Zhou G *et al.* (2024)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 52, Issue 4, Article number 14110
DOI:10.15835/nbha52414110
Research Article

Inhibition of seed germination and seedling growth of *Coix lacryma-jobi* L. in leachate prepared from rhizosphere soil under different continuous cropping years and concentrations

Guiqin ZHOU[1,2], Lei LI[1], Bi SONG[1,2,3*], Yi CHENG[1,3*], Min LIU[1,2], Yinying LIU[1], Dailing LIU[1,4]

[1]*Guizhou University, Huaxi, College of Agriculture, Guiyang, Guizhou 550025, China; 2607816402@qq.com (G.Z.); lilei080620@163.com (L.L.); songbi446@126.com (B.S.)(*corresponding author); ycheng3@gzu.edu.cn (Y.C.) (*corresponding author); 947119587@qq.com (M.L.); 18685618785@163.com (Y.L.); 675742533@qq.com (D.L.)*
[2]*Guizhou Key Laboratory of Propagation and Cultivation on Medicinal Plants, Huaxi, Guiyang, Guizhou 550025, China*
[3]*Key Laboratory of Molecular Breeding for Grain and Oil Crops in Guizhou Province, Huaxi, Guiyang, Guizhou 550025, China*
[4]*Key Laboratory of Functional Agriculture of Guizhou Provincial Higher Education Institutions, Huaxi, Guiyang, Guizhou 550025, China*

Abstract

Continuous planting of *Coix lacryma-jobi* L. has expanded, causing yield and quality drops. A key issue is autotoxicity due to unbalanced root-microbe interactions, but this is not well understood. In this study, we hypothesized that root exudates from *C. lacryma-jobi* impede seed germination and young plant growth, which is exacerbated in continuous cropping systems. Autotoxicity was simulated using rhizosphere soil leachate prepared from Xingren *C. lacryma-jobi* cultivated for different durations to investigate the effects on seed germination and young plant growth and explore the relationship between continuous cropping and autotoxicity. Four concentrations, 0.025 (C1), 0.050 (C2), 0.100 (C3), and 0.200 g/ml (C4), and three continuous cropping years, rotation (P0), continuous cropping for 4 years (P1), and continuous cropping for 6 years (P2), were set, with distilled water as the control, totaling 13 treatments. Seed germination and young plant growth tests were conducted. The results showed that the rhizosphere soil leachate significantly affected seed germination and young plant growth depending on continuous cropping years and leachate concentration. The inhibitory effect of P2 at C4 was the strongest. The synthesis effect (SE) of the rhizosphere soil leachate under different continuous cropping years and concentrations was P2 < P1 < P0, C1 < C2 < C3 < C4. The research results reveal key factors contributing to continuous cropping obstacles and provide a scientific basis for developing sustainable agricultural strategies. This will help increase crop yields in *C. lacryma-jobi* planting areas, improve soil health, and promote the development of more resilient agricultural systems.

Keywords: autotoxicity; *Coix lacryma-jobi*; continuous cropping years; rhizosphere soil leachate; seed; young plant

Abrreviations: Ci, intercellular CO2 concentration; Gs, stomatal conductance; Pn, net photosynthetic rate; RI, response index; SE, synthesis effect; Tr, transpiration rate

Received: 06 Sep 2024. Received in revised form: 22 Nov 2024. Accepted: 29 Nov 2024. Published online: 18 Dec 2024.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Coix lacryma-jobi L., known as Job's tears, is an ancient grain and medicine crop in China. Its seeds are not only well-off in proteins, fats, starches and a large number of mineral elements necessary for human and animals, but also have the functions that boost immunity, lower blood pressure, reduce blood sugar levels, and anti-tumor properties, and have high nutritional and medicinal value (Tang *et al.*, 2023), which is utilized in medicine, food, and feed, and have been officially included in China's list of "grain and medicine dual-purpose" crops (Liu *et al.*, 2024). The planting area and production of *C. lacryma-jobi* in China is estimated to be approximately 73,000 hm^2 and 2.2 million tons, respectively (Li *et al.*, 2023). Xingren City in Guizhou Province has the world's largest planting area and the highest total yield (Liu *et al.*, 2024; Liu *et al.*, 2024). Approximately 33,000 tons of material can be produced in the city's planting area, which exceeds 16,000 hm^2. It has been honored as the "Hometown of Chinese *C. lacryma-jobi* " by the China Grain Trade Association and the China Grain Economics Society (Bai *et al.*, 2024). Vigorously developing the *C. lacryma-jobi* industry is pivotal in guaranteeing the provision of traditional Chinese medicinal resources and augmenting rural revenues.

However, due to policy guidance and market demand, the phenomenon of continuous cropping of *C. lacryma-jobi* in Xingren City has become increasingly severe. Long-term continuous cropping is very easy to produce continuous cropping obstacles, causing soil acidification and compaction, reducing organic matter content, nutrient imbalance, resulting in a large accumulation of soil pathogenic bacteria and insect eggs, and causing soil-borne diseases such as wilt and root-knot nematode disease, leading to a decline in crop resilience and quality, reduced yields and planting benefits, and severely impacting the healthy development of the industry (Qi *et al.*, 2024). Studies show that after continuous cropping of *C. lacryma-jobi*, the total root length, average root diameter, total root surface area, and total root volume, as well as the soluble sugar, lysine, and amylopectin content in the grains, all decrease. For each additional year of continuous cropping, the yield of *C. lacryma-jobi* decreases by approximately 600 kg /hm^2 (Li *et al.*, 2023). Therefore, understanding the obstacles to implement continuous cropping systems has become an urgent concern.

The occurrence of continuous cropping obstacle is the consequence of the comprehensive action of various factors, such as the accumulation of plant autotoxic substances, soil microecological imbalance, oil-borne disease accumulation, and nutrient imbalance (Yang *et al.*, 2024). Autotoxicity caused by autotoxic substances is one of the crucial reasons for continuous cropping obstacles. Autotoxic substances are released through leaching, volatilization, root secretion, residue degradation and other pathways, triggering phytotoxicity to the same or following crops of the same species (Li *et al.*, 2024; Zou *et al.*, 2022). Autotoxicity is mainly manifested in morphological structure, physiological function and material metabolism of seeds and seedlings (Wu *et al.*, 2023). Some studies reported that the leachate prepared of the dried leaves of *Solidago canadensis* L. reduces the germination rate, seedlings growth, fresh weight and chlorophyll content of *Trifolium pratense* L. (Zandi *et al.*, 2020); Aqueous leachate from *Lapsana communis* L. subsp. *communis* roots and leaves increasingly inhibit radish (*Raphanus sativus* L. var. *radicula* Pers.) as concentrations rise. Diminishing seed the germination index, the vigor index, inhibiting the root growth, lower seedlings fresh and dry weight ratios, and increasing cell membrane instability and electrolyte leakage by its. (Możdżeń *et al.*, 2021). *Alfalfa* leaf extract reduces the germination rate, germination potential, dry-fresh ratio and seedlings vigor index of its seeds (Zhang *et al.*, 2024). The rhizosphere soil leachates exhibit pronounced autotoxicity, Continuous monoculture of potatoes (*Solanum tuberosum*) can lead to soil mineral imbalances, increased harmful underground microorganisms, and accumulation of root-secreted toxins in the rhizosphere, inhibiting the potato growth. In addition, the rhizosphere soil leachates from Lanzhou lily (*Lilium davidii* var. *unicolor*) (Huang *et al.*, 2020), *Panax notoginseng* (Xiang et al., 2022), and *Elymus sibiricus* L (Yang *et al.*, 2022) significantly inhibited seed germination and seedlings growth of their species under different continuous cropping durations. Studies on the rhizosphere soil leachates of P. *notoginseng* (Xiang *et al*., 2022), Lanzhou lily (Huang *et al*., 2020), and *Atractylodes lancea* (Wang *et al*., 2023) have revealed that the types and concentrations of autotoxins increased with increasing continuous cropping duration.

