

Allelopathic effects of aerial parts of *Descurainia sophia* L. on wheat

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ABSTRACT

The phytotoxicity of organic extracts obtained from aerial parts of flixweed (*Descurainia sophia* L.) were evaluated against seed germination and seedling growth of wheat (*Triticum aestivum* L.). The 96% ethanol extract was the most inhibitory to wheat growth. Four compounds (quercetin, isorhamnetin, xanthotoxol and xanthotoxin) were isolated and identified by physical and chemical methods. These compounds were inhibitory to wheat growth and could be the key allelochemicals in flixweed-infested wheat fields. Further investigations are required to demonstrate the mechanism by which quercetin and isorhamnetin are released into the rhizosphere soil by flixweed.

Keywords: Allelopathy, bioassay, *Descurainia Sophia* L., flixweed, inhibitory effects, organic extracts, release mechanism, seed germination, seedling growth, *Triticum aestivum* L., wheat.

INTRODUCTION

Allelopathy is the biochemical process, whereby living or dead plants release chemicals that exert a negative or positive effect on neighboring plants and microorganisms and thus may play an important role in controlling the growth and development of other plant spp. (9,14,16,26). The bio-chemical bio-interactions between the plants, between the plants and microbes and between the plant secondary metabolites and pests wounds are induced by the plant secondary metabolites (7,10). These secondary metabolites are natural treasure of biologically active chemicals (1). Allelochemicals may be water-soluble substances that are released into the rhizosphere soil through volatilization from the aerial plant parts, exudation from the plant root system of the plant and leachates (through rain, dew and fog washings) from the plant surface.

Flixweed (*Descurainia sophia* L.), Cruciferae (Brassicaceae) family (Fig. 1) is an annual herb. It originated in southern Europe and North Africa and is most problematic weed in wheat fields across the globe and is major weed in wheat growing regions in China (3,30). It quickly invades the cultivated wheat fields and causes severe reductions in wheat yields through competition for moisture and nutrients and perhaps also allelopathy (6). However, it is also widely used in folk medicine, in Middle Asia, a decoction of its aerial parts is used for throat diseases and as antipyretic for measles and smallpox (15). In addition

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to resource competition, allelopathic interactions are also significant factor by which weeds interfere with the growth and development of crop plants by releasing the allelochemicals into the surrounding environment (4,5,24,28). It is known that the phytotoxicity of allelochemicals released by weeds on crops is species-specific (11,23,25). An in-depth understanding of allelopathy in cropping systems may be useful to minimise the serious problems that these chemicals inflict on food safety, crop yields and the loss of crop diversity (29,31). Such knowledge can also be useful to develop the new environmentally safe biological control of pests for sustainable agriculture (12,18). Some interference mechanisms between flixweed and wheat have been suggested, however, the mechanisms and allelochemicals involved in the impact of flixweed on wheat are still unknown. Volatile oils of flixweed inhibits the seed germination and seedling growth of wheat cultivars by affecting the microbial community in the soil (15,17). Therefore, allelopathy appears to be an important factor in flixweed's successful interference with wheat.



Figure 1. Flixweed alone and flixweed growing with wheat crop.

This study aimed, to identify the key allelochemicals of flixweed that impact wheat growth by providing evidence of flixweed interference on seed germination and seedling growth of wheat cultivars mediated by soil allelopathy and the mechanism of release of these allelochemicals in soil.

MATERIALS AND METHODS

I. Plant material

The aerial parts of *Descurainia sophia* L. were collected in April 2010, at physiological maturity from wheat field in Liangxiang village (39°43' N and 116°60' E), Fangshan District, Beijing City, China. The plant was air-dried in shade and ground to fine powder and then passed through a 2-mm sieve and stored in a dark glass flask at 4°C. The selected wheat cultivar was 'Duokang 1', major cultivar in China and was obtained from the Institute of Crop Research, Chinese Academy of Agricultural Sciences, Beijing.

