

# Effect of leaf water extracts of four Asteraceae alien invasive plants on germination performance of *Lactuca sativa* L. under acid deposition

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Received: 2 December 2020/Accepted: 1 February 2021/Published online: 19 February 2021 © The Author(s), under exclusive licence to Springer Nature B.V. part of Springer Nature 2021

**Abstract** Allelopathy of alien invasive plants (AIP) on plant germination performance is essential for their successful invasion. However, the allelopathy of AIP may be reformed or even strengthened under acid deposition. AIP in Asteraceae covers the uppermost number of AIP species at the family level presently in China. It is necessary to estimate the allelopathy of multiple Asteraceae AIP under acid deposition to address the mechanism driving their successful invasion, especially under acid deposition. However, research in this area is very restricted presently. This study purposes to estimate the allelopathy of four Asteraceae AIP, i.e., Convza canadensis L. Crong., Erigeron annuus (L.) Pers., Aster subulatus Michx., and Bidens pilosa L., on germination performance of the cultivated Asteraceae plant species Lactuca sativa L. which is sensitive to allelochemicals under acid deposition with different levels of acidity. Of the four Asteraceae AIP, C. canadensis, E. annuus, and B. pilosa create noticeable allelopathy on germination performance of L. sativa. The allelopathy of the four

Communicated by Chuihua Kong.

C. Wang

Asteraceae AIP decreases in the following order: *E. annuus*, *C. canadensis*, *B. pilosa*, and *A. subulatus*. Acid deposition with a low level of acidity reduces the allelopathy of *C. canadensis*, *E. annuus*, and *B. pilosa*. Inversely, acid deposition with a high level of acidity elevates the allelopathy of *B. pilosa*. The progressively growing level of acid deposition with high acidity may facilitate the invasion process of *B. pilosa* via the improved level of allelopathy.

**Keywords** Allelochemicals · Germination performance · Growth fitness · Invasion process · *Lactuca sativa* L.

## Introduction

Currently, alien invasive plants (AIP) cause a significant effect on the ecosystem, especially the biodiversity and stability of plant community (Kiełtyk and Delimat 2019; Lyytinen and Lindström 2019; Wang et al. 2020a). Hence, the issues actuating the efficient colonization of AIP have converted one of the core issues of invasive ecologists recently. Several AIP can seriously endanger plant growth fitness, especially germination performance, mainly via the allelopathy mediated by the released allelochemicals (Wang et al. 2020b; Gris et al. 2019; He et al. 2019; Lyytinen and Lindström 2019; Wei et al. 2020). However, the

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germination performance controls the first stage of plant growth and population maintenance (Wang et al. 2020b; Gris et al. 2019; He et al. 2019; Lyytinen and Lindström 2019; Wei et al. 2020). Predictably, the reduced plant germination performance recruited by the raised allelopathy of AIP can significantly restrain their growth fitness (Wang et al. 2020b; Gris et al. 2019; He et al. 2019; Lyytinen and Lindström 2019; Wei et al. 2020). Further, AIP in Asteraceae covers the top of AIP species number at the family level currently in China (Wang et al. 2016a). Hence it is necessary to estimate the allelopathy of several Asteraceae AIP on plant germination performance to clarify the driving mechanism that regulates the successful invasion of Asteraceae AIP.

