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Allelopathic effects of rapeseed (Brassica juncea) on invasive weed Phalaris minor Retz

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Abstract

Background

Growing allelopathic crops to control invasive weeds is considered as an environmental friendly, and economically sustainable approach, understanding the reasons are essential for current and future green control of invasive weeds. *Phalaris minor* Retz. is one of the most troublesome invasive weeds worldwide. Our previous studies found that rapeseed(*Brassica juncea*)exhibited strong inhibitory effects of this weed in agroecosystems, but it is unknown whether allelopathic effects are involved in this process. In this study, we assessed the allelopathic effects of *B. juncea* to *P. minor*, then, allelochemicals were separated and identified from its leaves, further, biological activity and physiological responses by some individual compounds were investigated.

Results

Our results showed that water extracts from roots, stems, and leaves of *B. juncea* have strong allelopathic potential which can significantly inhibit the seedling growth of *P. minor*, and leaves have the strongest allelopathic inhibitory effects. Different solvents extracts from leaves water extracts of *B. juncea* significantly inhibited seed germination and seedling growth of *P. minor* at higher concentration (20 mg/mL), but no significantly inhibited at lower concentration (1.25 mg/mL), allelopathic effects of different solvents extracts were ranked in order from petroleum ether (strongest), ethyl acetate (moderate), N-butyl alcohol, and residual water (least). The petroleum ether extracts of *B. juncea* affected the protective oxidase activity and chlorophyll content, resulting in a significant increase of malondialdehyde (MDA) content and a significant reduced superoxide dismutase (SOD) activity and chlorophyll content of *P. minor*. Twenty-one components were identified by GS-MS, with proportion of 96.47% of petroleum ether extracts of *B. juncea*. The major components were erucic acid (EA) (19.08%), palmitic acid(PA) (14.16%), dibutyl phthalate(DP) (13.19%), ethyl palmitate(EP) (12.31%), linoleic acid(LA) (9.43%), and oleic acid(OA) (6.24%), among which components PA, EP, and LA exhibited strong inhibitory activity against *P. minor*.

Conclusions

Overall, these relults suggested that *B. juncea* has strong allelopathic inhibitory effect on *P. minor*, and PA, EP and LA were major compounds of petroleum ether extract of *B. juncea* and exhibited strong inhibitory activity against *P. minor*, suggested that *B. juncea* can be used for ecological control and developing environmentally friendly herbicides to control *P. minor*.

Background

Phalaris minor Retz., native to the Mediterranean region, is the most troublesome annual herbaceous plant of the family Gramineae [1,2]. At present, this invasive weed widely distributed in over 60 countries worldwide and caused serious harm to winter crops, especially wheat, often causing a reduction of over 30% in wheat production[1,3]. In the past several decades, many researchers have tried to control this weed through physical[4], chemical[5,6], biological[7,8] or ecological methods[9,10]. Currently, chemical herbicides, such as Isoproturon, Tralkoxydim, Diclofop-methyl *et al.*, have been used to control this weed through the world due to its convenience, low costs and labor saving[11]. However, excessive and long term use of herbicides with same modes of action caused adversely effects on environment, biodiversity and human health, resulting in rapid development of herbicide resistance, and posing a huge threat to weed sustainable management[12, 13]. Therefore, exploring environmentally friendly and economically sustainable alternative is urgent for efficient management of *P. minor*.

Some crops have strong weed-suppression ability, utilizing crop cultivars themselves to control invasive weeds has been regarded as a promising weed control option in agroecosystems[14]. For example, sweet potato plants were shown to have strong inhibitory effects on the invasive weed, *Mikania micrantha*, and applied them to achieve sustainable and effective management of this plant on sloping farmland [15]. Thus, identifying suitable crops with strong ability against weeds and low economic value is critical for the ecological control of invasive weeds. Rapeseed is the main winter crop in Yunnan Province and the second largest oil crop in the world[16]. Previous studies showed that rapeseed can suppress various weeds significantly, such as *Alopecurus japonicus* Steud., *Myosolon aquaticum* (L.) Mocnch., and *Lapsana apogonoides et al*. in agroecosystems[17]. Our former investigation found that *Brassica juncea* significantly reduced the density, frequency, cover, and importance value of *P. minor* in agroecosystems, which may have the potential to control this invasive weed[18]. Thus, *Brassica juncea* promises to provide effective sustainable weed management against *P. minor*.

Allelopathy is understood in terms of a plant producing certain chemical substances to affect the germination and growth of other plants, thus playing an important role in plant interspecific interactions[19, 20, 21]. Utilizing allelopathic crops to control invasive weed are considered an environmentally friendly, and economically sustainable approach due to the lack of introduction of synthetic chemical herbicides into the environment[22, 23]. In deed, allelochemicals are the main reason why plants can stimulate or inhibit the germination and/or growth of plants[24]. Thus, finding more crop germplasm resources with allelopathic activity, identifying allelochemicals, and revealing mechanism are helpful in developing weed management options for sustainable agriculture. Our previous studies showed that rapeseed (*B. juncea*) significantly inhibits the growth and reproduction of *P. minor* [18, 25], but the critical compositions of allelopathic effects in this process are still unclear, and relevant deeper research is urgently needed.

Here, we assessed the allelopathic functions of *B. juncea* to *P. minor*, characterized the physiological responses of this invasive weed to petroleum ether extract of *B. juncea*, further, the chemical composition from this extract was identified and the biological activity of some individual compounds was determined for its inhibitory effect on *P. minor*. The objectives of this study were to examine the

allelopathic effects of *B. juncea* on *P. minor* and uncover their mechanism from the interaction of allelochemicals and weed physiological responses. All these findings supported that some key components from *B. juncea* can be used for ecological control and developing environmentally friendly herbicides against *P. minor*.

