Original Research

# Nitrogen Deposition Elevated the Allelopathic Effects of Three Compositae Invasive Species on Indigenous *Lactuca sativa*

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# Abstract

It is essential to understand the ecological role and importance of allelochemicals on the growth of native plants. Our study hypothesized that nitrogen (N) intimidation would influence the secretion of allelochemicals in invasive plants, impacting native plants. In the experiment, the root and aboveground aqueous extracts of three invasive compositae (*Soildago Canadensis* L., *Erigeron Canadensis* L. and *Erigeron annuus*) were used to treat the native compositae (*Lactuca sativa*) seeds under different N concentrations 0 mgL<sup>-1</sup>, 50 mgL<sup>-1</sup>, 100 mgL<sup>-1</sup>, 200 mgL<sup>-1</sup> and distilled water added as an additional control (CK). The results showed that the *Lettuce* seeds (germination rate, germination potential, germination index, and vigor index) and seedlings (root height, plant height, fresh weight, and Malondialdehyde (MDA)) had different responses to different parts of the plants. At the same time, N addition elevated the allelopathy effects of three Compositae invasive species and affected the growth of the seedling of native *Lettuce*. This approach offered the opportunity to study the influence of allelochemicals of exotic plants with an additional N source and explain the mechanism of a successful invasion.

**Keywords**: allelopathy, aqueous extract, invasive compositae species, nitrogen concentration, seedling growth

## Introduction

The invasion of exotic plants is a major threat to natural ecosystems, causes major loss in biodiversity [1, 2], and substantially decreases the abundance of native species in a new habitat [3]. Accordingly, the topic of successful invasion and its mechanism is becoming a long-standing interest to environmentalists. In particular, the concept of allelopathy, such as several invasive species inhabited the growth of native species. It established its competitors by releasing

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the allelochemicals and has become the main reason for the invasion's success in the past decades [4-6]. There is now growing evidence of allelopathy for native plants in many plant invaders, and this allelopathic action can be a vital weapon for invasive plants [7, 8]. For example, a group of invasive plants called *Acacia dealbata Link* (silver wattle; Mimosaceae) introduced into southern Europe from Australian acacia has deeply changed the community structure [9]. Another exotic invader *Centaurea maculosa Lam* (spotted knapweed), which came from North America, also releases allelochemicals compounds to regulate native species seedling's establishment [10].

According to the "New Weapon Hypothesis," several exotic plants will produce unexplained allelochemicals in the native community [11]. Such new allelochemicals could inhibit the growth of native plants and thus increase the growth of invasive species [8, 12]. The seedling growth and germination of seeds are usually the critical processes of the inhabitant's recruitment and the ecological expansion of the invasive plant species [5, 12-14]. Accordingly, the strongly allelopathic effects elevated by the invasive plant species had led to a reduction in the growth performance and development of their co-occurring native species [15-17]. However, the research focused on donor and receptor biological characteristics, and the mechanism of allelopathy, especially under environmental intimidation, is still limited.

With the gradual increase in production and usage of chemical nitrogen fertilizers, fossil fuels burning, and growing breeding industry, etc., in recent years, the situation of atmospheric nitrogen deposition (N-deposition) has increased to a dangerous level all over the world [18-20]. Moreover, the increasing N-deposition trend will rise more and more with time [18, 20-22]. Currently, in many regions of China, the maximum levels of N-deposition have been noted on a global scale. The ever-increasing N-deposition can arouse the invasiveness of invasive plant species and noteworthy shifts in the plant's physiological ecological performance [23-25]. and Therefore, N-deposition has evolved into a major scientific issue in the research field of ecological sciences to explore and clarify how N-deposition affects the invasive plant species positively or negatively and their corresponding driving mechanism.

