis* on its own seed germination and seedling growth. In laboratory experiments, aqueous extracts (rhizomes, shoots, soil extracts) and rhizome exudates inhibited its own seed germination and seedling growth and this inhibitory effect increased with increase in aqueous extracts concentrations. The extracts from *A. sinensis* rhizomes were less inhibitory than those from shoots. The extracts of rhizome and shoot collected at the rhizome thickening stage were more inhibitory than those collected at seedling stage. The radicle growth was more sensitive to extract than shoot growth and seed germination. These results showed that *A. sinensis* had autotoxicity potential.

The pot experiments showed that plant parts and sterilized soil (previously cropped with *A. sinensis*) inhibited the seedling growth at 1.5% concentrations (g plant material/g soil), and confirmed the autotoxicity potential of *A. sinensis*. Thus autotoxicity is the main problem in continuous cropping of *A. sinensis*.

Key Words*: Angelica sinensis*, autotoxicity, aqueous extracts, rhizome exudates, seed germination, seedling growth

INTRODUCTION

Schreiner and Reed (20) first reported the autotoxicity in roots of wheat, oats and other crop plants, which exuded chemicals to inhibit the growth of their own seedlings. Autotoxicity is an intraspecific allelopathy, which occur when a plant specie releases the chemical substances that inhibit or delay the germination and growth of same plant species. The ecological significance of autotoxicity is seen in geographical distribution of plants and adaptation to induced dormancy (10). The autotoxicity drastically reduces the quality and quantity of crop plants in agriculture (21,27). It has been identified in many field crops [alfalfa (*Medicago sativa* L.) (10), rice (6), barley (4), wheat (22), asparagus (*Asparagus officinalis* L.) (28), cucurbit groups (29) and jara (*Cistus ladanifer* L.) (3)].

Angelica sinensis (Oliv.) Diels (family Apiaceae) is a well-known oriental herb used to treat the gynecological diseases in Traditional Chinese Medicine since ancient times (30). Besides, recently it has been widely used in cosmetic, health beverage and drinks (5,16) due to its multiple pharmacological effects. Hence to meet its demand, in last decades its continuous cropping area has increased sharply. *A. sinensis* farmers have found

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that the stunting, plant mortality and *Ditylenchus destructor* infestation occurs in its continuous cropping, these reduces the yield and quality (31,32). The continuous cropping has become the main deterrent in *A. sinensis* production.

A reduction in both crop yield and quality often occurs, when the same crop or cultivar is cultivated continuously on the same soil (29). It is a threat to agricultural production in many crops (28), owing to the buildup of pests, disorders in soil physical-chemical properties or autotoxicity (11,23,28). The autotoxicity is major problem in continuous cropping in cucurbit crops (1,29), *Cistus ladanifer* (3), alfalfa (21), cucumber (27) and wheat (26). However, whether autotoxicity exists in *A. sinensis* and what is the role of continuous cropping, it remains still unclear. Therefore, this sutdy aimed to determine, whether autotoxicity exists in *A. sinensis* continuous cropping problem and to determine the effect of autotoxicity on *A. sinensis* growth.

MATERIALS AND METHODS

LABORATORY BIOASSAYS

Experiment 1. Autotoxic bioassays potential of plant parts extracts

A. sinensis plants were harvested at seedling and rhizome thickening stage during 2006 from Minxian (103°34' E, 34°27' N), Gansu province, China. The plants were partitioned into shoots (leaves and stems) and rhizomes. The rhizomes were washed clean with distilled water. Then shoots and rhizomes were cut in 2 cm pieces and 100 g of each were kept in oven to dry at 80 ^{o}C.

Bioassay procedure of Wu *et al* (25) was used with slight modification. To prepare aqueous extract, 10 g shoot and rhizome samples were soaked separately (with occasional stirring) in 200 ml distilled water for 48 h at 20^{o}C. The extracted mixture was filtered through two layers of filter paper and the filtrate was centrifuged at 4000 rpm for 15 min at 10^{o}C. The supernatant of resulting filtrate was then vacuum-filtered through one layer of microfilter paper (0.45 μm). The filtrate was designated as full strength (100%), which has further diluted with distilled water to prepare desired concentrations (50%, 25% and 12.5% of original solution). A series of extract concentration are reported as 50 g, 25 g, 12.5 g and 6.25 g of dry *A. sinensis* tissue L^{-1}. Extracts were stored at 4 ^{o}C.