Materials and methods

Plant species

P. minor is an annual weed widely distributed in subtropical and temperate regions in Yunnan Province, Southwest China. The seeds of *P. minor* in this study were collected from the wheat fields in Songming County of Yunnan Province in April 2021, dried at room temperature and then kept at 4℃. During the experiments, once the dormancy of its seeds was released, the germination rate of this weed seeds exceeded 92%.

Rapeseed belongs to the *Brassica juncea* (chromosome number n = 18), and has been previously confirmed to have strong weed-suppressing ability[18]. The seeds were provided from the Rapeseed Technology Research Center of Industrial Crops Institute, Yunnan Academy of Agricultural Sciences (YAAS).

Evaluate the allelopathic potential of different tissues of B. juncea on P. minor

We pulled up the *B. juncea* seedling by the roots at the age of 45 d, cleaned them with water and allowed to air dry. A quantity of 50 g fresh leaves, stems, and roots of *B. juncea* were collected and crushed approximately 3 mm separately. Then, which soaked by distilled water 1 L and treated with an ultrasonic wave water bath (frequency: 35 KHZ) for 30 min every 12 h; 48 h later, the water extracts were filtered by Buchner funnel under the negative pressure conditions. The water extracts from leaf, stem, and root of *B. juncea* with concentration in 6.25 g/L, 12.5 g/L, 25 g/L, and 50 g/L were used in this experiment. For each treatment, 30 sterile seeds were evenly placed in a separate petri dishes (9 cm), and 10 mL of extract or distilled water (control) were added. The experiment had four replicates and distilled water as blank control. The petri dishes were sealed with parafilm to minimize evaporative loss of the solution, then place it into a culture chamber (25.0 ± 0.1℃, with a 12 /12 h day/night cycle). Samples were collected to determine the shoot height, root length, and fresh biomass on the tenth day post extracts treatment.

Extraction and separation of allelopathic activity components from B. juncea leaves and its bioactivity

Extraction and separation of allelopathic activity components from B. juncea leaves

Based on the experimental results of 2.2, we collected *B. juncea* leaves at seedling age of 45 d, dried them in the shade and crushed. A quantity of 1,500 g dried leaves power of *B. juncea* was soaked by distilled water in a 1:5 (w/w) solid-liquid ratio. Then, the method of ultrasonic extraction and filtration of

the leaves water extract is the same as experiment 2.2. Using the polarity gradient extraction method, the stepwise extractions of water extracts of *B. juncea* were performed using petroleum ether, ethyl acetate, and n-butanol alcohol for three times, remaining solution after different solvents extraction is residual water phase. Then, the corresponding solvent extraction solution was combined. Finally, the extract was vacuun and steam dried at low temperature, and stored at 4 ℃.

Bioassay of various solvent extracts of B. juncea on P. minor

To test the allelopathic effects of various solvent extracts of *B. juncea* on *P. minor*, we carried out bioassay experiments using the petri dish filter paper method [26]. The seeds of *P. minor* were used to test the allelopathic effects of different solvents extracts of *B. juncea*. The experiments were performed as following: Firstly, we weighed 1 g of different solvent phase extracts, then 1ml of acetone and 1ml of Tween-80 were added to the petroleum ether extract, ethyl acetate extract and n-butanol phase extract, respectively. The next step was to dilute the solvent extracts to 100 mL with distilled water. Residual water extracts were directly dissolved to 100 mL with distilled water as control. The petroleum ether extract, ethyl acetate extract, n-butanol extract with concentration in 1.25 mg/mL, 2.5 mg/mL, 5 mg/mL, 10 mg/mL, and 20 mg/mL were used in this experiment for each solvent phase. For each treatment, 30 sterile seeds were evenly placed in a separate petri dishes (9 cm), and 10 mL of extract or distilled water (control) were added. The experiment had four replicates, with 1% acetone and 1% Tween-80 aqueous solution as solvent control, and distilled water as blank control. The petri dishes were sealed with parafilm to minimize evaporative loss of the solution, then place it into a culture chamber (25.0 ± 0.1℃, with a 12 /12 h day/night cycle). The number of germinated seeds were counted every day, and samples were collected to determine the germination, shoot height, root length, and fresh biomass on the tenth day post extracts treatment.

Physiological effects of petroleum ether extract of B. juncea on P. minor

The method of the biological activity of petroleum ether extract from *B. juncea* to *P. minor* is the same as experiment 2.3.2. The physiological effects of petroleum ether extract from *B. juncea* on fresh leaves of *P. minor* were measured at the tenth day post petroleum ether extract. Weighed 0.2g fresh leaves of *P. minor*, added 1.8 mL of phosphate buffer solution(Ph 7.2), and grinded at low temperature(4 ℃). Then, centrifuged the obtained weed tissue solution at 3500 rpm for 10 minutes, and then take the supernatant for measurement. At last, the levels of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), malondialdehyde (MDA), and chlorophyll of petroleum ether extract of *B. juncea* to fresh leaves of *P. minor* were determined and analyzed in the laboratory[26, 27].

Identification of chemical composition of petroleum ether extract of B. juncea and the bioassay of several main components

The chemical composition of petroleum ether extract were analyzed using GC-MS methods due to the petroleum ether extract from fresh leaves of *B. juncea* exhibiting the strongest inhibitory effect on *P. minor* among the extracts tested. The GC-MS was performed using an Agilent 7890 gas chromatograph equipped with a quadrupole mass spectrometer (Agilent 5975 N, United States). A 1 μL aliquot of each

sample was injected into an HP–5MS fused silica capillary column (0.25 mm × 30 m i.d., film thickness 0.25 μm) with helium carrier gas flow rate at 1.0 mL/min in the splitless mode. The specific chromatographic and mass spectrometric conditions were presented according to the method reported by Shen[26].

According to the results of the “Identification of components from petroleum ether extract”, six main commercial compounds erucic acid (EA) (19.08%), palmitic acid (PA) (14.16%), dibutyl phthalate (DP) (13.19%), ethyl palmitate (EP) (12.31%), linoleic acid (LA) (9.43%), and oleic acid (OA) (6.24%) were selected for further bioassay experiments on *P. minor*. Five extract concentrations (0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL) and a control (distilled water) (CK) were used in this experiment for each compound. The seed selection, bioassay methods, and data collection of *P. minor* and experimental conditions same as experiment 2.3.