In recent studies, ecologists have paid much attention to overcome this problem by considering the environmental influence factors, i.e., nutrients, heavy metals, and N-deposition on invasive plant species and their driving mechanism. But these studies commonly emphasize the influences of one or two factors. However, the information on the impact of allelopathy of invasive plant species on their co-occurring native plant species under N-deposition is limited. Thus, this study aims to evaluate the allelopathic effects of three notorious invasive compositae species, i.e., *S. Canadensis, E. Canadensis, and E. annuus* (using

root and aboveground part aqueous extracts) on the cultivated crop lettuce, i.e., Lactuca sativa L. under N-deposition. We chose these species among all the compositae species because they were more exotic, competitive, and strong. Specifically, S. Canadensis and lettuce can live and grow together in the same habitat, particularly in the farmlands. Lettuce is one of the most common vegetables found in the agricultural farmlands, colonized by S. Canadensis. That is why lettuce is frequently used as experimental material in the field or greenhouse experiment of allelopathy because of its sensitive nature to the fluctuations in the environment facts [6, 22, 25, 26]. S. Canadensis is a perennial rhizomatic plant and belongs to Compositae. In the 1930s, it was introduced into China as an ornamental plant from North America and originally cultivated in Shanghai and Nanjing area, and later spread to the wild. In the 1980s began to spread to Jiangsu and Zhejiang area. The spread of S. canadensis causes serious damage to China's ecological environment and is considered an invasive malignant weed [16, 27]. E. canadensisi is an annual composite plant native to North America [28]. It is one of the most widely spread exotic invasive plants in China. The maturing parts of E. canadensisi have been regularly used as raw material in the folk-medicine. It was recommended in official medicine as a haemostypic, diuretic, antidiarrhoeic, adstringent, and anthelmintic [29, 30]. It contains many flavonoids and tannins besides essential oils, and aqueous extracts are rich in antioxidant and anti-platelet compounds [31, 32]. E. annuus is a member of the Compositae and is broadly spread all over the regions (urban and rural) of Korea [33]. Moreover, it has also been used extensively as a hypoglycemic drug in China [34].

The outcomes of the current study will give a solid foundation to understand better mechanism of a successful invasion of invasive plant species based on allelopathy under N-deposition. In addition, it will provide the support, practices, and guidance to prevent and control invasive plant species, particularly in the area riches by N-deposition. According to raised problems in recent time, we hypothesized that; 1) There could be noteworthy differences in the allelopathy of three Compositae invasive species on L. sativa; 2) nitrogen intimidation will influence the secretion of allelochemicals in invasive plants, which may impact native plants. Moreover, this approach offered the opportunity to study the influence of allelochemicals of exotic plants with an additional N source and explain the mechanism of a successful invasion.

## **Materials and Methods**

## Preparation of Materials

The Lettuce seeds (Manufacturer: Minshu Agricultural Science & Technology Co., Ltd., Fuzhou,

China) used in this experiment were brought from the local farmer market of Jinkou, Zhenjiang. The root and aboveground parts of Soildago Canadensis L., Erigeron Canadensis L., and Erigeron annuus were collected from the campus of Jiangsu University in Zhenjiang (32.21°N, 119.52°E), Jiangsu Province. The collected samples of root and aboveground parts were washed carefully and air-dried thoroughly at 25°C and then used to proceed with the following process processing. Air-dried root and aboveground samples were soaked in flasks containing distilled water about 800 mL for 48 h at room temperature. The next step is to filter the impurities, i.e., solid material in the root and above ground extracts, using cheesecloth and filtered with sterilized membrane filters (0.45 µm, Sangon Biotech, Shanghai, China). The prepared solution of all the aqueous extracts was placed in the refrigerator at four °C for processing. The final concentration of all the aqueous extracts was 100 gL<sup>-1</sup>.

The nitrogen (N) solution was prepared by adding an equal amount of (1)  $NH_4Cl$  (2)  $KNO_3$ , and (3) CO  $(NH_2)_2$  with an equal ratio (1:1:1). In the N solution, the ratio was set according to [16], parallel to the actual global-average ratio in the natural elements of atmospheric N-deposition. The concentration for N solution were prepared as; (1) 0 mgL<sup>-1</sup>; (2) 50 mgL<sup>-1</sup>; (3) 100 mgL<sup>-1</sup>; (4) 200 mgL<sup>-1</sup> and distilled water added as an additional control (CK).