Despite extensive research supporting the adverse effects of autotoxicity on seed germination and seedlings growth of crops, according to our review of literature, there are few reports on autotoxicity in *C. lacryma-jobi.* It is currently unclear how soil affects the growth of *C. lacryma-jobi.* on seeds and seedlings after continuous cropping. Therefore, we hypothesized that because the root system of *C. lacryma-jobi* continuously secretes high amounts of compounds, which accumulate in the soil contributes autotoxicity, which would impede seed germination and young plants growth, ultimately causing a decrease in yield and quality. This study collected rhizosphere soil from *C. lacryma-jobi* under rotated, 4-year continuous, and 6-year continuous cropping systems to prepare aqueous extracts at various concentrations. Seed germination and seedling growth experiments were conducted to the hypothesis, measuring germination parameters, root length, shoot length, root vigor, growth parameters, and photosynthesis parameters of *C. lacryma-jobi* young plants in order to explored the relationship between continuous cropping and autotoxicity.

Materials and Methods

Soil samples

In 2021, soil samples of the Xingren Xiaobaikuo variety of *C. lacryma-jobi* were selected under rotational cropping (P0), continuous cropping for 4 years (P1), and continuous cropping for 6 years (P2) in Chengbei, Xingren City, Guizhou Province (25°26′N, 105°11′E). During the harvest period, a 5-point sampling method was employed to randomly select and excavate the plants. A brush was used to collect the soil adhering to the roots within a radius of 4 mm, which was then sieved through a 40-mesh sieve and retained for subsequent use.

Experimental design

Preparation of rhizosphere soil leachate

The determination of the concentration of root soil leachate was modified according to the pre experimental method of Huang *et al.* (2020):

To prepare the rhizosphere soil leachate, 50 g rhizosphere soil was placed into a 500 ml conical flask, followed by the addition of 250 ml sterile water. The flask was sealed and immersed in a constant temperature incubator set at 40 °C for 24 h. The flasks were shaken once every 12 h. The mixture was centrifuged at 6400 rpm for 3 min. The supernatant was collected to obtain a stock solution with a concentration of 0.200 g/ml Subsequently, this stock solution was diluted to obtain solutions with mass concentrations of 0.025, 0.050, and 0.100 g/ml The prepared solutions were stored at 4 °C for use in seed germination and young plant growth experiments.

Seed germination experiment

Seed germination experiments were conducted using the double-layer filter paper method. The filter paper was sterilized, and in each Petri dish, 50 peeled *C. lacryma-jobi* seeds that were full, uniform in size, and mature were evenly arranged. The experiment was designed using a two-factor completely randomized design: four concentrations, 0.025 (C1), 0.050 (C2), 0.100 (C3), and 0.200 g/ml (C4), of rhizosphere soil leachate from P0, P1, and P2 were set, with distilled water used for the control (CK). This resulted in 13 treatments, with five replicates each, for a total of 65 Petri dishes. Initially, 10 ml treatment solution was added to each Petri dish, and every 24 h, using the constant weight method, the evaporation loss of the leachate was replenished. Seed disinfection and incubation in a light-temperature incubator were conducted as described in

Chao *et al.* (2022). Seed germination was recorded daily after the appearance of sprouts (radicle extend of 2 mm from the seed coat was considered as the germination), and the germination process lasted for 8 d.

Young plant growth experiment

The young plant experiment was conducted using plastic pots (18 cm in diameter and 15 cm in height). Each pot was filled with 1 kg soil, and 10 pre-soaked *C. lacryma-jobi* seeds were uniformly sown in each pot. The experimental design was consistent with that of the seed germination experiment, comprising 13 treatments with 15 pots per treatment for a total of 195 pots. When the seedlings reached the two-leaf stage, four seedlings were retained in each pot. Initially, 100 ml rhizosphere soil leachate was applied to each pot. Next, every 5 d, depending on the weather conditions and soil moisture levels, an equal amount of water and rhizosphere soil leachate was added. This process was repeated six times over 30 d. throughout the experiment, watering was adjusted based on weather conditions and soil moisture levels to maintain the soil moisture content in the pots at 80% of the field capacity.

Measurement indices and methods

Measurement of seed germination indices

The relevant indicator calculation formula is as follows(Bai et al., 2023):

Germination index = ∑(Gt/Dt), where Gt represents the number of germinated seeds on a particular day, and Dt represents the number of days since seeding.

Germination potential = (number of seeds germinated within 4 d/total number of seeds) × 100.

Germination rate = (number of seeds with normal germination at the end of the experiment/total number of seeds) × 100

Vigor index = germination index × Sx, where Sx is the length of the seed embryo root.

On the 4th day of seed germination, soluble sugars, soluble proteins, protease activity, and α-amylase activity were determined using the anthrone-sulfuric acid, Coomassie Brilliant Blue staining, 3,5-dinitrosalicylic acid (Qian *et al*., 2012), and Folin phenol (Lowry *et al*., 1951) methods, respectively.

Anthrone-sulfuric acid: 0.1000 g of leaf tissue and put in a 10ml centrifuge tube. Add 8 ml of 80% ethanol and incubate in a water bath at 80 °C for 30 minutes. Centrifuge at 8000 r/min for 5 minutes. Repeat this process twice, then combine the supernatants and transfer them to a 50 ml volumetric flask, making up to the mark with solvent. Take 1.0 ml of the supernatant and set in a 10 ml centrifuge tube. Add 1 ml of distilled water and 5 ml of anthrone-sulfuric acid reagent (0.2 g anthrone dissolved in 100 ml of 98% sulfuric acid), and incubate in a boiling water bath for 15 minutes. Cool the mixture and measure the absorbance at 630 nm. Prepare a standard curve using glucose.

Coomassie Brilliant Blue staining: 0.5 g fresh sample, it was grinded into homogenate with 5 ml distilled water or buffer solution, then centrifugalized at 10000 r/min for 10 min, taking 1.0 ml supernatant liquid into 10 ml centrifugal tube, adding 5 ml coomassie brilliant blue G-250 solution, after fully mixing, placed 2 min, then determined the absorbance value under 595 nm. Using bovine serum albumin to make standard curve.

3,5-dinitrosalicylic acid: Refer to the methods determined by Li *et al*. (2024).

Folin phenol: Refer to the methods determined by Qian e*t al*. (2012).

Measurement of young plant growth indices

At 10 and 30 d post-treatment, measurements were conducted to evaluate young plant root vitality, plant height, stem diameter, leaf area, stem leaf dry weight, and root dry weight. Additionally, calculations were performed to determine the root-to-shoot ratio, relative growth rate, response index (RI), and synthesis effect (SE).

Root vitality was determined using the triphenyl tetrazolium chloride method: 0.5 g root tip samples, put them into 25 ml centrifugal tubes, add 10 ml equal mixture of 0.4% TTC solution and 0.067 (1/15) mol/L phosphate buffer solution (PH=7), immerse the roots fully in the solution, keep them in dark at 37 ℃ for 2h, then add 2 ml 1 mol/L sulfuric acid (at the same time, do a blank experiment, add sulfuric acid first, then add root samples, the operation steps are the same), to stop the reaction. Take out the root tip segments, dry their surface with filter paper, put the colored root tip segments into a mortar, add 4 ml ethyl acetate, grind fully to extract triphenylformazan (TTF), slowly move the red TTF liquid into a 10 ml capacity bottle, wash the root residue in the mortar with a small amount of ethyl acetate, move the washing liquid into the capacity bottle together, and add ethyl acetate solution to the constant volume. Read the absorbance value of the root system to be tested under the spectrophotometer at 485 nm with the blank test as a reference.

Plant height was measured from the base of the main stem to the top of the plant by using a measuring tape. Stem diameter was measured using a Vernier caliper at the middle of the first internode of the main stem. The dry weight was determined for the three representative plants selected, and their stems and leaves were separated. After dehydration at 105 °C for 30 min and drying to a constant weight at 80 °C, dry weight was measured. The area of fully expanded leaves was determined using an LI-3100C desktop leaf area meter. The ratio of root dry weight to the sum of stem and leaf dry weights was calculated as follows: root-to-shoot ratio = root biomass / (stem biomass + leaf biomass); relative growth rate = $(\ln W2 - \ln W1) / (t2 - t1)$, where W1 and W2 are the initial and final dry weights, respectively, and t1 and t2 are the initial and final times (days), respectively.