Data Analyses

The germination rate (GR), germination energy (GE) and germination index (GI) were calculated using the following formulas[18]:

Germination rate (GR)=(Total number of germinated seeds/total number of tested seeds)×100%.

Germination energy (GE) =(Total number of seeds germinated from 3d to 6d/ total number of tested seeds) (where X is the total number of germinated seeds after X days).

Germination index (GI) =Σ (Gt/Dt), where Gt represents the germination numbers on day t and Dt represents the corresponding germination days.

The allelopathic response index (IR) was calculated using the following formulas: when T>C, RI = 1-C/T; when T < C, RI = T/C-1 (T < C) [28]. C is the control value and T is the treatment value, RI > 0 represents a stimulatory effect, RI < 0 represents an inhibitory effect, and the absolute value is consistent with the allelopathy intensity. The synthetical allelopathic index (RI_{SE}) was evaluated by calculating the arithmetic mean value of RI for 6 parameters, including the germination rate, germination energy, germination index, seedling height, root length, and biomass. Data was analyzed using the SPSS 23.0 software package (SPSS, Inc., Chicago, IL, USA). T-test and One-way Analysis of Variance (ANOVA) were used to analyze the significant difference between different treatment groups.

Results

Allelopathic effects of water extracts from various parts of B. juncea on P. minor

The value of the synthetical allelopathic response indexes (RI_{SE}) of water extracts from leaves, stems, and roots of *B. junce* to *P. minor* are less than 0 when the concentration of the inhibitor exceeds 12.5 g/L, its value of leaves is smallest, which showed that different parts of *B. juncea* have strong allelopathic inhibitory effects on the seedling growth (the seedling height, root length and biomass) of *P. minor*, and

leaves are the strongest than others (Table 1). At the concentration of 50 g/L, the inhibition of water extracts from leaves, stems, and roots of rapeseed on seedling height of *P. minor* were 40.38%, 24.24%, and 33.11%, root length were 55.69%, 28.36% and 41.36%, and biomass were 60.39%, 49.58% and 54.46%, and the synthetic allelopathic index (RI_{SE}) is -0.52, -0.34, and − 0.43.

Table 1
Allelopathic effects of water extracts from leaves, stems and roots of *B. juncea* on *P. minor*

Items	Different tissues of *B. juncea*	Concentration (g/L)			
		50.00	25.00	12.50	6.25
Seedling height inhibition%	Leaf	40.38 + 0.45^{a}	21.65 + 1.09^{b}	3.74 + 0.44^{c}	-1.08 + 1.39^{d}
	Stem	24.24 + 1.03^{a}	7.81 + 0.79^{b}	2.26 + 0.96^{c}	-6.39 + 0.63^{d}
	Root	33.11 + 1.79^{a}	13.37 + 1.60^{b}	2.16 + 0.88^{c}	-2.50 + 0.46^{d}
Root length inhibition%	Leaf	55.69 + 1.83^{a}	30.47 + 1.31^{b}	10.84 + 0.76^{c}	2.56 + 0.45^{d}
	Stem	28.36 + 1.68^{a}	16.45 + 1.26^{b}	3.73 + 0.40^{c}	-5.52 + 1.02^{d}
	Root	41.36 + 1.31^{a}	27.94 + 1.06^{b}	8.37 + 1.15^{c}	-2.47 + 0.67^{d}
Biomass Inhibition%	Leaf	60.39 + 1.64^{a}	42.53 + 1.45^{b}	18.82 + 1.24c	2.48 + 0.39^{d}
	Stem	49.58 + 1.42^{a}	34.70 + 1.27^{b}	1.33 + 0.40^{c}	-5.66 + 0.98^{d}
	Root	54.46 + 1.08^{a}	37.47 + 0.86^{b}	11.29 + 1.05^{c}	-1.93 + 0.59^{d}
Synthetical allelopathic response index	Leaf	-0.52 + 0.01^{c}	-0.32 + 0.01^{b}	-0.11 + 0.02^{a}	-0.01 + 0.06^{a}
	Stem	-0.34 + 0.05 ^{b}	-0.20 + 0.03^{b}	-0.02 + 0.07^{a}	0.06 + 0.06^{a}
	Root	-0.43 + 0.02^{c}	-0.26 + 0.03^{b}	-0.07 + 0.02^{a}	0.02 + 0.04^{a}

Data are expressed as mean ± standard error. Different letters within the same line under different concentrations significant differences at $P < 0.05$.

Germination and growth of P. minor upon treatments with different solvent extracts of B. juncea

The results showed that the germination rate and germination index of *P. minor* were inhibited by various solvent extracts of *B. juncea* ($P < 0.05$) (Fig. 1, A and C). The germination energy, seedling height, root length, and biomass of this invasive weed on different solvents extracts are characterized as "low promotion and high inhibition"(Fig. 1, B, D, E, F).. In addition, our data also showed that the inhibition extent on germination and growth of *P. minor* by different solvent extracts of *B. juncea* were ranked as following: petroleum ether extract > ethyl acetate extract > N-butyl alcohol extract > residual water extract under the same concentration(Fig. 1).

The germination rate and germination index of *P. minor* in all treated groups were lower than in the control groups at the concentration of 1.25 mg/mL, but there was no significant difference. As the concentration of solvent concentration extract increased, the germination rate and germination index of *P. minor* showed a corresponding decrease. The germination rate and germination index of *P. minor* in the four solvent extract treatment groups were significantly lower than in the control groups at a concentration of 20 mg/mL (Fig. 1).