II. Choice of extraction solvent

The air-dried *Descurainia sophia* L. were ground into a powder and 100 g sample was extracted with 1500 mL of petroleum ether, acetone, 95% ethanol or methanol (shaken for 3 days at 25°C and vacuum-filtered through filter paper at 0.05 MPa). The filtrate was concentrated under vacuum at a reduced pressure, yielding 16 g, 17 g, 13 g and 16 g of residue, respectively; all residues were stored at -4°C. Each residue was dissolved with the original extraction solvent to prepare a 100 g/L solution. Diluted solutions (50 g/L) were used to prepare the wheat bioassays. Wheat germination and seedling growth were evaluated using bioassays. The fractions showing the greatest inhibition of wheat germination and growth were further separated.

III. Initial separation of the allelochemicals

Based on the above tests results, 5 kg sample of *Descurainia sophia* L. was extracted thrice with 30 L 95% ethanol that exhibited the maximum inhibition of wheat germination and growth. The procedure was identical to that described in the previous section. The filtrate was subsequently evaporated to approx 1000 mL and transferred to a separatory funnel. Petroleum ether, chloroform, ethyl acetate and n-butanol (in ascending order of polarity) were used as solvents; each extraction solvent volume was 3-times greater than the sample volume. Samples were shaken vigorously for 15 min to ensure complete extraction and then distilled. The resulting sample was then combined with same extraction solvent and concentrated into a paste in a rotary evaporator. The concentrated compound was then diluted to 0.5 g/L, 1 g/L, and 10 g/L in methanol for the wheat bioassay.

IV. Bioassay

Seed germination: The wheat seeds were sterilized with 5% sodium hypochlorite for 10 min and thoroughly rinsed thrice with deionized water. They were then imbibed in deionized water at 21°C for 12 h and carefully blotted with paper towels. Three replicates of 20 seeds for each treatment were uniformly distributed in Petri dishes (10-cm dia) containing 50 g soil. A 5-mL aliquot of extract from the different phases (at 50 g/L concentration of solution) and 10 mL distilled water were added to each Petri dish. The same volume of distilled water containing the same concentration of the organic solvent served as control. The Petri dishes were subsequently placed in an illuminated growth chamber [25°C ± 1°C and 80 ± 2% RH (relative humidity) under a 12/12 h L/D photoperiod]. The seeds were considered germinated when the radicle was visible protruding through the lemma and palea. The germinated seeds were counted daily for 7 days and then shoot height and root length were measured (30 seedlings with radicle lengths of approximately 1 mm). Based on the number of germinated seeds, the germination index was calculated as under:

$$\text{Germination index (GI)} = \sum (Gt/Tt),$$

Where Gt : Number of germinated seeds on t th day and Tt : Time corresponding to Gt in days and then vigour index ($VI = GI \times \text{shoot height}$) were calculated (2).

Seedling growth: A 5-mL aliquot of extract from the different phases (diluted with water to concentration of 50 g/L solution) and 10 mL distilled water were added to each pot (5 cm × 5 cm) containing 100 g soil. Three replicates of 20 pre-germinated seeds for each treatment were evenly sown in each pot. The same volume of distilled water containing the same concentration of the organic solvent served as control. The pots containing the wheat seedlings were placed in a growth chamber [25°C during the 12 h light period and 20 °C during the 12 h dark period at 80 ± 2% RH under a 12/12 h L/D photoperiod]. The pots were watered once daily. The length of the seedling root and shoot were measured after 14 days. The shoots were then clipped at the juncture of the first root, and the dry weights were determined after drying for 48 h at 70°C.

