Allelopathic Effects of *Conyza canadensis* Water Extract on Seed Germination and Seedling Growth

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ABSTRACT

In order to investigate the effects of allelo-pathic substances of *Conyza canadensis* on seed germination and seedlings, this study was designed to explore the allelopathic effects of *Conyza canadensis* water extract (CCE) on pakchoi, spinach and lettuce. Germination rates of three crops (pakcholi, spinach, and lettuce) during the seed germination, root length, seedling height, and fresh weight of two crops (pakcholi and lettuce) during the seedling were measured under different concentrations of CCE treatment (20, 40, 60, 80, 100 g/L). The allelopathic effect index was also calculated. The results showed that CCE had a varying degree of inhibitory effect on the germination rate of the seeds of the three crops. Spinach and lettuce have significant allelopathic effects from *C.canadensis*. At higher concentration of CCE, the germination rate of spinach and lettuce significantly decreased ($P<0.01$). After germination, the root length, seedling height, and fresh weight of the crop were measured, and the allelopathic effects were also calculated. It was found that the CCE had a significant inhibitory effect on root length, but it showed a promoting effect on seedling height and fresh weight of pakchoi, and CCE had a "low promotion-high inhibition" effect on seedling height and fresh weight of lettuce.

Keywords: Seed germination; Conyza canadensis water extract; Allelopathy

1. INTRODUCTION

Allelopathy is mainly a phenomenon in which chemical substances produced and released by plants have a beneficial or adverse effect on their own or other biological life activities. Chemical substances released by plants can enter the environment through leaching and other forms, and are widely present in ecosystems[1,2].

Conyza canadensis is currently considered a typical invasive species and a typical pioneer species in ecological succession[3,4]. It grows and reproduces on a massive scale. It is easily grown in fields and orchards with a simple community composition. While altering the soil structure, *C. canadensis* usually has a different effect on the germination and growth of crops by secreting its own allelopathic chemicals into the environment [3,5]. Seed germination is an important link in the process of plant growth and development [1], and allelo-chemicals have a promoting or inhibiting effect on the germination of plant seeds and the growth and development of seedlings[6]. *C. canadensis* is often found on the edges of farmland, abandoned agricultural land, or abandoned mining land, so its effects on crops, especially the germination of crop seeds and seedlings, have an undeniable impact on agricultural production and sustainability[7].

Therefore, three crops (spinach, pakcholi, and lettuce) which are widely cultivated and having high demand in the market were selected to study the allelopathy of *C. canadensis* on its seed germination and seedling. It is expected to provide more theoretical support for the allelopathy of *C. canadensis* on crop seeds and seedlings.

2. MATERIAL AND METHODS

2.1 EXPERIMENTAL MATERIALS

C. canadensis was taken from the abandoned agricultural land at the southeast corner of Shandong agricultural engineering University (Zibo, Shandong) in October 2022, and dried naturally. *C. canadensis* water extract (CCE) were prepared using cleaned plants.
Spinach, pakchoi and lettuce' seeds were purchased from Zibo Seed Station.

2.2 EXPERIMENTAL DESIGN

2.2.1 Preparation of *Conyza canadensis* water extract

① Crushing. Cut the dried *C. canadensis* into approximately 5 mm pieces.

② Extraction. Immerse the crushed *C. canadensis* sample (100 g) in deionized water (900 ml) and stir well [8]. The samples were immersed at room temperature for 48 h and ultrasounded for 30 min.

③ Filter. The original liquor 100 g/L of CCE was obtained after coarse filtration with gauze and vacuum extraction, and stored at 4℃.

④ Preparation of experimental treatment solution. During the experiment, the original liquor was divided into different concentrations of treatment liquid (20 g/L, 40 g/L, 60 g/L, 80 g/L).

2.2.2 Seed pretreatment

Soaked and disinfected the seeds (about 200 seeds for each crop) with 75% alcohol for 5 min, then rinsed thoroughly with deionized water. Then the seeds were soaked in deionized water for 12 hours. Seeds with full and uniform were selected for the experiment.

2.2.3 Seed germination test

The soaked spinach, pakchoi and lettuce seeds were spread evenly in a pasteurized petri dish covered with filter paper. Five concentrations of CCE (20 g/L, 40 g/L, 60 g/L, 80 g/L, 100 g/L) were set and water was used as control (CK) for seed germination experiment. Three replicates were set for each treatment with 40 seeds per replicate. The culture was carried out in a constant temperature incubator at 25℃.

During the seed cultivation period, CCE (5 ml) applied to each petri dish daily, and added irregularly according to water consumption. The seed which considered to have germinated when its embryonic root breaks through the seed coat. Seed germination rates are regularly observed and recorded on a daily basis, with a germination period of 7 days.

After 7 days, 3-6 typical samples of each crop were selected for each treatment to determine root length, seedling height, and fresh weight [9].