Apart from the petroleum ether extract, the germination energy, seedling height, root length, and biomass of this invasive weed in other solvent extract treated groups were higher than that in the control groups at concentration of 1.25 mg/mL, but there were no significant differences. Biomass decreased with increasing concentration of various solvent phases, reaching the lowest at a solvent concentration of 20 mg/mL and significantly lower than the control except for the residual water phase (Fig. 1).

Allelopathic effects of different solvent extracts of B. juncea on the germination and growth of P. minor

The results showed that the allelopathy of various solvent extracts compared from the water extract of *B. juncea* on *P. minor* were obviously different, which generally presented as following: petroleum ether phase > ethyl acetate phase > N-butyl alcohol phase > residual water phase under the same concentration (Table 2). The synthetic allelopathic index (RI_{SE}) for the ethyl acetate phase, the N-butyl alcohol phase and the residual water phase for *P. minor* were greater than 0 except for petroleum ether phase at a concentration was 1.25 mg/mL. As the concentration increased, the value of synthetic allelopathic index (RI_{SE}) decreased, with the petroleum ether phase exhibiting the smallest values under the same conditions. When the concentration was 20 mg/mL, the value of the synthetic allelopathic index (RI_{SE}) on *P. minor* was − 0.819, which was significantly lower than other solvent phases. These results showed that petroleum ether had the strongest synthetic allelopathic index (RI_{SE}) to *P. minor*, and was significantly stronger than other solvent phases (Table 2).

Table 2
Synthetical allelopathic response index of different solvent extracts of *B. juncea* on *P. minor*

Solvent extracts	Synthetical allelopathic index				
	1.25 mg/mL	2.5 mg/mL	5 mg/mL	10 mg/mL	20 mg/mL
Petroleum ether	-0.033 ± 0.001^{Ca}	-0.131 ± 0.012^{Cb}	-0.278 ± 0.021^{Cc}	-0.532 ± 0.041^{Bd}	-0.819 ± 0.053^{De}
Ethyl acetate	0.008 ± 0.001^{Ba}	-0.093 ± 0.004^{Bb}	-0.241 ± 0.018^{Cc}	-0.445 ± 0.036^{Bd}	-0.648 ± 0.042^{Ce}
N-butyl alcohol	0.036 ± 0.003^{Aa}	-0.045 ± 0.006^{Ab}	-0.124 ± 0.016^{Bc}	-0.185 ± 0.006^{Ad}	-0.335 ± 0.011^{Be}
Residual water	0.038 ± 0.003^{Aa}	-0.033 ± 0.004^{Ab}	-0.078 ± 0.007^{Ac}	-0.111 ± 0.008^{Ac}	-0.147 ± 0.015^{Ad}

Different capital letters in the table indicate significant differences in the allelopathic comprehensive effect index of different substances on *P. minor* at the same concentration level (P<0.05), while different lowercase letters indicate significant differences in the allelopathic comprehensive effect index of the same component on *P. minor* at the same concentration level (P<0.05); The reverse is not significant.

Physiological effects of petroleum ether extract of B. juncea on new leaves of P. minor

The enzyme and chlorophyll characteristics of fresh leaves of *P. minor* varied significantly (P < 0.05) among concentrations of petroleum ether extract in contrast with the water extract of *B. juncea* (Table 3). Apart from the concentration of 1.25 mg/mL, the MDA content of fresh leaves of *P. minor* increased significantly with higher concentration of the petroleum ether phase. The enzyme activity of CAT and POD in new *P. minor* leaves initially increased and then decreased with increasing concentration of three components and lower than CK activity when the concentration was greater than 5 mg/mL. The SOD activity and Chlorophyll contents of fresh leaves of *P. minor* significantly declined with increasing concentration of petroleum ether phase from the water extract of *B. juncea* (Table 3).

Table 3
Antioxidant enzyme and Chlorophyll content of fresh leaves of *P. minor* affected by petroleum ether extract of *B. juncea*

Items	CK	1.25 mg/mL	2.5 mg/mL	5 mg/mL	10 mg/mL	20 mg/mL
MDA (μmol/g)	5.56 ± 0.24^{e}	5.69 ± 0.43^{e}	5.91 ± 0.32^{d}	6.12 ± 0.17^{c}	7.69 ± 0.18^{b}	8.76 ± 0.29^{a}
CAT (U/g)	151.91 ± 5.97^{b}	158.31 ± 6.24^{a}	149.34 ± 3.15^{b}	145.57 ± 4.06^{bc}	140.62 ± 3.92^{c}	131.57 ± 4.1^{7d}
POD (U/g)	214.72 ± 6.53^{b}	221.15 ± 5.42^{a}	218.65 ± 6.16^{a}	211.27 ± 4.95^{b}	205.83 ± 5.82^{c}	196.47 ± 6.14^{d}
SOD (U/g)	114.06 ± 3.95^{a}	96.23 ± 4.12^{b}	78.35 ± 2.67^{c}	51.84 ± 2.15^{d}	42.87 ± 2.03^{e}	33.96 ± 1.65^{f}
Chl (μg/mL)	6.46 ± 0.23^{a}	6.31 ± 0.21^{b}	5.31 ± 0.35^{c}	3.69 ± 0.29^{d}	2.75 ± 0.14^{e}	1.96 ± 0.25^{f}

Data are expressed as mean ± standard error. Different letters within the same line under different concentrations significant differences at $P < 0.05$.

Chemical components of petroleum ether extract of B. juncea were identified

Twenty-one components with a content greater than 0.5% were identified and detected in petroleum ether phase from the water extract of *B. juncea*, accounting for 96.47% of the total peak area (Fig. 2 and Table 4). The major components were erucic acid (19.08%), palmitic acid (14.16%), dibutyl phthalate (13.19%), ethyl palmitate (12.31%), linoleic acid (9.43%), and oleic acid (6.24%); the content of other compounds was less than 5% (Table 1). These five largest components: erucic acid, palmitic acid, dibutyl phthalate, ethyl palmitate, and linoleic acid were further confirmed with standard commercially available compounds through GC-MS (Fig. 2 and Table 5).