V. Isolation and identification of the allelochemicals

The fraction (563 g) with the strongest inhibition of wheat germination and growth in the initial separation of the allelochemicals was subjected to silica gel column chromatography (80 cm × 6 cm) using n-hexane/ethyl acetate (100:0, 10:1, 3:1 and 0:100; v/v) and ethyl acetate/methanol (10:1 and 4:1; v/v) mixtures as eluting solvents, and 50 ml fractions of each were collected. Column fractions were combined on the basis of their TLC patterns into two main fractions (A, B) for the 100:0 (v/v) n-hexane/ethyl acetate eluent, two main fractions (C-D) for the 10:1 (v/v) n-hexane/ethyl acetate eluent, three main fractions (E-G) for the 3:1 (v/v) n-hexane/ethyl acetate eluent, two main fractions (H, I) for the 0:100 (v/v) n-hexane/ethyl acetate eluent, three main fractions (J-L) for the 10:1 (v/v) ethyl acetate/methanol eluent, and two main fractions (M, N) for the 4:1 (v/v) ethyl acetate/methanol eluent. The 50 g/L concentrated fractions were prepared for the wheat bioassay (results shown in Fig. 4); the fractions F, I, J and K had the strongest inhibition of wheat growth of 3.1 g, 2.9 g, 3.3 g and 4.1 g, respectively. These fractions were further purified using a prepared Sephadex LH20 column (70 cm × 2 cm) eluted with methanol/chloroform (1:1), and 30 mL fractions were collected. The collected fractions were separately run through a Sephadex LH20 column to obtain pure compounds 1 (98 mg), 2 (87 mg), 3 (101 mg) and 4 (63 mg). The melting point, UV, HRMS (high resolution mass spectra) and NMR (nuclear magnetic resonance) of these compounds were then determined. The molecular formulae and structures were identified using a combination of spectral data and information from the literature (19).

VI. Bioassay of purified compounds

The quercetin, isorhamnetin, xanthotoxol and xanthotoxin were purchased from ChromaDex (USA) and dissolved in methanol. Concentrations of 30 mg/kg, 150 mg/kg, and 200 mg/kg were added to Petri dishes (10-cm dia) containing 150 g soil. The final concentrations of the compounds used for the wheat bioassay were 10, 50, 100 and 150 mg/kg in soil. The same volume of distilled water, which contained the same concentration of the organic solvent, served as the control. The biological activity of these substances was determined as described above.

Mechanism of allelochemicals release

To determine the mechanism of allelochemical release from *Descurainia sophia* L.

into the surrounding soil medium, the following experiments were done. The aerial parts of *Descurainia sophia* L. were collected at physiological maturity from the same field. A 250 g plant sample was chopped into pieces 10-15 cm in length and then 750 mL distilled water (v/v = 1:3) was added and left to infuse for 48 h. The filtrate was extracted with 1500 ml (v/v = 1:2) petroleum ether, ethyl acetate or chloroform. The filtrate was concentrated under vacuum at a reduced pressure and the dry residue was dissolved in 200 mL of acetonitrile and filtered with 0.22- μ m syringe filters for UPLC-MS/MS analysis.

Response Index

The effects of allelochemicals were evaluated using the method of Williamson and Richardson (27). The response index (RI) was determined as under:

$$\begin{aligned} &\text{If } T \geq C, \\ &\text{RI} = 1 - C / T, \\ &\text{and if } T < C, \text{ then RI} = T / C - 1, \end{aligned}$$

Where, T : Treatment response and C : Control response. The RI values ranged from +1 to -1 with positive values indicating stimulation and negative values indicating inhibition relative to the controls. The RI is easy to interpret upon visual inspection, because it is simply the proportional reduction of the treatment relative to the control when stimulation occurs (a negative RI) or of the control relative to the treatment when inhibition occurs (a positive RI). The absolute values of the RI varied directly with the allelopathic effects.

Statistical Analysis

Duncan's Multiple Range (DMR) test was applied to analyze the results of experiments evaluating the allelopathic effects of the extractions from the aerial parts of *Descurainia sophia* L. (28). The level of significance was set at $p < 0.05$. All values are the mean (\pm S.E.) of three replicates, and the data in a column followed by the same letter are not significantly different. All data were analyzed using the Statistical Package for Social Sciences (SPSS; version 17.0 for Windows).

RESULTS AND DISCUSSION

I. Choice of extraction solvent

It is necessary to demonstrate the inhibition of the target for allelopathic substances to be considered to be effective. The comparative overall effect of *Descurainia sophia* L. on seed germination and seedling growth is shown in Fig. 2. The four organic extracts obtained from *Descurainia sophia* L. inhibited the seed germination and seedling growth of wheat, but the 95% ethanol extract was most inhibitory to germination rate, vigour index, shoot and root length, shoot fresh weight, and shoot and root dry weight. Therefore, 95% ethanol was chosen as the extraction solvent.