Table 1 Treatment

Treatment	CCE (g/L)
CK	Deionized water
T1	20
T2	40
T3	60
T4	80
T5	100

2.3 PROCESSING OF TEST RESULTS

The germination rate was calculated as follows.

$$\text{Germination Rate (\%)} = \frac{Number\ of\ seeds\ germinated}{Number\ of\ seeds\ placed\ germinated} \times 100 \quad (1)$$

The response index (RI) is calculated as follows [1,2]:

$$RI = 1 - \frac{C}{T} \quad (2)$$

In Eq.2, C is the control value; T is the value of the treatment. RI represents the strength of allopathy, with a positive value indicating a boosting effect and a negative value indicating a inhibiting effect. Its absolute value reflects the strength of the allelopathy.

The synthetical allelopathic effects (SE) represents the arithmetic mean value of each test index RI of the same tested plant treated with CCE [1,10]:

$$SE = \frac{(RI_1 + RI_2 + RI_3 + RI_4)}{4} \quad (3)$$

In Eq.3, RI_1 to RI_4 are the allelopathic effect indices of germination rate, root length, seedling height and fresh weight, respectively.

2.4 DATA PROCESSING AND STATISTICAL ANALYSIS

Excel 2019 was used to perform mapping, variance analysis and significance testing on the experimental data. Single factor analysis of variance was used to compare the significance of differences between different treatments by SPSS 16.0.

3. RESULTS

3.1 THE EFFECT OF CCE ON THE GERMINATION RATE AND ALLELOPATHIC EFFECT INDEX OF CROP

As the germination of spinach seeds were seriously affected by the treatment of the concentration of water extract of each *C. canadensis*, at the end of the experiment, the germination of spinach seeds only broke through the seed coat and did not grow into seedlings, so the data of root length and seedling height could not be measured. Therefore, there are no other indicators for spinach determination data except GR.

Table 2 The Effect of CCE on the GR and Allelopathic Effect Index of Crop

Crops	Concentration (g/L)	GR (%)	RI_1	Root length (mm)	RI_2	Seedling Height (mm)	RI_3	Fresh weight (mg)	RI_4
Pakchoi	CK	100±0.00	-	58.55±8.04	-	37.75±4.30	-	30.65±3.65	-
	T1	98.33±0.47	-0.02	48.69±9.05	-0.17	48.51±7.01	0.22	41.37±4.88*	0.26
	T2	98.33±0.47	-0.02	32.88±7.81	-0.44	61.01±11.13**	0.38	55.78±4.74**	0.45
	T3	96.68±0.47*	-0.03	51.90±7.27	-0.11	61.21±9.73**	0.38	49.T1±5.64**	0.38
	T4	93.75±1.25*	-0.06	42.13±11.53	-0.28	73.19±13.60**	0.48	68.38±3.21**	0.55
	T5	96.68±0.47*	-0.03	30.33±5.33	-0.48	72.58±3.47**	0.48	54.60±3.65**	0.44
Spinach	CK	60.83±2.49	-	-	-	-	-	-	-
	T1	28.33±1.70**	-0.53	-	-	-	-	-	-
	T2	26.17±0.47**	-0.60	-	-	-	-	-	-
	T3	20.83±1.25**	-0.66	-	-	-	-	-	-
	T4	22.50±2.94**	-0.63	-	-	-	-	-	-
	T5	11.00±1.00*	-1.21	-	-	-	-	-	-
Lettuce	CK	79.17±2.36	-	36.72±7.80	-	37.60±5.51	-	13.85±1.68	-
	T1	67.50±2.16	-0.15	32.18±4.33	-0.12	47.08±5.76*	0.20	17.23±1.48*	0.20
	T2	64.17±2.87	-0.19	32.69±3.61	-0.11	42.53±3.37	0.12	20.15±1.41**	0.31
	T3	45.83±3.40*	-0.42	26.33±3.43**	-0.28	40.39±9.21	0.07	21.47±2.32**	0.35
	T4	10.83±1.86**	-0.86	13.51±4.55**	-0.63	26.39±8.82	-0.30	13.45±0.45	-0.03
	T5	7.50±0.82**	-0.91	12.69±4.87**	-0.65	14.59±16.32*	-0.61	3.28±0.65**	-0.76

Note: The value is the mean ± standard deviation, and "" represents a significant level P<0.05, "**" indicates a significant level of P<0.01.*

From the Table 2 it can be observed that the seed germination rates of the three crops were reduced to varying degrees after treatment, and all the allelopathic effect indices were negative. The GR of spinach and lettuce were significantly reduced compared to the control (P<0.05), except for pakchoi. The GR of spinach was extremely significantly lower than that of the control (P<0.01) under each treatment of different concentrations of CCE, while the lettuce GR was significantly reduced (P<0.01) when the concentration of CCE reached T4. Therefore, each concentration of CCE has a different inhibitory effect on GR in these crops.