Using the method proposed by Williamson and Richardson (1988), the response index (RI) was calculated, and based on this, the synthesis effect (SE) was derived. The formula for calculating the RI is as follows: $RI = 1 - CK/T$ ($T \geq CK$) or $RI = T/CK - 1$ ($T < CK$), where T represents the data of the treatment group, and CK represents the data of the control group. A positive RI value indicated a promoting effect; a negative RI value indicated an inhibitory effect. Calculating the germination potential, germination rate, germination index, seed vigor, root length, shoot length, soluble sugar content, soluble protein content, protease activity and α-amylase RI of seeds during germination period, as well as root vitality, height, stem diameter, leaf area, stem and leaf dry weight, root dry weight, root shoot ratio, relative growth rate and photosynthesis parameter RI of young plant.

The SE was computed as the average of the RI of various test items under the same treatment. An $SE > 0$ indicated a promoting effect, and an $SE < 0$ indicated an inhibitory effect. The magnitude of the absolute value represented the intensity of SE.

Measurement of young plant photosynthetic indices

At 25 d post-treatment, three representative young plant from each treatment were selected for measurement. On clear days between 09:00 and 12:00, the net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO2 concentration (Ci), and transpiration rate (Tr) of the second fully expanded leaf from the top of the plant were determined using an LI-6400XT portable photosynthetic instrument (LI-COR, Lincoln, NE, USA).

Statistical analysis

For each assay, all the experiments were repeated three times. The results are presented as means ± SE (the error bars represent standard errors of the mean ($n = 3$)). For statistical analysis, we performed ANOVA tests by using IBM SPSS Statistics 26 software. Differences between the treatments were tested using the least significant difference (LSD) test at the 0.05 and 0.01 probability level, and Origin 2021 was used for graphing.

Results

Effect of continuous cropping years and leachate concentration on seed germination, root and shoot length

Continuous cropping years significantly affected C. lacryma-jobi seed germination (Table 1). The continuous cropping years and leachate concentration had a significant effect on the seed germination potential, germination rate, vigor index, and root length. There was a highly significant interaction effect of concentration and continuous cropping years on the germination index, and continuous cropping years, concentration, and their interaction significantly influenced shoot length. At the same concentration, the germination potential, germination rate, and vigor index under P2 were significantly lower than those under P0, with no significant difference from those under P1, and P1 and P0 showed no significant differences. Root lengths under P2 and P1 were significantly lower than those under P0, with no significant difference between P2 and P1. Shoot lengths under P2 were significantly lower than those under P1 and P0 and shoot lengths under P1 were significantly lower than those under P0. For the same continuous cropping years, the germination rate initially increased and then decreased as the leachate concentration increased. This value peaked under C1, with a nonsignificant increase of 5.39% compared with that under CK, and the lowest value was observed under C4, which was significantly decreased by 9.08% compared with that under CK ($P < 0.05$). The germination index, germination potential, vigor index, root length, and shoot length all showed a continuously decreasing trend, reaching the lowest values under C4 and significantly decreasing by 15.98%, 18.80%, 39.53%, 27.52%, and 49.35%, respectively ($P < 0.05$), compared with those under CK.

Table 1. Impact of rhizosphere soil leachate from *Coix lacryma-jobi* under different continuous cropping years on seed germination

Factor	Level	Germination index (grains/d)	Germination potential (%)	Germination rate (%)	Vigor index	Root length (cm)	Shoot length (cm)
P	P0	17.42±2.00a	84.13±6.78a	91.07±4.71a	222.95±46.48a	12.67±1.35a	13.18±3.04a
	P1	17.82±1.61a	82.53±6.82a	90.00±5.86a	210.26±41.35ab	11.71±1.47b	12.55±2.98b
	P2	17.41±1.87a	79.47±9.81b	87.60±6.51b	194.20±47.95b	11.12±2.26b	10.97±3.91c
C	CK	18.90±0.09a	88.67±4.36a	90.67±2.65a	254.72±18.82a	13.48±1.06a	16.07±0.59a
	C1	18.33±2.21a	88.00±3.16a	95.56±1.67a	244.32±38.97a	13.29±0.89a	14.96±0.86b
	C2	18.23±0.91a	86.00±3.87a	93.56±2.96b	215.99±23.80b	11.85±1.14b	12.78±1.96c
	C3	16.42±0.60b	75.56±4.45b	85.56±4.10c	176.61±10.34c	10.78±0.90c	9.22±1.53d
	C4	15.88±2.01b	72.00±4.47c	82.44±3.84d	154.04±25.28d	9.77±1.64d	8.14±1.27e
F	P	0.605	6.5**	5.6**	11.448**	5.892**	35.284**
	C	11.293**	41.257**	31.921**	28.348**	31.749**	198.861**
	P*C	3.401**	1.08	0.784	1.647	0.805	3.64**

Note: Means with different superscript lowercase letters indicate significant differences between different concentrations and planting years at $P < 0.05$ (LSD test). ** indicates highly significant differences ($P < 0.01$).

Soluble sugar, soluble protein, α-amylase and protease activities during germination

The contents of soluble sugars, soluble proteins, and related enzyme activities during the seed germination period were significantly influenced by the different continuous cropping years and concentrations tested (Figure 1). At various concentrations, the contents of soluble sugars, soluble proteins, and related enzyme activities under P2 and P1 were lower than those under P0. The α-amylase and protease activities under P2 were significantly reduced by 28.44% and 41.44%, respectively, compared with those under P0 at C4, with no significant difference from those under P1. For the same continuous cropping years, the contents of soluble sugars, soluble proteins, protease activities, and α-amylase decreased with increasing concentrations of leachate (Figure 1). The contents of soluble sugars, soluble proteins, protease activities, and α-amylase of P0, P1, and P2 reach the lowest levels at concentration C4, significantly decreasing by 31.86%, 42.17%, 39.29%, and 30.2% (P0); 39.13%, 40.47%, 56.18%, and 58.84% (P1); and 68.64%, 53.98%, 66.23%, and 73.05% (P2) compared with those under CK ($P < 0.05$).