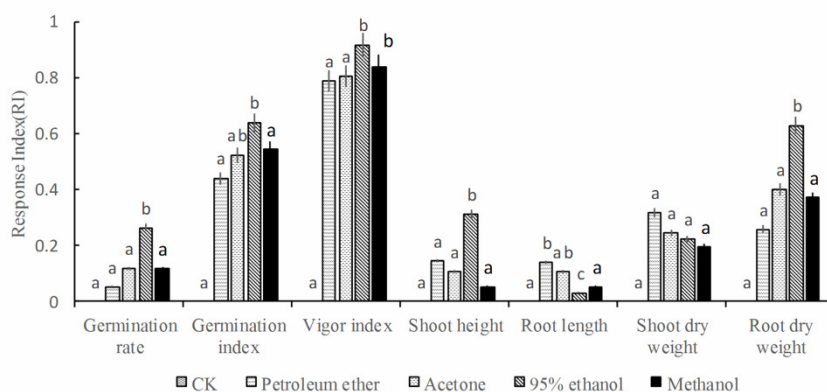


Figure 2. Phytotoxicity of organic extracts from the aerial part of *Descurainia sophia* L. on the germination and seedling growth of wheat cv Duokang 1.

II. Initial separation of allelochemicals

The inhibitory effects of 4-organic extracts (Petroleum ether, chloroform, ethyl acetate and n-butanol) obtained from the 95% ethanol extract on seed germination and seedling growth are given in Fig. 3. All values were negative, indicating that the four organic extracts inhibited the seed germination and seedling growth of wheat. Ethyl acetate proved the most inhibitory to the seed germination and seedling growth of wheat.

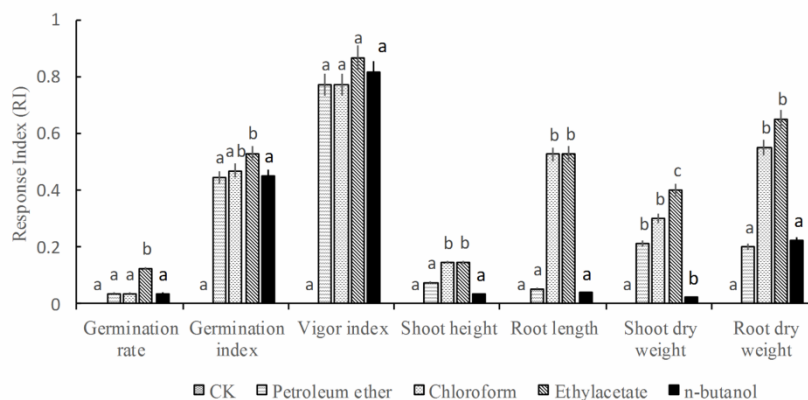


Figure 3. Phytotoxicity of the organic obtained from liquid-liquid partitioning of the 95% ethanol extract from *Descurainia sophia* L. on the germination and seedling growth of wheat cv Duokang 1.

III. Isolation and identification of allelochemicals

Column chromatography in silica gel yielded 14-major fractions. The fractions F, I, J and K showed the greatest inhibitory effects on the wheat growth (Table 1). The isolation of their bioactive compounds yielded two furocoumarins (compound 1 and 2) and two flavonoids (compounds 3 and 4) that were identified by comparison of their UV, HRMS and NMR spectra with those previously published (19). And the structure of these compounds was given in Fig.4.

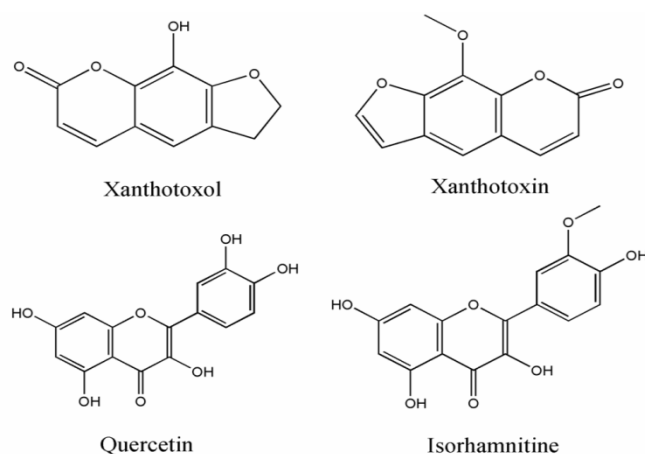


Figure 4. Structure of four compounds isolated from the aerial parts of *Descurainia sophia* L.