## **Determination of Parameters**

The concentration of the six kinds of aqueous extract was 100 gL<sup>-1</sup>. We set the concentration of mixed nitrogen as 0 mgL<sup>-1</sup>, 50 mgL<sup>-1</sup>, 100 mgL<sup>-1</sup>, 200 mgL<sup>-1</sup> and distilled water added as an additional control.

The incubation of lettuce seeds was conducted in the Petri dishes (9 cm). About thirty surface-sterilized Lettuce seeds with 1% sodium hypochlorite were sown on filter paper in Petri dishes containing 6mL mixed solution or 6mL distilled water (control), and five replicates were set for each treatment, resulting in a total of 125 petri dishes. All the Petri dishes were cultivated at room temperature (28°C) with a light: dark cycle of 16:8 hr for seven days, and the germination was measured during the experiment.

Germination rate was measured according to [35]

$$Germination rate (\%) = \left[\frac{\text{The 5th} - \text{day number of germinated seeds}}{\text{total number of seeds}}\right] \times 100 \tag{1}$$

Germination potential(%) was noted according to [36]

Germination potential (%) = 
$$\left[\frac{\text{The 4th} - \text{day number of germinated seeds}}{\text{total number of seeds}}\right] \times 100$$
(2)

Germination index (GI) = 
$$\sum$$
(Gt/Dt) (3)

Gt is the number of germination after (t) days, and Dt is the days of germination [37].

Vigor index (VI) = 
$$(\frac{1}{a} + \frac{2}{b} + \frac{3}{c} + \frac{4}{d} \dots \dots + \frac{x}{n} \times 100/S$$
 (4)

Where a, b, c... respectively, represent the number of seeds germinated after 1, 2, 3 days of imbibition, x is the number after (n) days, and (S) is the total number of germinated seeds [38].

Besides, we selected ten seedlings randomly from each Petri dish on the 4<sup>th</sup> day and measured the root length and plant height. Fresh weight was measured during the 6<sup>th</sup> day. On the 7<sup>th</sup> day, the MDA concentration of Lettuce seedlings was determined by a common method called the thiobarbituric acid-reactivesubstances (TBARS) assay [39].

## Statistical Analysis

Analysis of variance (ANOVA) was applied to differentiate the differences in the values of each parameter of lettuce among different treatments followed multiple comparisons test, by i.e.. Duncan'Duncan's multiple-range test and Tukey test. All statistical analyses were performed using IBM SPSS Statistics (version 25.0; IBM Corp., Armonk, NY, USA). Data were presented in means  $\pm$ SE at P<0.05. SCR is an aqueous extract of S. Canadensis root. SCA is an aqueous extract of S. Canadensis aboveground part. ECR is an aqueous extract of E. Canadensis root. ECA is an aqueous extract of E. Canadensis aboveground part. EAR is an aqueous extract of E. annuus root. EAA is an aqueous extract of E. annuus aboveground part.

## **Results and Discussion**

# Effect of Allelopathy of Three Invasive Compositae on Lettuce Seeds Germination and Seedlings Growth

Effect of allelopathy on Lettuce seeds germination and seedlings growth varied with a different type of aqueous extract (Fig. 1 and Fig. 2). SCR, ECA, and EAR showed a little negative effect on the Lettuce seed's germination rate, and SCA, ECR, and EAA significantly inhibited the seed's germination. The germination rate of EAA was under 10% (Fig. 1a). SCA and EAA suppressed the germination potential absolutely, and significant suppressive was found in ECR, ECA, and EAR (Fig. 1b). SCA, ECR, ECA, EAR, and EAA decreased the germination index, and EAA had the strongest effect (Fig. 1c). The vigor index was decreased by SCA, ECR, ECA, and EAA, and the vigor index of EAA was 0 (Fig. 1d).