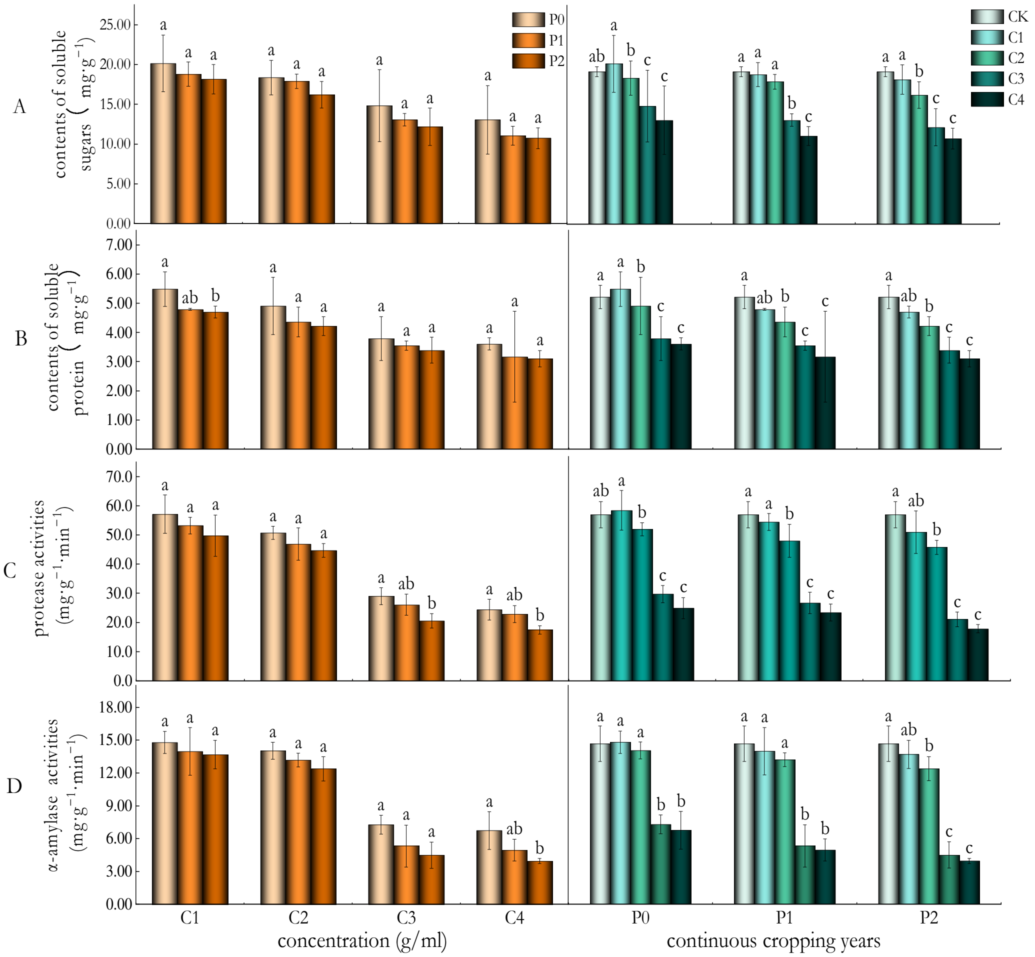

Figure 1. Effects of continuous cropping years on soluble sugars, soluble proteins, protease activity, and α-amylase activity of *Coix lacryma-jobi* seeds at the germination stage. Error bars are one standard error of the mean

Data values are the means ± SE of three independent biological samples. The error bars represent standard errors of the mean (n = 3). Different letters indicate significant differences according to LSD tests ($P < 0.05$).

Effect of continuous cropping years and leachate concentration on young plant growth

Effect on root vitality

Young plant root vitality decreased with increasing continuous cropping years and concentration (Figure 2). At various concentrations, the root vitality of young plant treated with rhizosphere soil leachate under P1 and P2 was lower than that under P0. At 10 d under C4, there was a significant difference between P2 and P0 ($P < 0.05$), and there was no significant difference between P1 and P0 or between P2 and P1. At 30 d under C4, young plant root vitality exhibited a significant decrease of 28.28% under P2 compared with that under P0 ($P < 0.05$) (Fig2 A). The vitality of P2 was 13.84% lower than that under P1, and the vitality under P1 was 16.76% lower than that under P0, but the differences were not significant. At 10 d post-treatment, under the same continuous cropping years, the lowest value was observed under C4, and the C4 treatment of P0, P1, and P2 showed significant decreases in root vitality (52.68%, 60.82%, and 73.81%, respectively) compared with those under CK. At 30 d post-treatment, there were significant decreases in root vitality of 46.81%, 55.72%, and 61.85% under C4 under P0, P1, and P2, respectively ($P < 0.05$), compared with that under CK (Figure 2 B).

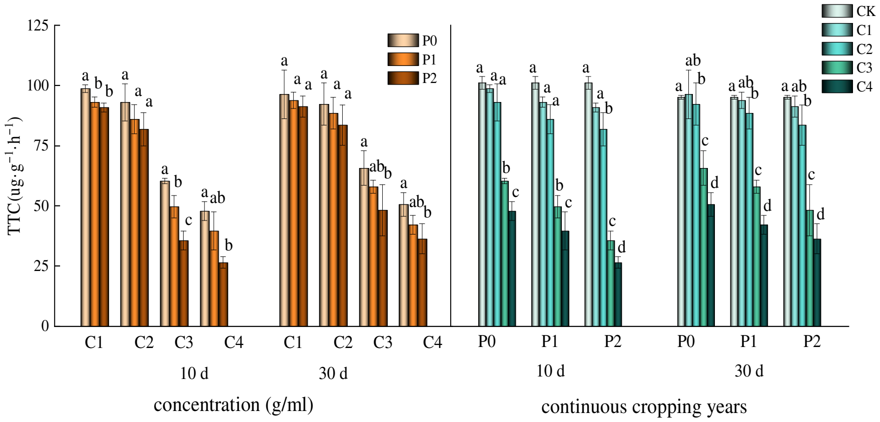

Figure 2. Effects of continuous cropping years and concentration on root vitality of *Coix lacryma-jobi* young plant

Error bars are one standard error of the mean. Data values are the means ± SE of three independent biological samples. The error bars represent standard errors of the mean ($n = 3$). Different letters indicate significant differences according to LSD tests ($P < 0.05$).

Effect on young plant growth

Table 2 details the impact of the duration of cultivation and the concentration of rhizosphere soil leachate on various growth parameters, including plant height, stem and leaf dry weights, root dry weight, and relative growth rate. There was a notable influence of continuous cropping years, leachate concentration, and their interaction on stem diameter and leaf area. Across the same concentrations tested, young plant growth metrics consistently decreased over prolonged continuous cropping years. P2 treatment consistently demonstrated the lowest values for plant height, stem diameter, leaf area, stem and leaf dry weights, root dry weight, root-to-shoot ratio, and relative growth rate. Significant reductions in plant height, root dry weight, and relative growth rate were evident under P2 compared with those under P0, with no significant differences observed between P1 and P0. Similarly, the stem diameter, leaf area, stem and leaf dry weights, and relative growth rate were significantly lower under P1 and P0 than under P0, although there were no significant differences in the relative growth rate. Notably, across all concentrations, C4 exhibited the most pronounced inhibitory effect on young plant growth after 30 d, with significant reductions observed in all growth parameters compared with those under the CK.

Table 2. Impact of rhizosphere soil leachate from *Coix lacryma-jobi* with varied n *Coix lacryma-jobi* young plant growth

Factor	Level	Height/cm		Stem thickness/mm		Leaf area（cm^2/plant）	
		10 d	30 d	10 d	30 d	10 d	30 d
P0	CK	41.63±1.31a	61.80±2.14a	7.34±0.65a	10.73±0.40ab	89.54±1.41a	238.85±11.03a
	C1	44.20±2.03a	64.03±6.22a	7.58±0.56a	11.68±1.08a	89.51±3.46a	237.52±6.56a
	C2	41.77±4.45a	61.47±1.10a	7.00±0.11a	10.38±0.82b	84.22±4.61b	232.41±12.79b
	C3	35.57±3.80b	56.40±5.62b	5.28±0.68b	8.73±0.18c	55.15±4.72c	189.46±5.73c
	C4	31.30±3.03b	53.03±3.15c	4.93±0.54b	7.91±0.17c	50.41±5.41d	175.19±6.46c
P1	CK	41.63±1.31a	61.80±2.14a	7.34±0.65ab	10.73±0.40a	89.54±1.41a	238.85±11.03a
	C1	39.97±1.32a	59.30±1.99a	7.05±0.12ab	9.33±0.54ab	85.50±4.11a	225.88±23.24ab
	C2	38.37±2.34a	54.33±3.59b	6.55±0.47b	8.74±0.28b	82.34±6.13b	219.23±4.01b
	C3	34.37±3.56b	50.93±0.67c	4.98±0.17c	7.97±0.16c	45.07±2.67c	166.49±11.30c
	C4	30.00±0.96c	48.87±4.13d	4.53±0.37c	7.47±0.73c	40.07±6.31c	163.02±4.31c
P2	CK	41.63±1.31a	61.80±2.14a	7.34±0.65a	10.73±0.40a	89.54±1.41a	238.85±11.03a
	C1	38.30±2.46ab	58.63±1.34ab	6.90±0.66a	9.17±0.33b	81.10±3.05b	212.13±25.85b
	C2	34.83±5.09b	52.67±1.68b	6.22±0.88b	8.18±0.43b	77.45±1.99b	210.03±11.46b
	C3	30.50±1.47c	48.97±1.93c	4.44±0.26c	7.13±0.60c	39.87±4.29c	142.38±10.18c
	C4	28.60±0.66d	48.30±2.89c	4.05±0.27c	6.42±0.83c	32.95±3.02d	139.20±12.82c
P	P0	59.35±5.35a		9.89±1.50a		214.68±28.35a	
	P1	55.05±5.48b		8.85±1.22b		202.69±33.89b	
	P2	54.07±5.68b		8.33±1.61c		188.52±42.93c	
C	CK	61.8±1.80a		10.73±0.33a		238.85±9.27a	
	C1	60.66±4.07a		10.06±1.33b		225.18±20.21b	
	C2	56.16±4.40b		9.10±1.07c		220.55±12.75b	
	C3	52.10±4.35c		7.94±0.74d		166.11±21.29c	
	C4	50.07±3.61c		7.27±0.84e		159.13±17.01c	
F	P	24.25**		60.95**		31.96**	
	C	48.79**		119.60**		149.90**	
	C*P	1.95		6.57**		2.98**	