Table 4
Chemical compounds of *B. juncea* petroleum ether fraction

No.	Name of compound	Molacular formula	Molecularion (m/z)	Percentage (%)
1	Para-xylene	C_8H_{10}	106	0.67%
2	m-Xylene	C_8H_{10}	106	2.14%
3	Boronal	$C_{14}H_{22}O$	206	1.27%
4	2,4-Di-t-butylphenol	$C_{14}H_{22}O$	238	1.14%
5	Palmitic acid	$C_{16}H_{22}O_4$	256	14.16%
6	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	268	0.98%
7	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	13.19%
8	Linoleic acid	$C_{18}H_{32}O_2$	280	9.43%
9	Oleic acid	$C_{18}H_{34}O_2$	282	6.24%
10	Ethyl palmitate	$C_{18}H_{36}O_2$	284	12.31%
11	Methyl Linolenate	$C_{19}H_{32}O_2$	292	2.04%
12	Phytol	$C_{20}H_{40}O$	296	1.09%
13	Ethyl linoleate	$C_{20}H_{36}O_2$	308	3.67%
14	Ethyl Stearate	$C_{20}H_{40}O_2$	312	1.67%
15	Erucic acid	$C_{22}H_{42}O_2$	338	19.08%
16	Ethyl icosanoate	$C_{22}H_{44}O_2$	340	1.24%
17	Desmesterol	$C_{27}H_{44}O$	385	0.94%
18	bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	396	1.24%
19	Stigmasterol	$C_{29}H_{48}O$	413	1.21%
20	beta-Sitosterol	$C_{29}H_{50}O$	415	0.75%
21	(20S)-20,21-[(tert-Butylboranediyl)bis(oxy)]-5α-pregnane-3α,11β-diol	$C_{25}H_{43}BO_4$	418	2.01%

3.6. Bioactivity of six components from B. juncea ether petroleum extract against the germination and growth of P. minor

To determine the inhibitory role of these six components, the germination and growth of *P. minor* were evaluated upon treatments with the components. The results showed that the inhibitory effects of seed germination and seedling growth of the six compounds against *P. minor* were different, the highest inhibition rates seed germination and seedling growth of *P. minor* were observed for linoleic acid, palmitic acid and ethyl palmitate, followed by dibutyl phthalate, with the lowest inhibition rates for erucic acid and oleic acid. The compounds of exhibited strong inhibitory activitis, and the inhibitory rate of seed germination and seedling growth was 100% at 2 mg/mL. The inhibitory effects of erucic acid and oleic acid on seed germination and seedling growth of *P. minor* were much weaker, and even promoted seed germination and seedling growth at a concentration of 0.125 mg/ mL (Table 5). In addition, for the differnet parts of the weed,we also found that the biomass of *P. minor* were most strongly inhibited, followed by shoot length and root length, finally germination rate was the least affected (Table 5).

Table 5
Effects of six components of *B. juncea* ether petroleum extract on the germination and growth of *P. minor*

Compounds	Concentration/ (mg mL-1)	Inhibitory rate(%)			
		Germination	Root length	Shoot length	Biomass
Erucic acid	0.125	1.52 ± 0.41d	-4.53 ± 1.22d	-1.37 ± 0.51e	-6.45 ± 0.69e
	0.25	9.07 ± 0.78c	12.16 ± 1.82c	13.05 ± 1.18d	27.30 ± 1.37d
	0.50	20.73 ± 1.22b	26.68 ± 3.08b	27.59 ± 1.43c	33.63 ± 1.39c
	1.00	24.26 ± 2.25ab	31.53 ± 2.40b	35.92 ± 1.38b	42.45 ± 1.19b
	2.00	26.60 ± 1.87a	43.58 ± 2.89a	44.81 ± 1.98a	47.41 ± 1.06a
Palmitic acid	0.125	13.42 ± 1.62d	7.48 ± 1.16e	12.69 ± 1.51e	24.37 ± 1.68e
	0.25	20.07 ± 0.85cd	27.66 ± 4.18d	30.55 ± 1.46d	39.07 ± 1.28d
	0.50	28.42 ± 1.07c	51.15 ± 2.79c	53.31 ± 1.10d	57.84 ± 1.30d
	1.00	56.30 ± 2.81b	82.34 ± 2.09b	84.65 ± 1.77b	78.81 ± 1.21b
	2.00	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Dibutyl phthalate	0.125	9.76 ± 1.03	11.58 ± 2.11c	16.78 ± 1.06e	22.78 ± 1.45e
	0.25	14.52 ± 1.23	19.06 ± 1.83c	23.80 ± 1.05d	33.11 ± 1.83d
	0.50	24.92 ± 2.63	46.95 ± 3.52b	48.96 ± 1.44c	55.50 ± 1.37c
	1.00	29.45 ± 2.11	53.91 ± 2.98b	57.87 ± 1.41b	62.49 ± 0.78b
	2.00	38.94 ± 4.37	78.29 ± 3.16a	80.34 ± 1.17a	88.61 ± 1.42a
Ethyl palmitate	0.125	9.18 ± 0.82d	24.76 ± 1.68e	14.80 ± 0.77e	28.71 ± 0.73e
	0.25	18.31 ± 1.78c	33.32 ± 1.76d	34.94 ± 0.92d	43.32 ± 1.31d