Xanthotoxol (Compound 1): White crystals, $R_f = 0.67$, m.p. = 253-255 °C; UV (λ_{max} in methanol): bands at 221, 244 and 300 nm; $^1\text{H-NMR}$ in deuterated DMSO (400 MHz): δ 8.21 (1H, d, $J = 9.5$ Hz, H-4), δ 8.13 (1H, d, $J = 3$ Hz, H-7), δ 7.65 (1H, s, H-5), δ 7.11 (d, 1H, $J = 3$ Hz, H-6) and δ 6.44 (1H, d, $J = 10$ Hz, H-3). EI-MS m/z (% rel. int.): M^+ at 202(100), 186(26), 185(18), 173(16), 145(11) and 71(10).

Xanthotoxin (Compound 2): White crystals, $R_f = 0.65$, m.p. = 145- 146 °C; UV (λ_{max} in methanol): 218, 249 and 299 nm. $^1\text{H NMR}$ in deuterated DMSO (400 MHz): δ 8.22 (1H, d, $J = 10$ Hz, H-4), δ 7.79 (1H, d, $J = 3$ Hz, H-7), δ 7.43 (1H, s, H-5), δ 6.91 (1H, d, $J = 3$ Hz, H-6), δ 6.41 (1H, d, $J = 10$ Hz, H-3) and δ 3.9 (3H, s, OCH₃). EI-mass m/z (% rel. int.): M^+ at 216(100), 201(22) and 173(56).

Quercetin (Compound 3): UV (λ_{max} in methanol): 255, 300 and 370; NaOMe: 290, 330 and 440; NaOAc: 260, 335 and 381; H₃BO₃: 260, 295 and 365; AlCl₃: 270, 312 and 445; HCl: 265, 305 and 425. $^1\text{H-NMR}$ (400 MHz, DMSO-*d*₆) δ (ppm): d 7.7 (1H, d, $J = 8.5$ Hz, H-2'), d 7.5 (1H, dd, $J = 8.5, J = 2.5$ Hz, H-6'), d 6.8 (1H, d, $J = 8.5$ Hz, H-5'), d 6.5 (1H, d, $J = 1.5$ Hz, H-6), and d 6.2 (1 H, d, $J = 1.5$ Hz, H-8).

Table 1. Allelopathic effect of 14 components of the first time column separations of ethyl acetate phase of the aerial part of *Descurainia sophia* L. extract on the germination and seedling growth of tested wheat (Duokang 1)