Table 2. Continued

Stem and leaf dry weight (g/plant)		Root dry weight (g/plant)		Root-crown ratio		Relative growth rate (g/g·d)	
10 d	30 d	10 d	30 d	10 d	30 d	10 d	30 d
0.342±0.019ab	2.103±0.042a	0.174±0.010a	0.465±0.032a	0.510±0.017a	0.221±0.011a	0.108±0.005ab	0.090±0.010a
0.379±0.048a	2.123±0.009a	0.177±0.006a	0.487±0.054a	0.537±0.147a	0.230±0.025a	0.116±0.009a	0.090±0.001a
0.334±0.070b	2.091±0.132a	0.173±0.006a	0.453±0.050a	0.491±0.086b	0.216±0.011b	0.106±0.013b	0.089±0.002a
0.290±0.080c	1.565±0.174b	0.134±0.008b	0.329±0.025b	0.484±0.123c	0.213±0.036b	0.088±0.019c	0.079±0.003b
0.246±0.009c	1.507±0.076b	0.121±0.025c	0.315±0.079b	0.471±0.049c	0.208±0.046c	0.074±0.009d	0.078±0.003b
0.342±0.019a	2.103±0.042a	0.174±0.010a	0.465±0.032a	0.510±0.017a	0.221±0.011a	0.108±0.005a	0.090±0.010a
0.337±0.064a	2.030±0.232a	0.170±0.021ab	0.443±0.028ab	0.521±0.138a	0.219±0.019ab	0.106±0.010a	0.088±0.003a
0.326±0.055a	1.939±0.064a	0.163±0.014b	0.399±0.005b	0.504±0.041a	0.206±0.006b	0.102±0.014a	0.087±0.001a
0.270±0.042b	1.506±0.017b	0.123±0.023c	0.277±0.056c	0.471±0.168b	0.184±0.035c	0.081±0.005b	0.078±0.001b
0.230±0.031b	1.428±0.189b	0.111±0.021c	0.257±0.036c	0.488±0.112b	0.185±0.051c	0.067±0.011c	0.076±0.003b
0.342±0.019a	2.103±0.042a	0.174±0.010a	0.465±0.032a	0.546±0.160a	0.221±0.011a	0.108±0.005a	0.090±0.010a
0.334±0.092a	1.957±0.057ab	0.165±0.011a	0.424±0.026a	0.529±0.190ab	0.217±0.017a	0.104±0.019a	0.087±0.001ab
0.310±0.086a	1.892±0.089b	0.160±0.001b	0.399±0.005b	0.510±0.017b	0.211±0.010a	0.098±0.018a	0.086±0.001b
0.236±0.054b	1.324±0.072c	0.100±0.007c	0.257±0.022c	0.489±0.062c	0.195±0.026b	0.065±0.015b	0.074±0.001c
0.196±0.020c	1.265±0.114c	0.099±0.009c	0.202±0.015d	0.441±0.111d	0.160±0.003c	0.052±0.008b	0.071±0.003c
1.10±0.82a		0.28±0.14a		0.36±0.16a		0.0918±0.0152a	
1.05±0.79b		0.26±0.13b		0.35±0.17a		0.0882±0.0146a	
1.00±0.77c		0.24±0.13b		0.35±0.17a		0.0835±0.0192b	
1.22±0.91a		0.32±0.15a		0.37±0.15a		0.0990±0.0102a	
1.19±0.87ab		0.31±0.15a		0.36±0.17a		0.0986±0.0136a	
1.15±0.85c		0.29±0.13b		0.37±0.18a		0.0947±0.0122a	
0.87±0.63d		0.20±0.09c		0.33±0.16a		0.0773±0.0112b	

0.81±0.61d	0.18±0.09c	0.34±0.17a	0.0696±0.0106c
10.43**	13.47**	0.06	7.13**
90.24**	87.18**	0.83	44.26**
0.89	1.24	0.13	0.87

Note: Means with different superscript lowercase letters indicate significant differences between different concentrations and planting years at P < 0.05 (LSD test). ** indicates highly significant differences (P < 0.01).

Effect of continuous cropping years and leachate concentration on photosynthetic characteristics

Both continuous cropping years and leachate concentration significantly affected the net Pn, Gs, and Tr of the young plant, and the concentration significantly affected the Ci (Figure 3). At the same concentration, Pn, Gs, and Tr gradually decreased with increasing continuous cropping years, and Ci exhibited the opposite trend. When the concentration exceeded C3, the Pn, Gs, and Tr under P2 were significantly lower than under P0, with no significant difference compared with those under P1. The Pn was significantly lower under P1 than under P0, Gs and Tr showed no significant difference, and Ci remained unaffected (fig3 A-D). Under the same continuous cropping years, Pn, Gs, and Tr decreased gradually with increasing concentration, reaching their lowest level under C4, and Ci exhibited the opposite trend. The Pn, Gs, and Tr under P0, P1, and P2 under C4 were significantly lower than those under CK by 27.24%, 47.13%, and 55.09% (Pn); 21.57%, 36.19%, and 51.00% (Gs); and 25.64%, 40.78%, and 49.96% (Tr), respectively, and the Ci was significantly higher by 67.35%, 86.73%, and 101.24% under P0, P1, and P2 under C4, respectively (P < 0.05) (Figure 3 E-H). Soil leachate promoted Ci, although there was no significant difference among the continuous cropping years (Figure 3 C). As Ci increased, intercellular CO2 pressure increased, resulting in a decrease in Pn.

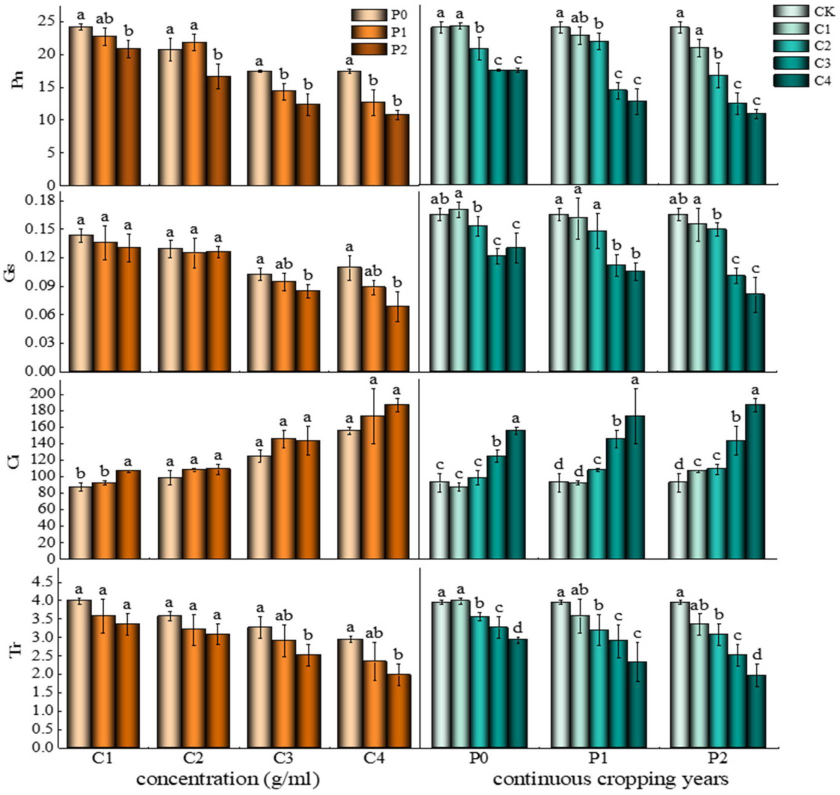

Figure 3. Effects of continuous cropping years and leachate concentration on photosynthesis of *Coix lacryma-jobi* young plant

Error bars are one standard error of the mean. Data values are the means ± SE of three independent biological samples. The error bars represent standard errors of the mean (n = 3). Different letters indicate significant differences according to LSD tests (P < 0.05).