Acid deposition is getting worse with the increasing intensity and frequency of atmospheric activities, especially the fast development of modern industry and the number of vehicles, etc. (Solberg et al. 2004; Xu et al. 2015a, b; Yu et al. 2017; Du et al. 2020). Specifically, China has become one of the three major areas polluted by acid deposition across the world currently (Wang et al. 2007; Xu et al. 2015a, b; Liu et al. 2017; Yu et al. 2017). Nevertheless, the steadily increasing acid deposition can trigger a profound influence on plant growth (Wu et al. 2013; Wang et al. 2018; Du et al. 2017; Liu et al. 2018a, b; Huang et al. 2019). Specifically, acid deposition can also rise the growth fitness of AIP (Wang et al. 2018) and the allelopathy of AIP on germination performance of plant species (Wang et al. 2012a, b, 2016b). Consequently, it is necessary to evaluate the allelopathy of numerous Asteraceae AIP on plant germination performance under acid deposition to illuminate the mechanism actuating the successful colonization of Asteraceae AIP especially in the context of acid deposition. However, research in this area is very limited presently. Specifically, most progress in the influences of acid deposition on the allelopathy of AIP focuses on the impacts of acid deposition on the allelopathy of one AIP species, but ignore the species differences in the allelopathy (Wang et al. 2012a, b, 2016b).

This study purposes to evaluate the allelopathy of four Asteraceae AIP, i.e., *Conyza canadensis* L. Cronq., *Erigeron annuus* (L.) Pers., *Aster subulatus* Michx., and *Bidens pilosa* L., using leaf extracts on germination performance of the cultivated Asteraceae plant species *Lactuca sativa* L. under acid deposition with different levels of acidity via a hydroponic culture method in 9 cm Petri dishes. In particular, the four Asteraceae AIP are all originated from North America (Wang et al. 2016a) and consequently they share a similar or even identical evolutionary process in the diffusion phase and subsequent invasion behavior in China supposedly. Meanwhile, the four Asteraceae AIP have entered the list of the most destructive AIP in China chiefly because of their remarkable influences on plant communities where the invasion process occurred. Further, the allelopathy of the four Asteraceae AIP on plant germination performance is vital for their successful invasion (Khanh et al. 2009; Djurdjević et al. 2012; Fabbro et al. 2014; He et al. 2019; Wei et al. 2020). As a commonly cultivated plant species in the region which has been invaded by the four Asteraceae AIP and also polluted by acid deposition, L. sativa is a bioindicator species for the study of the allelopathy of AIP on plant germination performance (Carvalhoa et al. 2019; Gris et al. 2019; Jmii et al. 2020; Wei et al. 2020).

We check the two following hypotheses: (I) allelopathy of the four Asteraceae AIP on germination performance of *L. sativa* may have significant interspecific differences and (II) acid deposition can strengthen the allelopathy of the four Asteraceae AIP on germination performance of *L. sativa*.

# Materials and methods

Preparation of the allelopathy solution and acidic solution

The mature leaves of four Asteraceae AIP (annual herbs and non-clonal plants), i.e., C. canadensis, E. annuus, A. subulatus, and B. pilosa, were randomly gathered from Zhenjiang (located at 32°21'N and 119°52'E) of Jiangsu, China in September 2019. The gathered leaves of the four Asteraceae AIP were mildly washed and subsequently air-dried drastically at about 25 °C. The air-dried leaves of the four Asteraceae AIP were soaked in sterile distilled water at about 25 °C for approximately 48 h to produce the allelopathy solution at 20 g  $L^{-1}$  (to imitate the status with plant invasion). Sterile distilled water was used as the treatment of control  $(0 \text{ mg } \text{L}^{-1})$  to imitate the status without plant invasion. The allelopathy solution of the four Asteraceae AIP was placed at approximately 4 °C not exceeding hebdomad.

The acidic solution was prepared to simulate acid deposition by blending 0.5 M  $H_2SO_4$  and 0.5 M  $HNO_3$  at 5:1 ratio with a gradient level of acidity, i.e., pH 5.6 and pH 4.5, with sterile distilled water as the treatment of control (pH 7.0) to imitate the status without acid deposition). Specifically, the pH of normal rainfall without pollution is about 5.6 (Mishra et al. 2012; Wang et al. 2016b, 2018). Further, the acidic solution at pH 4.5 imitated the near-annual mean pH value of actual rainfall at Zhenjiang (Wang et al. 2007, 2016b, 2018; Yu et al. 2017). Further, the ratio of  $SO_4^{2-}$  and  $NO_3^{-}$  was about 5:1 for the actual rainfall at Zhenjiang (Wang et al. 2017).