	0.50	22.31 ± 2.29c	57.96 ± 1.59c	59.73 ± 1.38c	75.02 ± 1.41c
	1.00	41.93 ± 4.39b	76.64 ± 2.39b	68.86 ± 1.92b	88.52 ± 1.22b
	2.00	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Linoleic acid	0.125	23.60 ± 2.68	20.87 ± 2.42e	17.99 ± 0.91e	16.49 ± 1.37e
	0.25	31.86 ± 4.45	42.93 ± 1.49d	40.11 ± 1.62d	50.94 ± 2.23d
	0.50	46.90 ± 2.26	48.96 ± 2.04c	57.34 ± 1.49c	66.21 ± 1.08c
	1.00	62.25 ± 3.27	80.29 ± 2.27b	71.24 ± 1.25b	89.92 ± 1.35b
	2.00	100.00 ± 0.00	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Oleic acid	0.125	-1.54 ± 0.39c	-2.80 ± 0.64c	-1.55 ± 0.83c	-2.40 ± 0.45d
	0.25	9.87 ± 2.31b	8.25 ± 1.14b	8.37 ± 1.08b	10.79 ± 0.93c
	0.50	12.98 ± 2.89ab	18.91 ± 1.39a	17.60 ± 0.92a	19.67 ± 0.87b
	1.00	11.75 ± 1.82ab	21.34 ± 1.45a	20.31 ± 0.61a	20.79 ± 2.32b
	2.00	17.85 ± 1.01a	20.39 ± 1.11a	19.49 ± 1.18a	26.16 ± 0.79a

Data are expressed as mean ± standard error. Different letters within the same column under same compound signify significant differences at $P < 0.05$.

Discussion

P. minor is the most serious weed occuring in winter farmland in southern China and many other world regions. However, how best to control this weed using ecologically sustainable approaches is a serious challenge. Our study showed that the *B. juncea* has strong allelopathic inhibitory effects on *P. minor*, a mechanism that may play an important role on the ecological management of this invasive weed[9, 18]. Many wild and cultivated crops with allelopathic potential exhibits strong ability to limit weed germination and emergence[20, 29]. Understanding allelopathic effects and its mechanisms of allelopathic crops may have many useful applications in the development of sustainable agricultural systems. Our previous study showed that *B. juncea* demonstrated significant suppression of *P. minor* through its competitive ability, and considered as an excellent crop for controlling this weed in

agroecosystems[25]. In fact, allelopathy plays an important role in plant interspecific interactions, but specific information about the allelopathic effects of *B. juncea* against *P*. minor was limited prior to the present study. The current study is the first to report the allelopathic effects of *B. juncea* on *P. minor*, including production of allelochemical by *B. juncea* and the physiological response of *P. minor* in this process. Knowledge from our study may be useful for the development and utilization of allelopathic rapeseed varieties of B. juncea .

Allelopathy can alter plant growth and development at different stages, such as seed germination, seedling growth, flowering and fruiting of adult plants, and results in the suppression of plant population[30, 31]. Our study here showed that seed germination and seedling growth of *P. minor* were slightly promoted (but not significantly) at low concentration (1.25 mg/mL) and significantly inhibited at high concentrations (20 mg/mL) by various solvent extracts of *B. juncea*. Allelopathy generally exhibits "low promotion and high inhibition" on seed germination and seedling growth of the target species, with stimulatory or inhibitory effect as a function of concentration, as seen in the current study [26, 31]. Our previous study also showed that *B. juncea* exhibits strong weed-suppressing ability on *P. minor* through its competitive ability, significantly suppressing its morphological and physiological characteristics and reducing the density of the weed in the crop field. Seed germination is crucial to species regeneration, which is closely linked with the population density and ecological niche of plants in the ecosystem[32]. Privious studies also suggested that decrease of germination rate and germination energy would reduce richness of weed populations in the ecosystem, while a decrease in germination potential would extenec germination time and delay emergence, seriously impacting the competitiveness of plants for both aboveground and underground resources[9]. Our current results perhaps provide the clearest evidence to help clarify why the population of *P. minor* in *B. juncea* field were significiant lower than in other crop fields.

Allelopathy can also affects the physiological processes of plants, such as the function and activity of enzymes[26], photosynthesis[33], and the metabolism of proteins and nucleic acids [34]. Under adverse conditions, the content of reactive oxygen species and free radicals (OH^-, H_2O_2, O^{2-}) of plants will increase, causing damage to biological functional macromolecules such as amino acids, proteins, and sugars, thereby damaging plant cells [35]. Antioxidant enzyme system (CAT, POD, SOD) can protect plant cells from damage by clearing or weakening the toxic effects of free radicals[36]. MDA is caused by membrane lipid peroxidation of plants in stress conditions[37]. Usually, the higher the content of MDA in plants, the greater the degree of damage to plant cell membranes [26]. We observed that petroleum ether extracts of *B. juncea* significantly increased MDA content, suggesting that allelochemicals of *B. juncea* damage the cellular membrane of *P. minor*. In addition, our study also shows that petroleum ether extracts significantly reduced the SOD activity, which indicates that the petroleum ether phase of rapeseed leads to the inhibition of SOD activity in *P. minor*, resulting in the disruption of the balance between the production and elimination of free radicals, and damaging cells.

At present, known allelopathic substances mainly include phenols, quinones, coumarins, favonoids, terpenes, sugars, glycosides, alkaloids and non-protein amino acids[29]. Due to differences in the polarity

of allelochemicals, different extracts of the same medicinal materials often contains diverse components then results in different allelopathy effects. In this study, we found different solvent extracts of *B. juncea* make different allelopathic inhibitory effects on germination and growth of *P. minor*, and petroleum ether extract had the best allelopathic inhibition effects. In addition, twenty-one components were identified by GS-MS, constituting 96.47% of petroleum ether extract of B. juncea. The major components were erucic acid (19.08%), palmitic acid (14.16%), dibutyl phthalate (13.19%), ethyl palmitate (12.31%), linoleic acid (9.43%), oleic acid (6.24%) and ethyl linoleate (3.67%). Currently, palmitic acid, dibutyl phthalate, ethyl palmitate and linoleic acid, oleic acid and ethyl linoleate all have been reported to have strong allelopathic effects on various weeds[26]. Liu *et al.* reported that oleic acid, palmitic acid, erucic acid, linoleic acid, stearic acid, eicosenoic acid, and behenic acid were possible allelochemicals in rapeseed, which is similar to our research results[38]. Zhang *et al.* detected the root exudates of rapeseed and discovered hydrocarbons, alcohols, esters, and organic acids from it[39]. All these results support that differences in allelopathic potential among crops result from different types and concentrations of allelopathic chemicals produced and released by different crop varieties.