Comprehensive allelopathic effects of rhizosphere soil leachate on germination and growth

To comprehensively evaluate the autotoxic effects of *C. lacryma-jobi* rhizosphere soil leachate on seed germination and young plant growth, we assessed allelopathic effects by calculating the RI of seed germination and young plant growth indices, resulting in an SE. The results indicated that except for P0 under C1, which showed a positive SE, promoting seed germination and young plant growth, all other treatments exhibited negative SE values, indicating inhibition of seed germination and young plant growth (Figure 4). Under C1, the SE under P2 and P1 was significantly lower than that under P0 ($P < 0.01$), with that under P2 being significantly lower than that under P1, and that under P1 being significantly lower than that under P0 ($P < 0.05$). Under C2 and C3, the SE under P2 was significantly lower than that under P1 and P0 ($P < 0.01$), with that under P1 being significantly lower than that under P0 ($P < 0.05$). Under C4, the SE under P2 was significantly lower than that under P1 and P0, and that under P1 was significantly lower than that under P0 ($P < 0.01$) (Figure 4 A). For each continuous cropping years, the SE under P0 showed an initial increase, followed by a decrease with increasing concentration, and that under P1 and P2 showed a continuous decrease, reaching the lowest values at C4 (Figure 4 B). Extremely significant differences in SE were observed among all treatment concentrations for all continuous cropping years ($P < 0.01$). The ranking of the comprehensive allelopathic effects of *C. lacryma-jobi* rhizosphere soil leachate on seed germination and young plant growth varied as follows: P2 < P1 < P0, with inhibitory effects showing an opposite trend to that of SE.

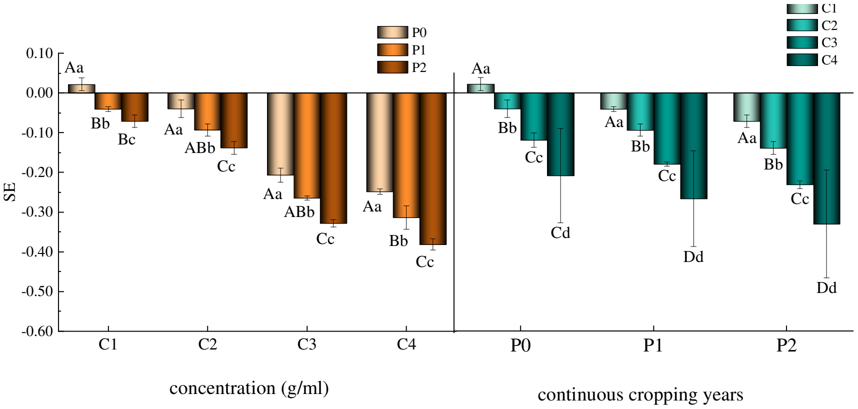

Figure 4. Synthesis effects of continuous cropping years and leachate concentration of *Coix lacryma-jobi* on seed germination and young plant growth

Error bars are one standard error of the mean. Data values are the means ± SE of three independent biological samples. The error bars represent standard errors of the mean ($n = 3$). Different lowercase letters indicate significant differences ($P < 0.05$), and different uppercase letters indicate highly significant differences ($P < 0.01$).

Discussion

Effect of rhizosphere soil leachate from different planting years on seed germination、*root and shoot length*

With the limited soil resources, increased intensive cultivation, and economic benefits, continuous cropping in the same field is widespread in China and even the world (Xiong *et al.*, 2024). Continuous cropping is prone to cause continuous cropping obstacles, and one of the key causes of continuous cropping obstacles is the autotoxic effect caused by the accumulation of autotoxic substances in continuous cropping soil (Qiu *et al.*, 2024). Autotoxicity is one of the reasons that restrict the sustainable development of *C. lacryma-jobi* industry. It is of great significance to explore the autotoxicity of *C. lacryma-jobi* to overcome the obstacles of continuous cropping. Under the influence of leachate prepared from the rhizosphere soil of *C. lacryma-jobi*, the seed germination potential, germination rate, germination index, root length, and shoot length all decreased with the increase in continuous cropping years and concentration. This result is similar to the findings in studies on wheat (*Elymus sibiricus*) (Yang *et al.*, 2022), Lanzhou lily (Huang *et al.*, 2020), *Polygonum capitatum* Buch. Ham ex Don (Liu *et al.*, 2019) and tea trees (*Camellia sinensis*) (Ye *et al.*, 2016). The inhibitory effect of continuous cropping for 6 years was the most obvious at 0.200 g/ml, which may be due to the differences in the types and quantities of autotoxic substances in the in the leachate prepared from rhizosphere soil(Huang *et al.*, 2020). This study also demonstrated that the SE leachate Prepared from the rhizosphere soil under the same concentration was smaller in the rotation treatment compared to the 6-year continuous cropping treatment. It states that the types and quantities of autotoxic substances in the rhizosphere soil of 6-year continuous cropping were more than that of rotation. Seed germination requires a substantial amount of energy and materials derived from the oxidation of stored materials, of which soluble sugars and starch play crucial roles *(*Rajjou *et al.*, 2012; Zhang *et al.*, 2020). The leachate prepared from rhizosphere soil reduced the contents of soluble protein and soluble sugar and inhibited the activities of protease and α -amylase. These findings are similar to those observed in melon (*Cucumis melo* L.)(Zhang *et al.*, 2020). The results indicate that the rhizosphere soil of *C. lacryma-jobi* has an autotoxic effect on seed germination. Seed germination is affected not only by autotoxicity but also by salt stress. Papastylianou (2018) et al. studied the effects of salt stress (NaCl) on the seed germination of Black Cumin (*Nigella sativa* L.) and found that salt stress inhibits the germination parameters of black cumin seeds.

Effect of rhizosphere soil leachates from different planting years on young plant growth

Seedling growth is one of the two critical stages in the plant growth cycle that determines whether the plant can grow normally (Papastylianou *et al.*, 2018).The accumulation of autotoxins in the soil can directly damage roots and inhibit plant nutrient uptake, and enzyme activity, profoundly affecting plant growth and development (Zhang *et al.*, 2022). Root exudates from peony (Zhang *et al.*, 2022) and *alfalfa* (Wang *et al.*, 2022) inhibit seedling growth. Root exudates from peony (Zhang *et al.*, 2022), cucumber(Huang et al., 2020), and alfalfa (Zhang *et al.*, 2022)disrupt the expression of genes related to ROS scavenging in root tips, reducing root vitality and root cell length. This leads to a decrease in root length, root surface area, and root volume, thereby inhibiting root growth. Our results indicated that rhizosphere soil leachates from *C. lacryma-jobi* inhibit root vitality, young plant height, stem diameter, leaf area, stem and leaf dry weights, and photosynthesis. Sensitivity to autotoxins was the highest in roots, followed by leaves and stems, suggesting that autotoxins mainly inhibit young plant growth by suppressing root elongation. The degree of inhibition was positively correlated with continuous cropping years, leachate concentration, and treatment day, while the SE was negatively correlated with continuous cropping years, indicating that long planting periods are detrimental to seed germination and young plant growth. These results demonstrate the autotoxic effects of *C. lacryma-jobi*. rhizosphere soil on seed germination and young plant growth, with the autotoxic effects becoming more pronounced with increasing continuous cropping years. This is consistent with the findings of studies on *Medicago truncatula* (Wang *et al.*, 2022), ginseng *(Angelica sinensis*) (Xin *et al.*, 2019), *Pinellia ternate* (Thnub.)(Liu *et al.*, 2018), licorice (*Glycyrrhiza uralensis*) (Ren *et al.*, 2017), Lanzhou lily (Wu *et al.*, 2015). The results indicate that the rhizosphere soil of *C. lacryma-jobi* has an autotoxic effect on young plant