***Germination*：** The seeds of *A. sinensis* were surface-sterilized in H_2O_2 (0.1%) for 20 min, rinsed many times and imbibed in deionized water for 24 h at 25^{o}C. 25 seeds were sown in Petri dishes (9 cm dia) lined with two layers of filter paper. Five ml extract from shoots or rhizomes (as per treatment) were added to each Petri dish and distilled water (5 ml) was used as control. All Petri dishes were covered with lids and placed in incubator at 20^{o}C for 20 days. Only seeds with 1 mm emerged radicle were considered germinated. The number of germinated seeds and emerged cotyledons were recorded daily. Each treatment was replicated four times. The data were presented as percentage differences from the control. Thus zero represents control; positive values represent stimulation and negative values represent inhibition.

Seedling growth **:** Twenty five pre-germinated seeds were placed on filter papers in Petri dish (9 cm dia) containing 5 ml of aqueous extract or distilled water (control). The Petri dishes were placed in an incubator 12/12 h light, darkness at 20^{o}C. The radicle and shoot length was measured after 10 days. Each treatment was replicated four times. The data were processed by the same methods in germination trial.

Experiment 2. Autotoxicity potential of rhizome exudates

The *A. sinensis* was used both as the donor and receiver. The equal-compartment-agar-method (ECAM) was used to determine the autotoxicity of *A. sinensis* rhizome exudates, because this method successfully separates the competition from allelopathy (24,26). *A. sinensis* seeds were surface-sterilized by soaking in 0.1% H_2O_2 for 20 min, followed by 5 rinses in sterilized distilled water. Then they were soaked in sterilized water for imbibition of water at 20^{o}C for 24 h and rinsed with fresh sterilized water. The *A. sinensis* seeds were germinated for 6-days at 20^{o}C.

Fifty uniform pre-germinated seeds (donor) were selected and aseptically sown on the agar surface with the embryo up in 3-rows on one half of 15 cm Petri dishes pre-filled with 200 ml of 0.3% water agar. Petri dishes with its cover were placed in an incubator at 20^{o}C. After the growth of *A. sinensis* seedlings for 6 days, fifty pre-germinated seeds (receiver) were aseptically sown on the agar surface in 3-rows on the other side of Petri dishes. A piece of pre-autoclaved white paperboard was inserted across the centre and down the middle of the Petri dishes with the lower edge of the paperboard kept 1 cm above the agar surface. The entire Petri dish was divided into two equal compartments that were occupied separately by donor and receiver seedlings. All autotoxins produced and released by donor *A. sinensis* seedlings were able to diffuse throughout the agar medium to affect the growth of receiver *A. sinensis*. After sowing of receiver *A. sinensis*, the Petri dishes were again covered and placed back in the incubator to grow for 10 days. The growth of each receiver *A. sinensis* without the donor *A. sinensis* plants was treated as control. The radicle and shoot length of receiver *A. sinensis* seedlings were measured after 10 days growth of receiver *A. sinensis* in the incubator.

Experiment 3. Autotoxicity potential of soil extracts

Soil samples were collected by soil auger (0-15 cm depth) from each of six fields in October, 2006, in Minxian, Gansu province, China. The samples were collected from 1-year *A. sinensis* stand and a spring wheat stand. Then the samples were sieved through 1 mm sieve remove the plant residues and stones.

Eighty g fresh soil samples were extracted by soaking with occasional stirring in 200 ml distilled water for 48 h at 20^{o}C. The extracted mixture was filtered through two layers of filter paper and the resulting filtrate was supernatant at 4000 rpm for 15 min at 10^{o}C. The supernatant of resulting filtrate was then vacuum-filtered through one layer of microfilter paper (0.45 μm). The filtrate of the supernatant was designated as full strength (100%), which was further diluted with distilled water to prepare desired concentrations (50%, 25% and 12.5% that of the original solution). A series of extract concentration are reported as 400 g, 200 g, 100 g and 50 g of fresh soil L^{-1}. Extracts were stored at 4 ^{o}C.

***Germination and seedling growth*:**

These were determined using the methods as in Experiment 1.

POT CULTURE

Experiment 4. Autotoxicity potential of shoots and rhizomes

*A. sinensi*s plants were collected at harvest in 2007 from Minxian, Gansu province, China. The plants were separated into shoots and rhizomes, and rhizomes were cleaned with distilled water. The shoots and rhizomes were air-dried, ground and passed through 1 mm sieve before adding to soil in pots.