SCR and ECR had no obvious effect on the root length of Lettuce seedlings, while SCA, ECA, and EAR inhibited the root length significantly (Fig. 2a). EAA negatively affected Lettuce seedlings, and seedlings



Fig. 1. The effect of aqueous extract of three compositae invasive species on native *Lettuce* seeds Germination rate a), Germination potential b), Germination index c), and Vigor index d). And the concentration of different kinds of the aqueous extract is 100 gL<sup>-1</sup>. Mean values with different superscript letters indicate a significant difference (P<0.05).



Fig. 2. The effect of aqueous extract of three compositae invasive species on native *Lettuce* seedlings i.e., Root height a), Plant height b), Fresh weight c), and MDA d). And the concentration of different kinds of the aqueous extract is 100 gL<sup>-1</sup>. Mean values with different superscript letters indicate a significant difference (P < 0.05).

couldn't grow normally. The plant height was decreased prominently by ECR and ECA, and SCA inhibits the plant height, while SCR and EAR had little inhibition effect (Fig. 2b). SCA decreased fresh weight, but SCR, ECR, ECA, and EAR presented acceleration (Fig. 2c). All the aqueous extract increased the MDA concentration of Lettuce seedlings, especially SCA and ECA (Fig. 2d).

# Effect of Nitrogen Deposition on Lettuce Seeds Germination and Seedlings Growth

Mixed N in different aqueous extracts affected the Lettuce seed's germination to a different extent (Fig. 3). Different concentrations of mixed N affected the Lettuce seed's germination rate without discernable difference in the same aqueous extract, except that mixed N in ECR increased the germination rate (Fig. 3a). Germination potential was inhibited by mixed N in SCR and ECA, but the inhibition differed distinctly with different N concentrations. At the concentration of 50 mgL<sup>-1</sup> and 200 mgL<sup>-1</sup>, germination potential significantly decreased in ECR and EAR while there was no difference at 100 mgL<sup>-1</sup> (Fig. 3b). Mixed N in SCR negatively affected the Lettuce seeds germination index, but there was no difference with different concentrations.

In contrast, the germination index was increased by ECR. The 50 mgL<sup>-1</sup> and 100 mgL<sup>-1</sup> mixed N increased germination index slightly while 200 mgL<sup>-1</sup> presented an obvious decrease. And EAR showed the same tendency at 200 mgL<sup>-1</sup> of mixed N (Fig. 3c). The ECR significantly increased the vigor index while ECA had the opposite effect (Fig. 3d).

The Lettuce seedlings in SCR and EAR at the concentration of 50 mgL<sup>-1</sup> of mixed N had an increase in their root length compared to control values, but SCR showed inhibition at 100 mgL<sup>-1</sup> and EAR at 200 mgL<sup>-1</sup>. 200 mgL<sup>-1</sup> mixed N caused a decrease in ECR and ECA (Fig. 4a). Mixed N in SCR had no significant difference in plant height of Lettuce seedlings. ECR showed a slight increase at 100 mgL<sup>-1</sup> and a pronounced decrease at 200 mgL<sup>-1</sup>. The decrease was much more significant at 100 mgL<sup>-1</sup> and 200 mgL<sup>-1</sup> than 50 mgL<sup>-1</sup> in ECA. An obvious decrease was found in EAR at 200 mgL<sup>-1</sup> (Fig. 4b). SCR and ECR significantly increased fresh weight while ECA decreased it (Fig. 4c). A slight increase of MDA was found at 50 mgL<sup>-1</sup> and 100 mgL<sup>-1</sup> in SCR, but significant

inhibition was shown at 200 mgL<sup>-1</sup>. The significant difference was presented in ECA. At 100 mgL<sup>-1</sup>, an obvious increase of MDA was found in the EAR. SCA and ECR showed no significant difference (Fig. 4d).