Effect of rhizosphere soil leachates from different planting years on young plant growth photosynthesis parameters

Photosynthesis is the physiological and metabolic foundation for the formation of plant biomass (Chen *et al.*, 2009), which is susceptible to the effects of allelopathic autotoxicity (Zhang *et al.*, 2022). Inhibition or destruction of the synthesis mechanism and accelerated decomposition of photosynthetic pigment may be the main effects of allelopathy or autotoxicity on plant photosynthesis (Zhang *et al.*, 2022). The results showed that rhizosphere soil leachates from *C. lacryma-jobi* reduced the photosynthetic rate, stomatal conductance and transpiration rate of young plant, which were the lowest under the treatment of 0.200g/ml continuous cropping for 6 years, and significantly increased the intercellular CO_2 concentration (Figure 3). It is speculated that non-stomatal limitations reduce the amount of CO_2 consumed by the plant, leading to a significant accumulation of CO_2 within the cells. This reduces the accumulation of organic matter and affects young plant growth. Ye *et al*. (2016) collected soil samples from continuous cropping for 4 years, 9 years, and 30 years, respectively. Soil extracts were prepared, determined soil toxicity by seed germination, and determined photosynthetic parameters of seedlings after transplanting them into continuously cropped soil for one year. The results indicated that continuously cropped soil reduced the photosynthesis of tea young plant. Wang *et al*. (2022) conducted seedling growth experiments using root exudates of alfalfa at different concentrations. The results showed that the transpiration rate decreased by 59.65% to 73.80%, the net photosynthetic rate decreased by 80.72% to 86.08%, and the stomatal conductance decreased by 65.6% to 77.98%. Zhang (2022) et al. treated seedlings with leachate prepared from melon roots at different concentrations. The results showed that the net photosynthetic rate, stomatal conductance, and transpiration rate of the seedlings decreased as the concentration of the extract increased, indicating that the water extract of melon roots has an autotoxic effect on the photosynthesis of the seedlings. Our results of this experiment showed that the rhizosphere soil of *C. lacryma-jobi* inhibited the photosynthesis of young plant, which was similar to the results of tea trees, Melon and *Medicago truncatula*. The results indicate that the rhizosphere soil of *C. lacryma-jobi* has an autotoxic effect on photosynthesis.

In the future

Vanillin, dibutyl phthalate, ferulic acid and dioctyl phthalate have been identified from the rhizosphere soil leachates from *Medicago sativa* (Zhang *et al*., 2024), *Solanum tuberosum* (Ma et al., 2023), *Pinellia ternata* (Xiang *et al*., 2022), *Angelica sinensis* (Xin *et al*., 2019) and *Lilium davidii* var. unicolor (Wu *et al*., 2015), and their toxicity has been determined to be autotoxic. Currently, measures to alleviate replant problems in agriculture include suitable crop rotation, intercropping, proper fertilization, plant growth regulators, and functional microorganisms (Zhang *et al*., 2024). The research findings provide guidance for our future studies on the mechanisms of autotoxicity and the alleviation of replant problems in continuous cropping of *C. lacryma-jobi*.

Conclusions

Rhizosphere soil leachates from *C. lacryma-jobi*. inhibit seed germination and young plant growth. The SE of the rhizosphere soil leachates from *C. lacryma-jobi*. under different continuous cropping years followed the sequence: continuous cropping for 6 years < continuous cropping for 4 years < rotation. These findings confirm our hypothesis that continuous cropping of *C. lacryma-jobi*. induces autotoxicity. The research results reveal key factors contributing to continuous cropping obstacles and provide a scientific basis for developing sustainable agricultural strategies. This will help increase crop yields in *C. lacryma-jobi* planting areas, improve soil health, and promote the development of more resilient agricultural systems.

Authors' Contributions

Guiqin Zhou: Data curation, Investigation, Methodology. Lei Li: Data curation, Investigation. Bi Song: Conceptualization, Data curation, Investigation, Writing – review & editing, Funding acquisition. Yi Cheng: Data curation, Investigation. Min Liu: Data curation, Investigation. Yinying Liu: Data curation, Investigation. Dailing Liu: Data curation, Investigation. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (32160516), Guizhou Science and Technology Program (Qiankehe Support [2021] General 270), the Science and Technology Innovation Talent Team for Research on Cultivation and Physiology and Ecology of Special Cereal and Oil Crops in Guizhou Province (Qiankehe Platform Talent Project [2019]5613), Guizhou Key Laboratory of Molecular Breeding of Cereal and Oil Crops Project (Qianke Hezhong Cited Land [2023]008), and the Key Laboratory Project of Functional Agriculture in Guizhou Higher Education Institutions (Guizhou Education and Technology [2023]007). We would like to thank Editage (*www.editage.cn*) for English language editing.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Bai GW, Sharentuya, Ji YY, Wu, Xing ZY, Z., Xing YK, … Liu J (2023). Effects of salt and drought stress on seed germination of *Morus alba*. Journal of Inner Mongolia Forestry Science and Technology 49(04):16-20. *https://doi.org/ 10.3969/j.issn.1007-4066.2023.04.004*

Bai QY, Chen DP, Liu L, Zhou L, Che Y (2024). Research on e-commerce to help agriculture based on rural revitalization strategy—taking *Xingren coix* seed as an example. Grain Oil and Feed Technology 04:109-111.

Chen YG, Zhang M, Wang DL, Zhu SD (2009). Effects of the Continuous cropping soil on chlorophyll contents and photosynthesis characteristics of the melon. Journal of Anhui Agricultural Sciences 37(03):956-958. *https://doi.org/ 10.3969/j.issn.0517-6611.2009.03.019*

Huang YF, Zhang EH, Zhang XH, Wang HZ, Wang Q, Liu QL, … Xu SR (2020). Autotoxicity of water extracts from continuous cropping soil of *Lilium davidii* var. *unicolor* Salisb. Journal of Northwest A & F University (Natural Science Edition) 48(07):84-93 *https://doi.org/10.13207/j.cnki.jnwafu.2020.07.010*

Li HT, Lv JM, Su Y, Wu YY (2023). Appropriate sodium bicarbonate concentration enhances the intracellular water metabolism, nutrient transport and photosynthesis capacities of *Coix lacryma-jobi* L. Agronomy 13(7). *https://doi.org/10.3390/agronomy13071790*

Li J, Li J, Yang P, P., Fu HB, Yang YC, Liu CW (2024). Screening of germplasm and construction of evaluation system for autotoxicity tolerance during seed germination in cucumber. Agronomy 14(5):1081. *https://doi.org/10.3390/agronomy14051081*

Li L, Fu JF, Wei S, Zeng T, Wei HL, H., Zhang J, … Song B (2023). Effects of different continuous cropping years on soil characteristics, yield and quality of *Coix lacryma-jobi* L. Soil and Fertilizer Sciences in China (06):87-96.

Li PT, Zhang JP, Liu YH, Niu ZZ, Zhao TT, Sun SG, … Wang ZX (2024). Fermentation condition optimization of chicken bone with *Bacillus subtilis* for production of antioxidant peptides. Food Research and Development 45(21):108-117.