The dried and grounded shoots and rhizomes were homogeneously mixed in soil. Each pot contained 10 kg soil. Based on the results from preliminary experiment, the shoots and rhizomes were added in soil at 0, 1.5%, 3.0% and 4.5% (w/w). Four plants were planted in each pot. Each treatment was replicated 8-times. Plants in the 4 pots were harvested after 3-months and in the rest remaining pots after 5-months.

Experiment 5. Autotoxicity potential of soil previously cropped with *A. sinensis*

Soil samples were collected in October, 2007 from 1-year *A. sinensis* stand and from spring wheat stand (hereinafter referred to as *A. sinensis* soil and wheat soil, respectively), from Minxian, Gansu province, China. The samples were sieved through 1 mm sieve to remove the plant residues and stones. Then the samples were air-dried and steam sterilised. *A. sinensis* was grown in pots filled with this soil, 4-plants were sown per pot. Treatments were replicated 8-times. Plants in 4 pots were harvested after 3-months and remaining after 5-months.

Statistical Analysis

All experimental data were subjected to analysis of variance (ANOVA) using SPSS and the treatment means were tested using least significant difference (LSD) at $P < 0.05$ level.

RESULTS

Autotoxicity of shoots and rhizomes extracts

The aqueous extracts of *A. sinensis* rhizomes and shoots (at seedling stage and rhizome thickening) significantly inhibited the seed germination and cotyledon emergence and this inhibitory effect increased with increase in aqueous extracts concentrations (Figure 1). The aqueous extracts of rhizomes and shoots collected at the rhizome thickening stage were more inhibitory to seed germination and cotyledon emergence than those collected at seedling stage. The shoot extracts were more inhibitory to seed germination and cotyledon emergence than rhizome extracts. The shoot extract caused 45.52% inhibition in seed germination as compared to 10.69% inhibition from the rhizome extracts. Rhizome and shoot extracts were more inhibitory to cotyledon emergence than

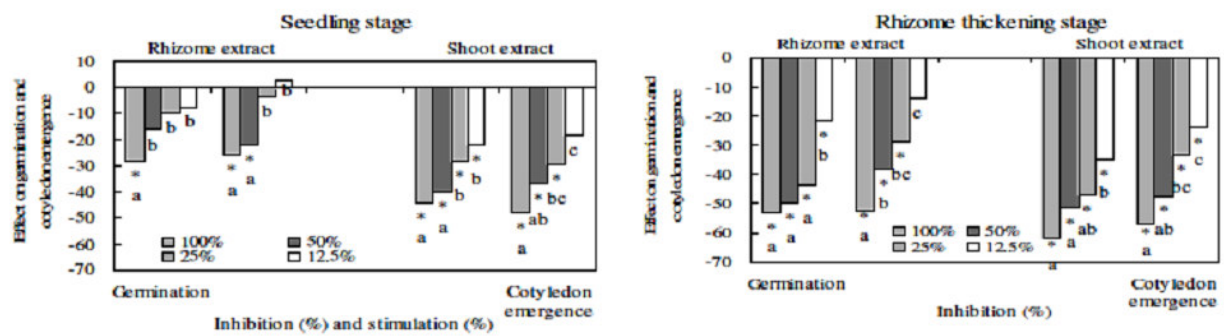

Figure 1. Effects of rhizomes and shoots extracts from seedling stage and rhizome thickening. Notes as in Table 2 on Germination (%) and cotyledon emergence of *A. sinensis* seeds in laboratory bioassay.