Allelochemicals are released by plants through various ways such as leaching by rain, volatilization from flower, secretion from root and decomposition from residual biomass [40]. At present, most known allelochemical, such as phenols, quinones, coumarins, and favonoids are soluble in water[41]. In this study, we found that the petroleum ether extracts revealed the allelochemicals of *B. juncea* which have the strongest allelopathic activity to *P. minor*, and twenty-one components were identified by GS-MS, constituting 96.47% of petroleum ether extract of research results, and almost all of them are insoluble in water. Different extract of the same medicinal materials often obtains diverse components with different effects[26]. In this study, water extraction followed by solvent gradient extraction was used for the extraction and separation of allelochemicals from *B. juncea*. However, in order to improve the yield, we use ultrasonic-assisted extraction and negative pressure extraction filtration, which may lead to some insoluble in water and suspended chemical substances in solution to be extracted in the form of micro-particles. This may better explain why the allelochemicals in this study are mostly insoluble in water. Ma et al. [42] also detected dibutyl phthalate, palmitic acid, linolenic acid, Linoleic acid and other substances in the petroleum ether extract of the aqueous extract of *Alternanthera philoxeroides*. Therefore, we believe that although some insoluble substances were found in our rapeseed water extract, the results obtained are still reliable.

Conclusion

Water extracts from roots, stems, and leaves of *B. juncea* have strong allelopathic potential which can significantly inhibit the seedling growth of *P. minor*, and leaves have the strongest allelopathic inhibitory effects. Four solvent extracts (petroleum ether extract, ethyl acetate extract, n-butanol extraction extract and residual water extract) from leaves water extracts of *B. juncea* exhibited strong inhibitory activity against *P. minor* at the concentration of 20 mg/mL, and the allelopathy activity of petroleum ether extract is the strongest one. Twenty-one compounds were identified by GS-MS from the petroleum ether extract of *B. juncea*. The major components were erucic acid (19.08%), palmitic acid (14.16%), dibutyl

phthalate (13.19%), ethyl palmitate (12.31%), linoleic acid (9.43%), and oleic acid (6.24%). These compounds exhibited strong inhibitory activity against *P. minor*, and the highest inhibition rates seed germination and seedling growth of *P. minor* were seen of palmitic acid(PA), ethyl palmitate(EP), anase impacts of petroleum ether extract were tested about the antioxidant activity and chlorophyll characteristics of fresh leaves of *P. minor*, we found that the SOD enzyme activity and Chl contents were significantly reduced, and MDA contents were significantly increased. These results showed that the allelochemicals of *B. juncea* break the balance of oxidative free radicals of *P. minor*, causing damage to the cell membrane and obstructing photosynthesis, exhibited strong allelopathic inhibitory activity on the seed germination and seedling growth against *P. minor*. Thus, our research not only improved our understanding that how to use allelopathic crop to ecological control invasive weeds in farmland, but also has significant implications for the breeding of new allelopathic varieties and the development of new green pesticides.

Declarations

Author contributions

Dong L, Xu G and Zhang F conceived and designed the experiments; Xu G, Shen S, Zhang Y and Yang Y performed the experiments; Xu G, Shen S and Jin G analyzed the data and wrote draft; David RC and Feng Z designed and edited the manuscript for style; Dong L and Feng Z commented on manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data set supporting the results of this article is available in the Dryad Digital Repository.

Ethics approval and consent to participate

All aspects of the study comply with institutional, national, and international guidelines. All experiments were conducted on non-regulated organisms. The study site was rented by Agricultural Environment and Resource Research Institute, Yunnan Academy of Agricultural Sciences and no permits are required to collect samples.

Consent for publication

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of this manuscript and in the decision to publish these results.