# The Interaction of the Allelochemicals of Three Invasive Compositae and Nitrogen Deposition on Lettuce Seeds Germination and Seedlings Growth

The interaction of the allelochemicals and mixed N decreased the *Lettuce* seed's germination rate to some extent (Fig. 5a). This decrease was substantial and more profound with a further increase of mixed N concentration in SCR and ECA. The interaction to germination rate was significantly different at 0 mgL<sup>-1</sup>, but it was not at other N concentrations in ECR (Fig. 5a). The interaction decreased germination potential and germination index. Still, there was no significant difference between different Ν concentrations except the germination potential of ECR at 0 mgL<sup>-1</sup> of N (Fig. 5b and Fig. 6a). The vigor index of SCR and EAR did not show much significant change with the increase of N. However, SCR and ECA presented an obvious decrease, and ECR decreased sharply only under aqueous extract treatment (Fig. 6b).

The root length was inhibited at high N concentration, but treating with 50 mgL<sup>-1</sup> N in SCR showed a sharp increase, and treating without nitrogen in EAR decreased even more than treating 50 mgL<sup>-1</sup> N (Fig. 7a). The interaction reduced

0mg/L

b) 100<sub>1</sub> 50mg/L 100 a) а a a a 100mg/L ∔ааа Germination potential (%) 200mg/L Germination rate(%) aaba 5( 25 24 aaaa d) c) a <sup>aa</sup>a a<sup>a a</sup> 50-10 а 40 8 Germination index 30 Vigor index 20abaa 10 2 0 0 SCR SCA ECR ECA EAR EAA SCR SCA ECR ECA EAR EAA

Fig. 3. The effect of nitrogen deposition on *Lettuce* seeds Germination rate a), Germination potential b), Germination index c), and Vigor index d). Mean values with different superscript letters indicate a significant difference (P < 0.05).



Fig. 4. The effect of nitrogen deposition on Root height a), Plant height b), Fresh weight c), and MDA d) of *Lettuce* seedlings. Mean values with different superscript letters indicate a significant difference (P<0.05).

the plant height seriously in ECA, EAR, and ECR. But in ECR, seedlings treated with 100 mgL<sup>-1</sup> N were not significantly different than control (Fig. 7b). The fresh weight of Lettuce seedlings improved in SCR and EAR, and at 50 mgL<sup>-1</sup> N concentration, it peaked. A similar trend can be observed in ECA and ECR, but the fresh weight was decreased slightly at 0 mgL<sup>-1</sup> N in ECR and 200 mgL<sup>-1</sup> N in ECA. However, SCA significantly reduced the fresh weight (Fig. 8a). All the treatments increased MDA concentration except 200 mgL<sup>-1</sup> N in SCR. ECA improved sharply without N treatment, and with the nitrogen increasing, the MDA decreased. The MDA concentration peaked at 100 mgL<sup>-1</sup> N in the EAR (Fig. 8b).

Germination rate, germination potential, germination index, vigor index, root length, plant height, fresh weight, and MDA concentration were evaluated allelochemicals and mixed nitrogen effects



Fig. 5. The interaction between allelochemicals of three compositae invasive species and nitrogen deposition on native *Lettuce* seeds Germination rate a) and Germination potential b). Mean values with different superscript letters indicate a significant difference (*P*<0.05).



Fig. 6. The interaction between allelochemicals of three compositae invasive species and nitrogen deposition on native *Lettuce* seeds Germination index a) and Vigor index b). Mean values with different superscript letters indicate a significant difference (P < 0.05).



Fig. 7. The interaction between allelochemicals of three compositae invasive species and nitrogen deposition on native *Lettuce* seedlings Root height a) and Plant height b). Mean values with different superscript letters indicate a significant difference (P < 0.05).

on Lettuce seeds germination and seedlings growth. The results partially support our original hypothesis that the allelochemicals of three invasive compositae plants and additional mixed nitrogen were responsible for influences on native seeds and seedlings' growth.