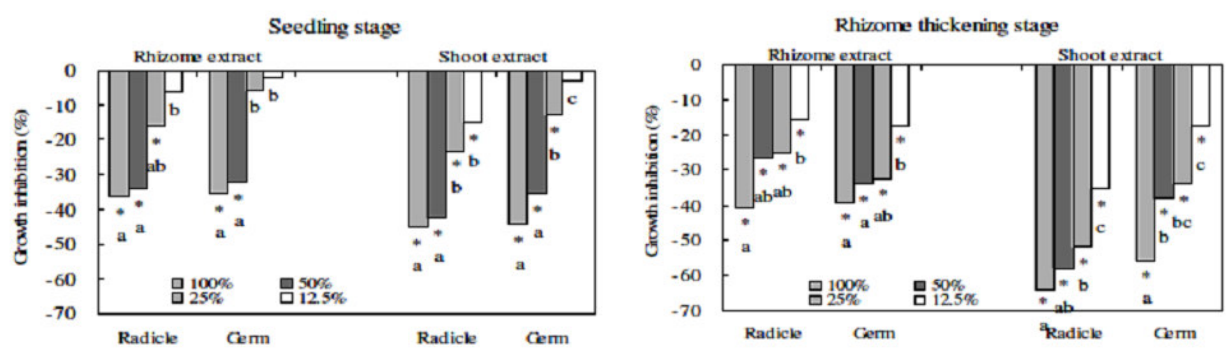

Figure 2. Effects of rhizomes and shoots extracts from seedling stage and from rhizome thickening stage on radicle and shoot length of *A.sinensis* plants in laboratory bioassays. Notes as in Table 2.

seed germination. The rhizome extracts decreased the cotyledon emergence and seed germination by 19.45% and 10.69%, respectively.

The response of seedling growth to aqueous extracts of *A. sinensis* rhizomes and shoots was similar to seed germination. All concentrations of rhizome and shoot extracts of *A. sinensis* decreased the radicle and shoot elongation of *A. sinensis* seedlings and the inhibition increased with increase in extracts concentration (Figure 2). The aqueous extracts from rhizome thickening and seedling stage showed different inhibitory effects. The aqueous extracts from rhizome thickening caused more reduction in radicle length and shoot length than aqueous extracts from seedling stage. The inhibitory effect of shoot extracts on radicle and shoot length (41.86% and 30.13%) was greater than rhizome extracts (25.01% and 24.73%). The shoot extracts caused 50.07% inhibition in radicle elongation, while, the rhizome extracts caused 18.68% inhibition in shoot elongation. Both extracts caused greater inhibition in radicle elongation than in shoot elongation. The extracts did not cause specific morphological changes or deformations (swelling or branching).

Autotoxicity of rhizome exudates

The rhizome exudates had significant autotoxic effect on the radicle and shoot growth (Table 1). The autotoxic effect of rhizome exudates caused 34.81% and 27.27% reduction in radicle and shoot growth suggesting that the radicle was more sensitive to own rhizome exudates than shoot.

Table 1. Effects of rhizome exudates from *A. sinensis* plants on radicle and germ length of *A. sinensis*

Treatment	Radicle length (cm)	Growth inhibition (%)	Germ length (cm)	Growth inhibition (%)
CK(Control)	1.81a	-	2.64a	
Rhizome exudate (50 plants donors/ 50 plant receivers)	1.18b	34.81	1.92b	27.27

Growth inhibition (%) was calculated as (control-data with donor)/control×100. The results are expressed in % inhibition over the control. *, Significant inhibition over the control (*P<0.05*). abc, difference between concentrations in the same parameter, identical letters mean no significant differences.

Autotoxicity of soil extracts

The extracts of *A. sinensis* soil significantly inhibited the seed germination and early seedling growth than extracts of spring wheat soil and this inhibitory effect increased with the increase in soil extracts concentration (Figure 3). Compared with distilled control, extracts of *A. sinensis* soil inhibited the seed germination (18.94%-51.52%), cotyledon emergence (1.20%-31.06%), radicle elongation (33.75%-66.25%) and shoot elongation (20.35%-39.68%). At the same concentration of soil extracts, the response of radicle growth was more sensitive than seed germination, cotyledon emergence and shoot growth. Contrarily, the extracts of spring wheat soil slightly stimulated the seed germination and early seedling growth of *A. sinensis*.

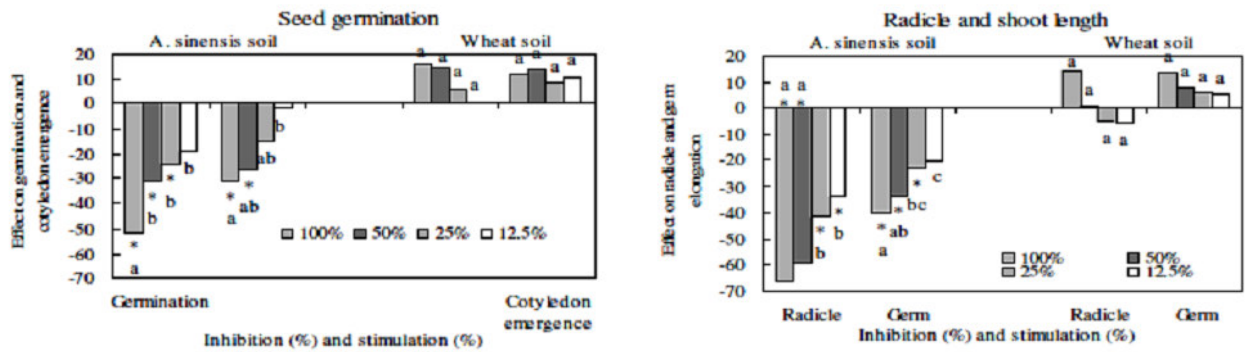

Figure 3. Effects of applied soil extracts of *A. sinensis* cropped soil and wheat soil on the germination, radicle and shoot length of plants. Notes as in Table 2.