Liu JK, Lyu PL, Wu C, Liu F, Zhao X, Tang H. (2024). The effects of different soil substrates on the growth and root coixol content of local *Coix* varieties in China. Agronomy 14(8):1792. *https://doi.org/10.3390/agronomy14081792*

Liu JK, Yan ZQ, Li XZ, Jin H, Yang XY, X., Xie M, … Qin B (2018). Characterization of allelochemicals from the rhizosphere soil of *Pinellia ternate* (Thnub.) and their inhibition activity on protective enzymes. Applied Soil Ecology 125:301-306. *https://doi.org/10.1016/j.apsoil.2018.01.001*

Liu M, Zeng T, Wei S, Fu JF, Cheng Y, Liu DL, & Song B. (2024). Effects of nitrogen, phosphorus and potassium fertilizers on the characteristics of grain filling and fruiting in different parts of *Coix lacryma-jobi* L. Chinese Journal of Eco-Agriculture 32(03):405-420. *https://link.cnki.net/urlid/13.1432.S.20231101.1044.001*

Liu Y, Liu Y, Wanng XX, Yang D, Zhao YP, Hu MF (2019). Allelopathic autotoxicity of aqueous extracts from *Polygonum capitatum* whole plant and its rhizosphere soil. Jiangsu Agricultural Sciences 47(17):266-270 *https://link.cnki.net/doi/10.15889/j.issn.1002-1302.2019.17.065*

Możdżeń K, Barabasz KB, Stachurs SA, Turisová I, Zandi P (2021). Germination and growth of radish under influence of nipplewort aqueous extracts. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 49(1):12195-12195. *https://doi.org/10.15835/nbha49112195*

Papastylianou P, Bakogianni NN, Travlos I, Roussis L. (2018). Sensitivity of seed germination to salt stress in black cumin (*Nigella sativa* L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca 46(1):202-205. *https://doi.org/10.15835/nbha46110861*

Qi L, Sun QM, Xu-WJ, Cheng XJ, Guo ZS, Chen LD (2024). Effects of biochar synergized with dazomet fumigation on growth of continuous cropping watermelon. China Cucurbits and Vegetables 37(11):107-113. *https://link.cnki.net/doi/10.16861/j.cnki.zggc.2024.0043*

Qian ZG, Pan SK, Wu SJ (2012). Determination via hydrolysis with α-amylase of curdlan in the presence of proteins. Carbohydrate Polymers 88(1):389-390. *https://doi.org/10.1016/j.carbpol.2011.11.084*

Qiu YJ, Shao XL, Chen YY, Huang YF, Chen HG, Yang FD, … Gao J (2024). Research on effects of continuous cropping on the growth, physiological characteristics and autotoxic substances of *Codonopsis pilosula* var. *modesta*. Acta Prataculturae Sinica 1-11. *https://doi.org/ 10.11686/cyxb2024068*

Rajjou L, L., Duval M, Gallardo K, Catusse J, Bally J, Job C, Job D (2012). Seed germination and vigor. Annual Review of Plant Biology 63(1):507-533. *https://doi.org/10.1146/annurev-arplant-042811-105550*

Ren X, Yan ZQ, He XF, Li XZ, Qin B (2017). Allelochemicals from rhizosphere soils of *Glycyrrhiza uralensis* Fisch: Discovery of the autotoxic compounds of a traditional herbal medicine. Industrial Crops and Products 97:302-307. *https://doi.org/10.1016/j.indcrop.2016.12.035*

Tang WJ, Xu B, Cai L, Ao MH, Ding N, Wei SH (2023). Effects of different forms of nitrogen fertilizer on yield components and yield formation of coix (*Coix lacryma-jobi* L.). Journal of Yunnan Agricultural University (Natural Science) 38(04):652-658. *https://link.cnki.net/urlid/53.1044.S.20230817.1447*

Wang C, C., Liu Z, Wang ZC, Pang WH, Zhang L, L., Wen ZZ, … Yang C (2022). Effects of autotoxicity and allelopathy on seed germination and seedling growth in *Medicago truncatula*. Frontiers in Plant Science 13:908426. *https://doi.org/10.3389/fpls.2022.908426*

Wang FY, Zhan P, Zhang XM, Xia PG, P, Liang ZS (2023). Unraveling rotational remedies: Deciphering the autotoxicity of *Panax notoginseng* saponins. Industrial Crops and Products 206:117601. *https://doi.org/10.1016/j.indcrop.2023.117601*

Wu B, Shi SL, Zhang HH, Du YY, Jing F (2023). Study on the key autotoxic substances of alfalfa and their effects. Plants 12(18):3263. *https://doi.org/10.3390/plants12183263*

Wu ZJ, Xie Z, Yang L, Wang RY, Guo Z, Z., Zhang Y, … Kutcher H (2015). Identification of autotoxins from root exudates of Lanzhou lily (*Lilium davidii* var. *unicolor*). Allelopathy Journal 35:35-48.

Xiang W, Chen JH, Zhang FY, Huang RS, Li LB (2022). Autotoxicity in *Panax notoginseng* of root exudate sand their allelochemicals. Frontiers in Plant Science 13. *https://doi.org/10.3389/fpls.2022.1020626*

Xin AY, Li XZ, Jin H, Yang XY, Zhao RM, Liu JK, Qin B (2019). The accumulation of reactive oxygen species in root tips caused by autotoxic allelochemicals – A significant factor for replant problem of *Angelica sinensis* (Oliv.) Diels. Industrial Crops and Products 138:111432. *https://doi.org/10.1016/j.indcrop.2019.05.081*

Xiong JX, Du LS, Li NN, Wu XT, Xiang Y, Li S, … Chao HJ (2024). *Pigmentiphaga kullae* CHJ604 improved the growth of tobacco by degrading allelochemicals and xenobiotics in continuous cropping obstacles. Journal of Hazardous Materials 465:133466. *https://doi.org/10.1016/j.jhazmat.2024.133466*

Yang H, Su JL, Qi J (2022). Autotoxicity effect of water extracts from rhizosphere soil of *Elymus sibiricus* in different planting years on seed germination, physiological characteristics and phytohormones of seedlings. PeerJ 10:e13768. *https://doi.org/10.7717/peerj.13768*

Yang RX, Liu P, Liu CY, Ren WG, Chen YQ, Zhou BY (2024). Research progress on causes and prevention measures of the continuous cropping obstacles of *Paeonia suffruticosa*. Northern Horticulture (14):129-135.

Ye JH, Wang HB, Yang XY, Zhang Q, LI JY, Jia XL, … He HB (2016). Autotoxicity of the soil of consecutively cultured tea plantations on tea (*Camellia sinensis*) seedlings. Acta Physiologiae Plantarum 38(8):195. *https://doi.org/10.1007/s11738-016-2216-5*

Zandi P, Barabasz KB, Stachurska SA, Puła J, Możdżeń K (2020). Allelopathic effect of invasive Canadian goldenrod (*Solidago canadensis* L.) on early growth of red clover (*Trifolium pratense* L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca 48(4):2060-2071. *https://doi.org/10.15835/nbha48412081*

Zhang DL, Sun LM, Xie AQ, Li X, Li Y, Liu ZM, Sun X (2022). Effects of residual broken roots on the growth and rhizosphere soil of herbaceous peony. European Journal of Horticultural Science 87(3). *https://doi.org/10.17660/eJHS.2022/035*

Zhang JY, Han HP, Yin Q, Xu LB, Zhang Y, Wang LD, Lin KL (2024). Advanced in continuous cropping obstacles of alfalfa. Chinese Journal of Grassland 46(06):135-143 doi: *https://link.cnki.net/doi/10.16742/j.zgcdxb.20230275*

Zhang ZZ, Fan JR, Wu JH, Zhang LZ, Wang JR, Zhang BB, Wang PGF (2020). Alleviating effect of silicon on melon seed germination under autotoxicity stress. Ecotoxicology and Environmental Safety 188:109901. https://doi.org/10.1016/j.ecoenv.2019.109901

Zhang ZZ, Wu JH, Xi YP, Zhang LZ, Gao Q, Wang PGF (2022). Effects of autotoxicity on seed germination, gas exchange attributes and chlorophyll fluorescence in melon seedlings. Journal of Plant Growth Regulation 41(3):993-1003. *https://doi.org/10.1007/s00344-021-10355-w*

Zou P, Guo YZ, Ding S, Song ZW, Cui HY, H., Zhang Y, … Chen XM (2022). Autotoxicity of endogenous organic acid stress in two *Ganoderma lucidum* cultivars. Molecules 27(19):6734. *https://doi.org/10.3390/molecules27196734*