Experimental design of the germination performance of *L. sativa* 

The experiment of germination performance of *L. sativa* included fifteen treatment combinations (triplicates per treatment combination) with all independent and combined treatment combinations of the allelopathy solution of the four Asteraceae AIP and the acidic solution with a gradient level of acidity. All of the experimental design of germination performance of *L. sativa* is presented in Table 1.

The seeds of *L. sativa* (cultivar name: cv. Xingmiao-Hongdajiang) were obtained in a local farm produce fair. Specifically, thirty seeds of *L. sativa*  which were full and uniform in size were placed in Petri dishes (9 cm) from December 2 to 12, 2019 at approximately 25 °C for 8 d at the condition of 12 h light per day. Further, the light intensity was set to 27.5  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. Meanwhile, 0.5 mL of sterile deionized water, allelopathy solution of the four Asteraceae AIP, and/or acidic solution were added per Petri dish every day. Specifically, allelopathy solution of the four Asteraceae AIP and acidic solution in the combined treatments were mixed in equal proportions (i.e., 1:1). Meanwhile, the final concentration of allelopathy solution of the four Asteraceae

AIP in the independent and combined treatment combinations was all set to 20 g  $L^{-1}$ . More descriptions about the experiment of germination performance of *L. sativa* are included in our former reports (Wei et al. 2020).

Measurement of the germination performance indices of *L. sativa* 

After the hydroponic cultivation for 8 d, ten seedlings of *L. sativa* per Petri dish (from thirty seedlings of *L. sativa* for one treatment combination) were randomly chosen to evaluate the values of germination performance indices of *L. sativa*. The assay-determining indices of *L. sativa* in this study is the same as in our former research (Wang et al. 2020b).

Table 1 Experimental design of the germination performance of Lactuca sativa L.

No	Treatment combinations	Concentration
I	Control (sterile distilled water)	$0 \text{ g } \text{L}^{-1}$
II	Conyza canadensis L. Cronq. leaf extract	$20 \text{ g L}^{-1}$
III	Erigeron annuus (L.) Pers. leaf extract	$20 \text{ g L}^{-1}$
IV	Aster subulatus Michx. leaf extract	$20 \text{ g L}^{-1}$
V	Bidens pilosa L. leaf extract	20 g L
VI	Acidic solution at pH 5.6	$0.5 \text{ M H}_2\text{SO}_4$ and $0.5 \text{ M HNO}_3$ at ratio of $5:1$
VII	Acidic solution at pH 4.5	$0.5 \text{ M H}_2\text{SO}_4$ and $0.5 \text{ M HNO}_3$ at ratio of $5:1$
VII	Combined C. canadensis leaf extract and acidic solution at pH 5.6	
IX	Combined E. annuus leaf extract and acidic solution at pH 5.6	
Х	Combined A. subulatus leaf extract and acidic solution at pH 5.6	
XI	Combined B. pilosa leaf extract and acidic solution at pH 5.6	
XII	Combined C. canadensis leaf extract and acidic solution at pH 4.5	
XIII	Combined E. annuus leaf extract and acidic solution at pH 4.5	
XIV	Combined A. subulatus leaf extract and acidic solution at pH 4.5	
XV	Combined B. pilosa leaf extract and acidic solution at pH 4.5	