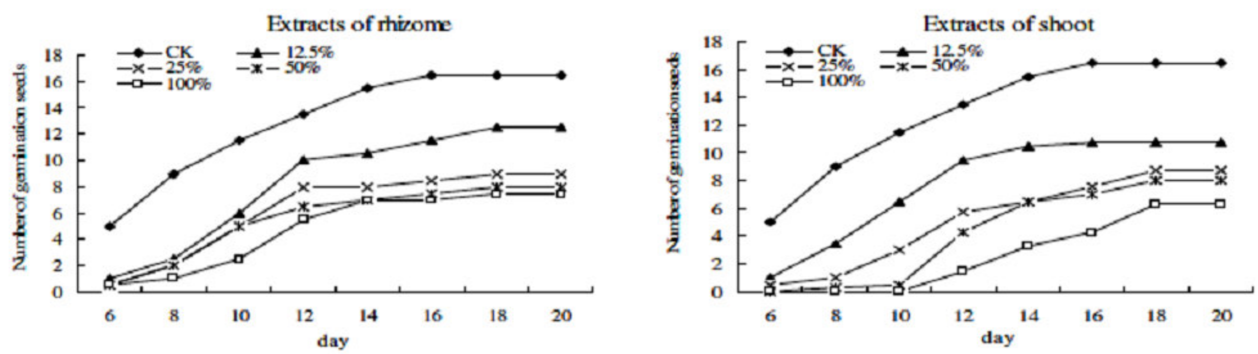

Figure 4. Effects of extracts of *A.sinensis* rhizomes and shoots from rhizome thickening stage on number of *A.sinensis* seeds germinated. Each point represents the mean of four replicate experiments.

Autotoxicity of *A. sinensis* shoots and rhizomes in pot culture

The shoots and rhizomes of *A. sinensis* added in pots significantly inhibited the shoot and rhizome growth of *A. sinensis* 3- and 5-months after application (Table 2 and 3). The inhibitory effect increased with the increase in doses of shoots and rhizomes added to soil. Rhizomes caused inhibition in wheat growth (19.63 %) than shoots. The shoots and rhizomes treatment caused the leaves yellowing and reduced the growth of plants, indicating the strong autotoxic effects of *A. sinensis*.

Table 2. Inhibitory and stimulatory effects of *A. sinensis* shoots on its own seedling growth.

Shoot (% w/w) (g shoot/100g soil)	After 3-months		After 5-months	
	Shoot dry weight (%)	Rhizome dry weight (%)	Shoot dry weight (%)	Rhizome dry weight (%)
1.5	-4.17c	1.08a	6.36c	-2.49c
3.0	16.67b*	10.81a	25.56b*	21.83b*
4.5	33.33a*	13.51a	43.35a*	53.33a*

Growth inhibition (%) was calculated as (control-data with extract)/control×100. The results are expressed in inhibition (%) over the control. *Significant inhibition over the control (*P<0.05*). Identical letters mean no significant differences.

Table 3. Inhibitory effects of *A. sinensis* rhizomes on its own seedling growth

Rhizome (% w/w) (g rhizome/100g soil)	After 3-months		After 5-months	
	Shoot dry weight (%)	Rhizome dry weight (%)	Shoot dry weight (%)	Rhizome dry weight (%)
1.5	5.77b*	4.50b	14.25c	9.15c
3.0	27.88a*	14.41b	36.04b*	35.76b*
4.5	37.50a*	16.22a*	52.65a*	59.88a*

Growth inhibition (%) was calculated as (control-data with extract)/control×100. The results are expressed in inhibition (%) over the control. *Significant inhibition over the control (*P<0.05*). Identical letters mean no significant differences.