Fractions	Response Index (RI)						
	Germination rate	Germination index	Vigor index	Shoot height	Root length	Shoot DW	Root DW
A	-0.031±0.80a	-0.425±0.15a	-0.609±0.41a	-0.107±0.25a	-0.087±0.41a	-0.252±0.46a	-0.283±0.26a
B	-0.080±0.20b	-0.461±0.14a	-0.640±0.36a	-0.111±0.32a	-0.081±0.52ab	-0.252±0.45a	-0.355±0.89ab
C	-0.080±0.48b	-0.457±0.11a	-0.642±0.35a	-0.135±0.15ab	-0.093±0.54ab	-0.278±0.41a	-0.337±0.96ab
D	-0.107±0.24bc	-0.474±0.14a	-0.663±0.34a	-0.151±0.17abc	-0.091±0.49ab	-0.270±0.29a	-0.305±0.29ab
E	-0.093±0.30bc	-0.474±0.14a	-0.687±0.29a	-0.143±0.09abc	-0.078±0.84ab	-0.261±0.26a	-0.290±0.36a
F	-0.361±0.05d	-0.626±0.08b	-0.854±0.12a	-0.390±0.05e	-0.294±0.07c	-0.487±0.07a	-0.546±0.06b
G	-0.121±0.14bc	-0.484±0.12a	-0.686±0.29a	-0.158±0.33bc	-0.089±0.56ab	-0.243±0.20a	-0.332±0.20ab
H	-0.100±0.19bc	-0.478±0.15a	-0.681±0.31a	-0.124±0.39ab	-0.083±0.70ab	-0.261±0.39a	-0.332±0.20ab
I	-0.128±0.12c	-0.486±0.12a	-0.690±0.29a	-0.203±0.23d	-0.107±0.58b	-0.330±0.33a	-0.337±0.28ab
J	-0.128±0.12c	-0.492±0.13a	-0.697±0.28a	-0.188±0.14cd	-0.113±0.54b	-0.278±0.32a	-0.359±0.89ab
K	-0.216±0.12d	-0.525±0.13a	-0.700±0.29a	-0.192±0.14cd	-0.121±0.54b	-0.288±0.32a	-0.341±0.17ab
L	-0.121±0.25bc	-0.481±0.12a	-0.700±0.27a	-0.142±0.27abc	-0.10±0.28ab	-0.252±0.37a	-0.283±0.26a
M	-0.107±0.43bc	-0.475±0.12a	-0.700±0.28a	-0.147±0.20abc	-0.097±0.53ab	-0.209±0.17a	-0.272±0.10a
N	-0.114±0.33bc	-0.478±0.14a	-0.687±0.29a	-0.135±0.15ab	-0.093±0.54ab	-0.278±0.41a	-0.328±0.96ab

DW : Dry weight.

Isorhamnetin (Compound 4): UV (λ_{\max} in MeOH): 253, 267 and 370; NaOMe: 271, 328 and 435; NaOAc: 259, 270 and 391; H₃BO₃: 257, 272 and 360; AlCl₃: 253, 268 and 410; HCl: 252, 269 and 359. ¹H-NMR (400 MHz, DMSO-d₆) δ (ppm): δ 7.9 (1H, d, J = 2 Hz, H-2'), δ 7.45 (1H, dd, J = 2, 8.5, J=2.5 Hz, H-6'), δ 6.9 (1H, d, J = 8.5 Hz, H-5'), δ 6.35 (1H, d, J = 2 Hz, H-8), δ 6.15 (1 H, d, J=2 Hz, H-6) and 3.8 (s, OMe).

IV. Bioassays of identified compounds

The biological activities of the 4-compounds (xanthoxol, xanthotoxin, quercetin and isorhamnetin) were determined using wheat germination and seedling growth (Fig. 5). The four compounds had different inhibitory effects on the germination and seedling growth. The experimental results showed that xanthoxol was significantly inhibitory to wheat seed germination, while the inhibitory effect of xanthotoxin was not evident. In seed growth experiments, we compared the influence of the different compounds on the same index and the same compounds on different indices. The results showed that the four compounds had inhibitory effects on the root length of wheat at concentrations as low as 50 mg/kg, and the inhibitory effects were enhanced when the concentrations of the compounds increased. The xanthotoxin was most inhibitory to wheat root length at 150 mg/kg. To determine the impact of 4-compounds on the shoot length of wheat, we found that the inhibitory effects of 4-compounds were enhanced, when their concentrations were increased. The 4-compounds showed the significantly inhibited the wheat shoot growth. The inhibitory effects of 4-compounds on dry weight of wheat also gradually enhanced when the concentrations of the compounds was increased. At the minimum concentration, the inhibitory effects of xanthotoxin and quercetin were weak. Nevertheless, when the concentrations of 4-compounds were 50 mg/kg, the inhibitory effects were visible. Experiment to further validate that the *Descurainia Sophia* L. had strong allelopathic potential and could cause different degrees of influence on the surrounding plants by releasing allelochemicals.

Descurainia sophia L. allelopathy can be achieved by allelochemicals produced and released into the environment. The allelopathic activity test showed that the four compounds from *Descurainia sophia* L. could play a role as allelochemicals for the defense of wheat against other plants. Nevertheless, there was insufficient evidence of allelopathic activity to explain how other plants are affected in the process of the wild oats growth, and it is not known what allelochemicals besides these five compounds can be released into the ambient environment by *Descurainia sophia* L.