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Figures

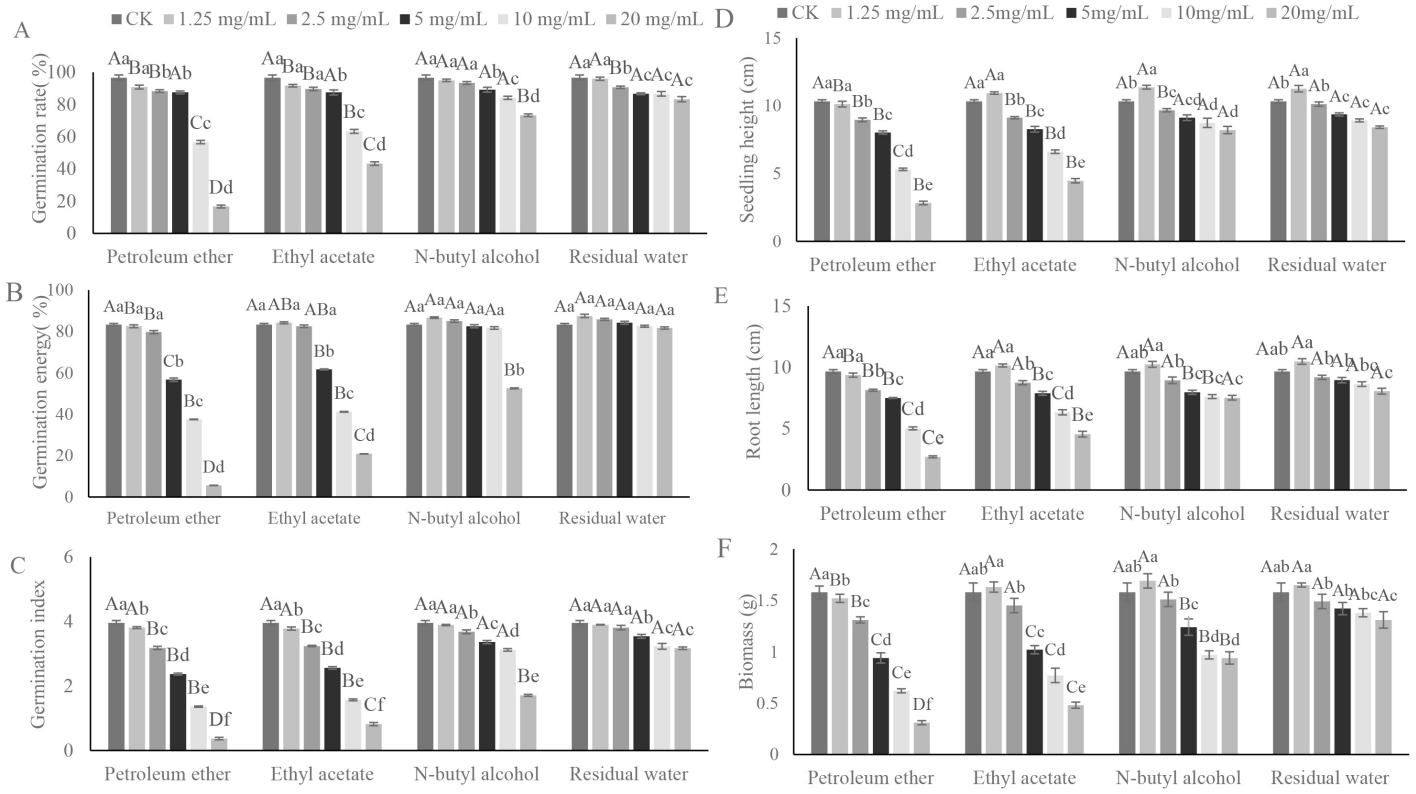


Figure 1

Effects of different solvents extracts of *B. juncea* on seedling growth of *P. minor*

A , B, C, D, E and F represent the germination rate, germination energy,, germination index seedling height, root length and biomass of *P. minor* relative to different solvents extracts of *B. juncea*. Different capital letters in the figure indicate significant differences in seed germination and the seedling growth of *P. minor* between different solvent phases with the same concentration ($P<0.05$); different lowercase letters indicate significant differences between different concentrations of the same solvent extract phase ($P<0.05$).

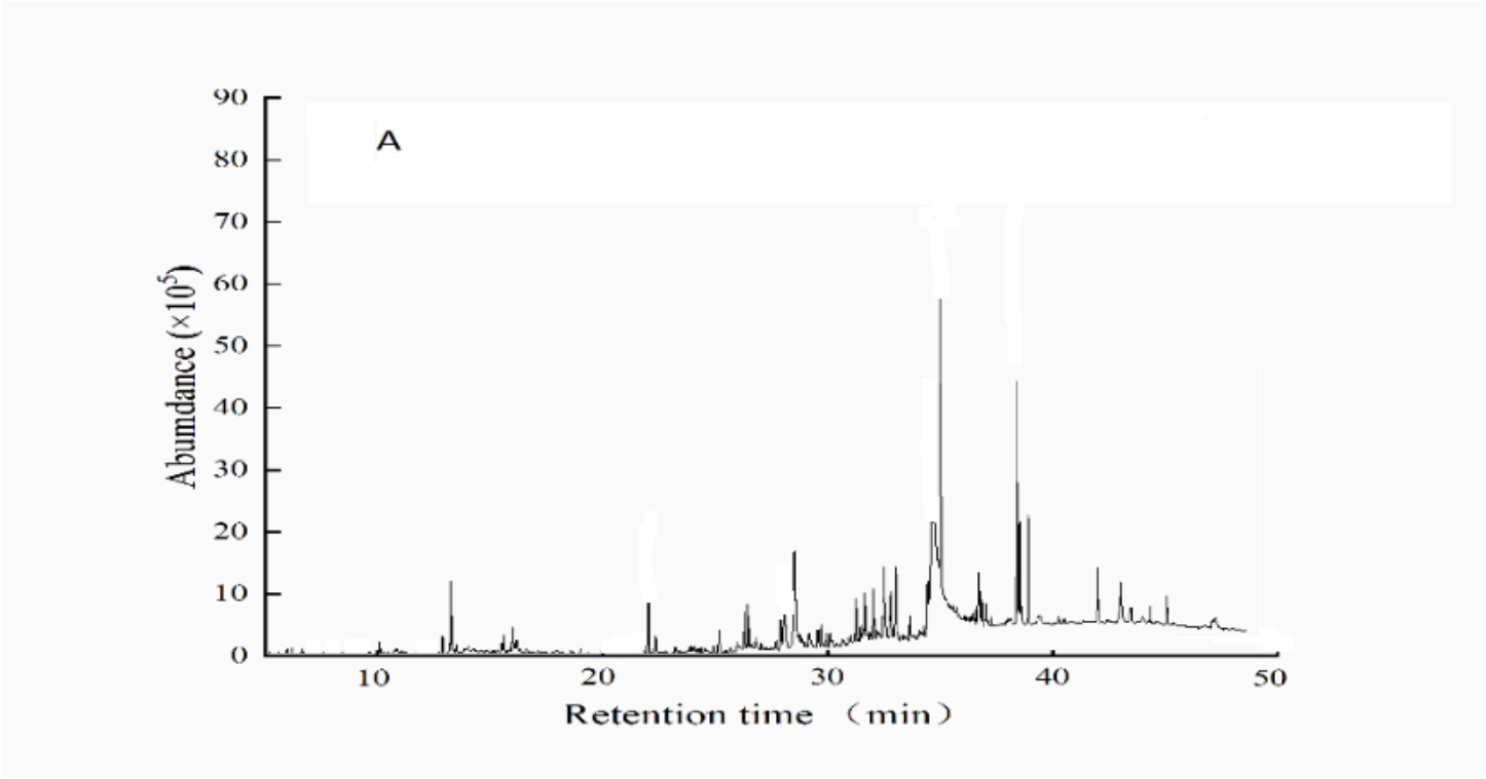


Figure 2

Total ion current diagram of *B. juncea*. petroleum ether extract