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◄ Fig. 1 Seed germination indices of L. sativa. Bars (means and SE) with different letters represent a significant difference (P < 0.05). Abbreviations: CK, Control; pH4.5, acidic solution at pH 4.5; pH5.6, acidic solution at pH 5.6; CC, C. canadensis leaf extract; CCpH4.5, combined C. canadensis leaf extract and acidic solution at pH 4.5; CCpH5.6, combined C. canadensis leaf extract and acidic solution at pH 5.6; EA, E. annuus leaf extract; EApH4.5, combined E. annuus leaf extract and acidic solution at pH 4.5; EApH5.6, combined E. annuus leaf extract and acidic solution at pH 5.6; AS, A. subulatus leaf extract; ASpH4.5, combined A. subulatus leaf extract and acidic solution at pH 4.5; ASpH5.6, combined A. subulatus leaf extract and acidic solution at pH 5.6; BP, B. pilosa L. leaf extract; BPpH4.5, combined B. pilosa leaf extract and acidic solution at pH 4.5; BPpH5.6, combined B. pilosa leaf extract and acidic solution at pH 5.6

#### Statistical analyses

Differences in germination performance indices of *L. sativa* among the treatment combinations were characterized by ANOVA with Tukey's test for the operation of multiple comparisons. The threshold of

statistically significant differences was set at  $P \le 0.05$ . IBM SPSS Statistics (version 25.0) was used for statistical analyses.

### Results

Influences of allelopathy solution of the four Asteraceae AIP and acidic solution on germination performance of L. sativa compared with control

All seed germination indices and root length of *L.* sativa were reduced under *C. canadensis* and *E.* annuus leaf extracts (P < 0.05; Figs. 1a–f and 2b). Germination index, germination rate index, germination vigor index, and root length of *L. sativa* were declined under *B. pilosa* leaf extract (P < 0.05; Figs. 1c–e and 2b). Root length of *L. sativa* was decreased under *A. subulatus* leaf extract (P < 0.05; Fig. 2b). However, leaf length and fresh weight were



Fig. 1 continued



**Fig. 2** Seedling growth indices of *L. sativa*. Bars (means and SE) with different letters representing a significant difference (P < 0.05). Abbreviations have identical meanings as labeled in Fig. 1. "ns" means no significant difference (P > 0.05)

increased under A. subulatus leaf extract (P < 0.05; Fig. 2c, f).

Germination percentage and germination potential of *L. sativa* under *C. canadensis* and *E. annuus* leaf extracts were less than those under *A. subulatus* and *B. pilosa* leaf extracts (P < 0.05; Fig. 1a, b). Germination index, germination rate index, germination vigor index, and promptness index of *L. sativa* under *C. canadensis*, *E. annuus*, and *B. pilosa* leaf extracts were less than those under *A. subulatus* leaf extracts were less than those under *A. subulatus* leaf extract (P < 0.05; Fig. 1c–f). Leaf length of *L. sativa* under *E. annuus* leaf extract was less than that under *A. subulatus* leaf extract (P < 0.05; Fig. 2c). Fresh weight of *L. sativa* under *E. annuus* leaf extract was less than that under *A. subulatus* leaf extracts (P < 0.05; Fig. 2c). Fresh

The independent acidic solution did not significantly impact the germination performance of *L. sativa* (Figs. 1a–f and 2a–h).

All seed germination indices of L. sativa were decreased under the combined C. canadensis leaf extract and acidic solution at pH4.5, the combined E. annuus leaf extract and acidic solution at pH5.6, and the combined B. pilosa leaf extract and acidic solution at pH4.5 (P < 0.05; Fig. 1a–f). Root length of L. sativa was declined under all combined treatment combinations of the allelopathy solution of the four Asteraceae AIP and acidic solution (P < 0.05; Fig. 2b). Germination index, germination rate index, and germination vigor index of L. sativa were decreased under the combined E. annuus leaf extract and acidic solution at pH4.5 (P < 0.05; Fig. 1c-e). Germination index and germination vigor index of L. sativa were attenuated under the combined C. canadensis leaf extract and acidic solution at pH5.6 and the combined B. pilosa leaf extract and acidic solution at pH5.6 (P < 0.05; Fig. 1c, e). However, leaf length of L. sativa was increased under the combined C. canadensis leaf extract and acidic solution at pH5.6, the combined A. subulatus leaf extract and acidic solution at pH5.6, the combined B. pilosa leaf extract and acidic solution at pH5.6, and the combined A. subulatus leaf extract and acidic solution at pH4.5 (P < 0.05; Fig. 2c). Similarly, fresh weight of *L. sativa* was increased under the combined *C. canadensis* leaf extract and acidic solution at pH5.6 (P < 0.05; Fig. 2f). Moisture content of *L. sativa* was increased under the combined *A. subulatus* leaf extract and acidic solution at pH4.5 (P < 0.05; Fig. 2h).