V. Mechanism of allelochemical release

The aerial parts of *Descurainia sophia* L. contained four compounds that were phytotoxic on wheat. These substances could be key allelochemicals associated with the invasion of *Descurainia sophia* L. in wheat fields. There are many ways in which allelochemicals can be released into the environment, such as root exudations and volatile compounds. Rose et al. noted that water-soluble plant extracts could leach into the soil and influence plant growth and microbial activity (22). Several explanations are tentatively proposed to account for this phenomenon. First, repeated washing by rain and interception by dew could play a major role in the transport of allelochemicals. The impact of raindrops

can cause large quantities of water-soluble substances to be released into the soil and consequently impact microbial growth. In this case, there could be inhibitory effects associated with the crude extract. Second, a wide range of secondary chemicals can be exuded from the plant roots; these compounds could directly and indirectly affect both the activity and the community composition of the soil microbiota (13,21). Water extracts from plant materials typically contain water-soluble carbohydrates. Thus, our results indicate that quercetin and isorhamnetin were released by washing of rain, fog or dew.

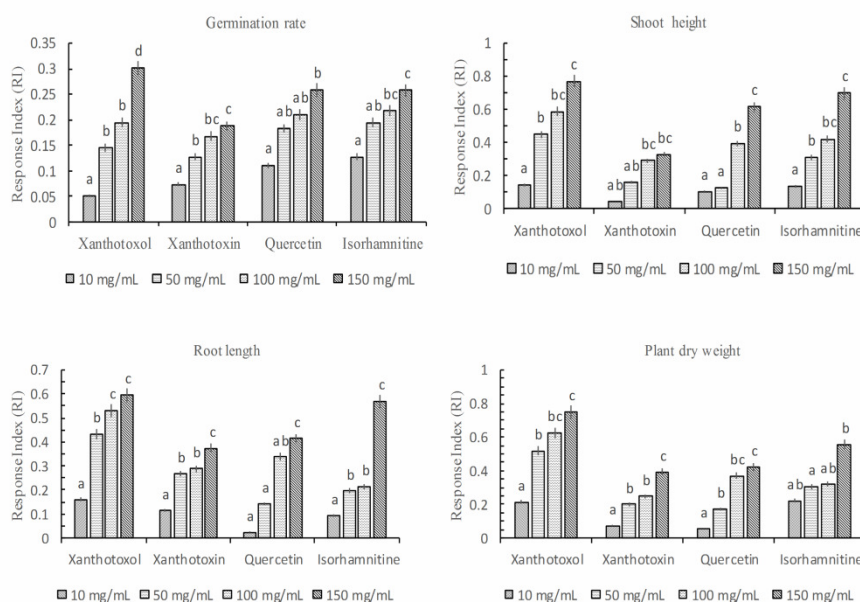


Figure 5. Allelopathic effects of 4 identified compounds on the germination and seedling growth of wheat cv Duokang 1.

The 4-compounds were identified using UPLC-MS/MS. The identity of the analyte was confirmed by the retention time and the major mass signals of the mass spectra of standards that had been identified in section isolates; quercetin and isorhamnetin were found in the chloroform phase. The results indicated that quercetin and isorhamnetin were released into the environment by washing of rain, dew or fog.

In this study, we have demonstrated complex biotic and abiotic interactions between *Descurainia sophia* L. and crop plants. The interference of *Descurainia sophia* L. with wheat growth involves several mechanisms, but the allelopathic mechanism in which *Descurainia sophia* L. releases allelochemicals into the soil to inhibit wheat growth is a particularly important one. It is not known what other allelochemicals, in addition to the four compounds identified, may be released into the environment by *Descurainia sophia* L. Answers to these questions could potentially lead to the development of natural bio-control

chemicals such as herbicides and insecticides and result in improved ecosystem management (4,8,20). Finally, as discussed above, the data indicate that the four extraction solvents have an inhibitory effect on wheat seed germination and seedling growth. However, we have no additional analysis of the extracts from the other three extraction solvents. Further study is needed regarding the release mechanism of the other two allelochemicals.

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