Autotoxicity of soil previously used for growing *A. sinensis*

The dry weight of *A. sinensis* after 3 and 5 months growth in soil previously cropped with *A. sinensis* was significantly lower than growth in control soil. The inhibitory effect was more pronounced at 5-months than at 3-months (Table 4).

Table 4. Effects of soil previously used for growing *A. sinensis* on its own seedling growth.

Treatment	After 3-months		After 5-months	
	Shoot dry weight (%)	Rhizome dry weight (%)	Shoot dry weight (%)	Rhizome dry weight (%)
A.sinensis soil	35.42*	15.70*	36.04*	39.44*

Growth inhibition (%) was calculated as (control-data with extract)/control×100. The results are expressed in inhibition (%) over the control. *Significant inhibition over the control (*P<0.05*). Identical letters mean no significant differences.

DISCUSSION

The autotoxicity often causes reduction in crop yield and problem in resowing or re-establishing plants in field due to low seed germination and poor seedling growth, and it has been verified in taro (2), alfalfa (9,12-15) and pearl millet (19). This study shows that the aqueous extracts from shoots and rhizomes of *A. sinensis* inhibited the seed germination and cotyledon emergence, radicle and germ elongation of itself in the laboratory experiments, and this inhibitory effects increased with the increase in concentrations of aqueous extracts. The results are similar with alfalfa plant (7). The shoot extracts at the 50 $mg{\cdot}ml^{-1}$ concentration delayed the seed germination till 10^{th} day (Figure 4). In subsequent pot experiments, the shoot and rhizome extracts of *A. sinensis* inhibited the growth of its own seedlings at low concentrations (1.5% g plant material/g soil). This indicated that *A. sinensis* has an allelopathic potential against itself.

The response of seed germination and seedling growth to same extract concentration was different, indicating that radicle is more sensitive than shoot and seed germination, this is in agreement with alfalfa results (17), where radicle length was more sensitive to aqueous extracts than seed germination or coleoptile length. Futher, aqueous extracts from various plant parts and development stages also influence the autotoxicity (Figure 1, 2). Extracts from *A. sinensis* rhizomes were less inhibitory than shoots extracts. The extracts obtained from the rhizome thickening of growth were more autotoxic than those from the seedling stage of growth, this is in accord with results of alfalfa and barley (7,8,12,18). Thus extracts from crop shoots, rhizomes and roots show different autotoxicity potential than extracts from various development stages.

Leaching (aqueous extracts) and root exudation are two main modes of release of autotoxic substances into the environment. Besides the aqueous extracts of *A. sinensis* plants, rhizome exudates of *A. sinensis* also show autotoxicity potential, indicating that rhizome exudates inhibited the seedling growth (Table 1), it has also been found in asparagus (28), cucurbits (29) and wheat (26). It is therefore possible that the autotoxins (both exuded from living *A. sinensis* plants and leached from *A. sinensis* residues) may accumulate in the soil and then influence the healthy growth of next *A. sinensis* crop.

Although aqueous extracts and rhizome exudation from *A. sinensis* show autotoxic potential, but they do not inhibit the growth of next *A. sinensis* crop, if they are not released (leached or exuded) into soil or they do not accumulate in soil to certain concentration inhibitory to *A. sinensis* growth. This study further confirmed that soil extracts exhibit autotoxicity potential by inhibiting the seed germination at moderate concentration (100 $mg{\cdot}ml^{-1}$) and seedling growth at low concentration (with 50 $mg{\cdot}ml^{-1}$). It may be concluded that the soil in which *A. sinensis* had been grown contains water-soluble substances to inhibit its own seed germination and growth and is most inhibitory to radicle elongation in laboratory conditions. The results of our pot experiment further confirmed that soil previously used for *A. sinensis* growing, inhibits the growth of *A. sinensis* seedlings at low concentrations (1.5% plant material/g soil), indicating that autotoxicity is main deterrent in continuous cropping of *A. sinensis* under field conditions. However, reduction in its own growth observed from the soil extract, could not be matched with the reduction observed from plant parts extract in experiment. This suggests that toxins of microbial origin may be partly involved.

CONCLUSIONS

This study provides evidence of autotoxicity potential of *A. sinensis* in laboratory and pot experiments, which partly explains the yield reduction of *A. sinensis* in the field, when it was replanted. Autotoxicity is a complex problem in agriculture, hence, more studies are need to identify the phytotoxic substances in the plant extracts and exudates and their fluctuation in rhizosphere, to elucidate the possible role of micro-organisms in the phytotoxicity.

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