Pakchoi and spinach showed different characteristics in terms of root length, seedling height and fresh weight after treated with CCE. The root length values of pakchoi and lettuce decreased to varying degrees after treated with CCE, and the allelopathic effect index was negative. Among them, the root length of lettuce decreased significantly when CCE reached T3 compared to the control (P<0.01). But the CCE had a different effect on the height of the seedlings in both crops.

Pakchoi has a gradual increase in seed height with increasing CCE concentration and a positive allelopathy index. When the CCE concentration reached T2, the seedling height of pakchoi was significantly different from that of the control, which indicated that the

application of CCE to pakchoi had a promoting effect on the seedling height. After treatment with CCE, the characteristics of seedling height changes in lettuce are different from those in pakchoi, reflecting the characteristics of "low promotion and high inhibition". At CCE concentrations ranging from T1 to T3, CCE promotes the seedling height of lettuce, and increased in seedling height at T1 concentration was significantly different from the control ($P<0.05$). However, at high CCE concentrations, there was a significant decrease in seedling height of the lettuce compared to the control at a concentration.

After the CCE treatment, the fresh weight change properties of pakchoi and lettuce are similar to seedling heights. Pakchoi has a gradual increase in fresh weight with increasing CCE concentration and a positive allelopathy index. After the CCE concentration reached T2, the value of fresh weight of pakchoi was significantly different from that of the control ($P<0.01$). The lettuce showed the characteristics of "low promotion and high suppression" after the treatment of CCE. At the CCE concentration of T1 to T3, the fresh weight of the lettuce was significantly increased ($P<0.05$). However, at high concentrations, the fresh weight of the lettuce decreased significantly, and the difference between T5 and the control was extremely significant ($P<0.01$).

3.2 The comprehensive allelopathic effects of different CCE on crops

Table 3 The synthetical allelopathic effects of different CCE on crops

Test plants		Concentration (g/L)					
		CK	T1	T2	T3	T4	T5
SE	Pakchoi	-	0.07	0.09	0.16	0.17	0.10
	Lettuce	-	0.03	0.03	0.07	-0.46	-0.73

Table 3 shows the synthetical allelopathic effects (SE) of different concentrations of CCE on various indicators of pakchoi and lettuce. The SE of CCE on pakchoi was positive. This suggests that CCE has an overall beneficial effect on the germination of pakchoi seeds. The SE of lettuce was found to be positive at low concentrations and negative at high concentrations of CEE, thus it was suggested that low concentrations of CCE had a positive effect on seed germination in lettuce, however, high concentrations of CCE had a negative effect on seed germination in lettuce.

4.DISCUSSION

The CCE has different degrees of allelopathic effects on seed germination and seedling growth [11]. The water extract of *Conyza canadensis* contains phenolic allelopathic substances [12]. Phenolic substances can damage the root tip cell structure of plants, thereby affecting the absorption of nutrients by plants [13]. Studies have shown that excessive levels of phenolic substances can inhibit root growth in plants [14]. In this study, all concentrations of CCE suppressed the GR of the three crops and also affected the root growth of pakchoi and lettuce, in agreement with the above results. However, at the same time, the CCE also showed a promoting effect on the growth of crop height and fresh weight. In particular, for the pakchoi seedling height and fresh weight promotion, significant differences with the control can be achieved. The response of seedling height and fresh weight of lettuce to the CCE was characterized by "low promotion and high inhibition" [15]. This may be due to that the allelopathic substance of CCE can somehow promote cell growth and cell division in the root tip of lettuce [16]. The different responses of different crops to the CCE may be related to the crop itself [16]-[18]. For example, *Avena sativa* aqueous extract significantly inhibited root growth and promoted seedling height on seeds of *Elymus nutans* and lettuce [2],[17]. The water immersion of the invasive plant (*Ambrosia*

trifida.L) had a promoting effect on the plant freshness of pakchoi and lettuce [18]. The effect of CCE on the fresh weight of lettuce is consistent with the research results of Zhang Ruiqi [16] et al., which showed "low promotion, high inhibition" in the effect of herb water extract on *Amygdalus pedunculata*. The results are consistent with the effect of Zhang Xiangyu on *Cirsium setosum* [19][20]. In this study, the allelopathic substances of *C. canadensis*, had different effects on crops, and the individual biomass of some crops tended to increase under the influence of the allelopathic substances. Therefore, in addition to some ecological restoration functions, it may have a possible ecological value in promoting crop biomass growth.

5.CONCLUSION

CCE has a varying degree of inhibitory effect on crop GR and root growth, but it can significantly promote the growth of pakchoi seedling heights and fresh weight. The seedling height and fresh weight of lettuce are promoted at low CCE concentrations and promoted at high CCE concentrations.

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