Influences of the combined allelopathy solution of the four Asteraceae AIP and acidic solution on germination performance of *L. sativa* compared with the independent allelopathy solution of the four Asteraceae AIP

All seed germination indices of L. sativa under the combined C. canadensis leaf extract and acidic solution at pH5.6, the combined E. annuus leaf extract and acidic solution at pH4.5, and the combined E. annuus leaf extract and acidic solution at pH5.6 were higher than those under C. canadensis and E. annuus leaf extracts, respectively (P < 0.05; Fig. 1a–f). Germination index, germination rate index, and germination vigor index of L. sativa under the combined B. pilosa leaf extract and acidic solution at pH5.6 were higher than those under B. pilosa leaf extract (P < 0.05; Fig. 1c–e). Green leaf area and fresh weight of L. sativa under the combined C. canadensis leaf extract and acidic solution at pH5.6 and the combined E. annuus leaf extract and acidic solution at pH5.6 were higher than those under C. canadensis and E. annuus leaf extracts, respectively (P < 0.05; Fig. 2e, f). Inversely, germination percentage, germination potential, germination index, germination vigor index, and promptness index of L. sativa under the combined B. pilosa leaf extract and acidic solution at pH4.5 were less than those under B. pilosa leaf extract (P < 0.05; Figs. 1a-c, e-f).

# Discussion

As expected, the four Asteraceae AIP, particularly *C. canadensis*, *E. annuus*, and *B. pilosa*, form evident allelopathy on germination performance of *L. sativa*, especially on germination competitiveness, seed viability and germination uniformity, germination rate and vitality, germination responsiveness to the external environment, and seedling competitiveness for water and inorganic salt absorption in this study. Hence the growth fitness of *L. sativa* can be



Fig. 2 continued

remarkably attenuated under the allelopathy mediated by the four Asteraceae AIP. The most likely factor may be due to the created allelochemicals formed by AIP which can incur harmful influences, e.g., disrupting nutrient absorption efficiency and intensity on plant growth and development (Wang et al. 2020b; Gris et al. 2019; He et al. 2019; Lyytinen and Lindström 2019; Wei et al. 2020).

Further, there are noteworthy interspecific differences in the allelopathy of the four Asteraceae AIP on germination performance of L. sativa, especially on germination competitiveness, seed viability and germination uniformity, germination rate and vitality, germination responsiveness to the external environment, and seedling growth competitiveness, in this study. Further, the allelopathy of C. canadensis and E. annuus is noticeably superior to those of A. subulatus and *B. pilosa* in this study. Thus, the importance of allelopathy of C. canadensis and E. annuus is markedly greater than that of A. subulatus and B. pilosa. Interestingly, A. subulatus leaf extract does not display noteworthy allelopathy on germination performance of L. sativa in this study. Thus, the allelopathy of A. subulatus does not show a vital role in its successful colonization. Largely, the allelopathy of the four Asteraceae AIP on germination performance of L. sativa distinctly declines in the following order: E. annuus, C. canadensis, B. pilosa, and A. subulatus in this study. The key cause may be because of the diversification in the types of secondary substances, i.e., allelochemicals, and their corresponding relative content among the four Asteraceae AIP supposedly. The results confirm the first hypothesis.

Although the independent acid deposition does not markedly affect germination performance of L. sativa, the combined allelopathy of the four Asteraceae AIP (particularly *B. pilosa*) and acid deposition trigger a significant negative influence on germination performance of L. sativa, especially on germination competitiveness, seed viability and germination uniformity, germination rate and vitality, germination responsiveness to the external environment, and seedling competitiveness for water and inorganic salt absorption, in this study. Thus, the growth fitness of L. sativa will be significantly decreased under the condition when the plant invasion was polluted by acid deposition.

The acid deposition may be increasingly worse with the growing intensity and frequency of atmospheric activities in current periods and is estimated to upsurge in upcoming years. Hence, the allelopathy of AIP may be changed and even strengthened under the condition with the increasing level of acid deposition. Further, nitrogen, which is one of the main constituents of acid deposition, can influence and even expedite plant metabolic process (Throop and Lerdau 2004; Luo et al. 2008; Fallovo et al. 2011; Yang et al. 2014; Sun et al. 2020). Interestingly, the combined allelopathy of C. canadensis, E. annuus, and B. pilosa and acid deposition at pH 5.6 can promote germination performance (especially germination competitiveness, seed viability and germination uniformity, germination rate and vitality, germination responsiveness to the external environment, seedling competitiveness for sunlight capture, leaf photosynthetic area, and seedling growth competitiveness) of L. sativa compared with only leaf extracts in this study. Thus, acid deposition with a low level of acidity decreases the allelopathy of C. canadensis, E. annuus, and B. pilosa on germination performance of L. sativa. The foremost issue may be credited to the nutrient fertilization (especially nitrogen) mediated by the nutrition elements in an acid deposition with a low level of acidity. Further, the increased level of nutrition can lift the capability of plant species to resist hostile environments (Hassan et al. 2005, 2008; Xu et al. 2015a, b; Xiong et al. 2018; Tariq et al. 2019). Inversely, the combined allelopathy of B. pilosa and acid deposition at pH 4.5 synergistically affect germination performance of L. sativa, especially on germination competitiveness, seed viability and germination uniformity, germination rate and vitality, and germination responsiveness to the external environment. Accordingly, acid deposition with a high level of acidity strengthens the allelopathy of *B. pilosa* on germination performance of *L. sativa*. The reason may be owed to the increased acidity under acid deposition with a high level of acidity which is poisonous to plant growth. Meanwhile, the high level of acidity recruited by acid deposition can increase the leaching process of acid-soluble substances (Zhang et al. 2007; Wang et al. 2016b; Pabian et al. 2012; Xu et al. 2015a, b), such as phenolics (mainly polyphenols), which is one of the most abundant allelochemicals in AIP (Li et al. 2010; Zhang et al. 2011; Djurdjević et al. 2012; Gomaa et al. 2014; Harrison et al. 2017; Marksa et al. 2020). Earlier outcomes also identify that acid deposition can also increase the allelopathy of AIP on plant germination performance

(Wang et al. 2012a, b, 2016b). Thus, the consequences confirm the second hypothesis partially.

In brief, the progressively growing level of acid deposition with high acidity in the environment can be good for the invasion process of *B. pilosa* via the enhanced allelopathy on plant germination performance.

Acknowledgements We are very grateful to the anonymous reviewers for the insightful and constructive comments that greatly improved this manuscript

Author contributions CW—conceived and designed this study. HC, SW, and MW—performed the experiments. SW, MW, and YY—analyzed the data. CW—wrote the manuscript. All authors provided editorial advice.

**Funding** This study was funded by Open Science Research Fund of State Key Laboratory of Pollution Control and Resource Reuse (Tongji University), China (Grant No.: PCRRF19009).

**Data availability** All data generated or analyzed during this study are included in this article.

Code availability Not applicable.

#### **Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Consent to participate** Informed consent was obtained from all individual participants included in the study.

**Consent for publication** All authors vouch that the work has not been published elsewhere, completely, in part, or in any other form, and that the manuscript has not been submitted to another journal. All other authors have read the manuscript and have agreed to submit it in its current form for consideration for publication in the Journal.

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