The Lettuce seeds and seedlings had different responses to different aqueous extracts in the experiment. It showed that allelochemicals are varied with different exotic invasive plants and different parts of the plants. Compared to different parts of the exotic plants, the allelopathic inhibitory effects of the extract from aboveground parts were stronger than root aqueous extracts. However, some other researchers found that the root and underground stem of *S*. *Canadensis* is prosperous and can form an underground density system. Therefore, many allelochemicals release into the surrounding environment and inhibit the growth of other species [40, 41]. The high concentration of *E. canadensisi* aqueous extract can significantly reduce the radicle length of *Brassica pekinensis* L., *Cichorium endivia* L., *and Daucus carota* L. Still, the effects were not obvious or even promoted at low concentrations. Different extracts of *E. canadensisi* could increase the hypocotyl length but sometimes inhibit at high concentrations [42]. Another research study found the allopathic impact of seed germination inhibition by applying Amaranthus species extract to the growth of radicle duration. In other research, the



Fig. 8. The interaction between allelochemicals of three compositae invasive species and nitrogen deposition on native *Lettuce* seedlings, Fresh weight a), and MDA b). Mean values with different superscript letters indicate a significant difference (P<0.05).

allopathic effect has been observed in the inhibition of seed germination and the development of the radicle length by adding plant Amaranthus species extract [43]. Many more examples showed different allelopathic effects by invasive plants; the leaf extracts from an invasive Lonicera maackii inhibit the native herb Blephilia hirsuta [44] and inhibit the growth of Mimosa pigra and Vigna radiata seedling [45]. Allelopathic effects of Conyza canadensis have reduced the growth of non-invasive crop Lactusa sativa [4, 46, 47], an aqueous extract from invasive S. canadensis leaf has significantly allelopathic effects on the coexisting native plants [48]. In our experiment, allelochemicals released by three invasive compositae plant's root and aboveground part extract with low N-concentration may maintain the reactive oxygen molecules in plant cell extension and didn't affect the seed germination and seedling growth of lettuce, but high concentration negatively affects the seed germination and seedling growth of lettuce. Therefore, N-deposition elevates the allelopathic effects of three invasive compositae species.

Many toxic compounds impede the plant growth rate, growing allélopathic phenolic concentration (vanillic acid. o-hydroxyphenyl acetic, p-hydroxybenzoic acid, camminic acid, ferulic and P-Cumaric acid) in various plants are found to be present in the seed. In Brassica rapa subsp, for example. Chinensis (L.) Hanelt, Echinochloa crus-galli, and Chenopodium album seedlings. Therefore, many toxic compounds are found that inhibit the plant growth rate, increasing the concentration of allelopathic phenolics (vanillic acid. *o*-hydroxyphenyl acetic, *p*-hydroxybenzoic acid, camminic acid, ferulic and p-coumaric acids) in different plants. For example, in Brassica rapa subsp. Chinensis (L.) Hanelt, Cunninghamia lanceolate, Echinochloa crus-galli and Chenopodium album seedlings [49]. The results were similar to what we have found. The allelopathy of SCA and EAA was so strong that the plant height of SCA was unable to measure, and the seedlings treated with EAA were inhibited. Besides, different nitrogen concentrations affected Lettuce seeds and seedlings to a different extent. The early research represented that the biomass of Fraxinus mandshurica increasing with the increase of nitrogen concentration [50]. The exotic invasive plant S. Canadensis has a strong biological interference ability. Its resource competition ability is stable under different nitrogen levels, but the allelopathy is enhanced with the nitrogen level decline [51]. The water-soluble allelochemicals of M. micrantha increased the soil nitrification rates (both  $NO_3^{-}$  and  $NH_4^{+}$ ) under three concentrations (0.005 g ml<sup>-1</sup>, 0.025 g ml<sup>-1</sup>, 0.100 g ml<sup>-1</sup>), through which the M. micrantha successfully invaded and established in the new environment [52]. Thus, the effects of allelopathy under N-deposition on the growth development of lettuce are negatively affected with increasing concentration. Consequently, the outcomes of our study were reliable with the study's hypothesis.

### Conclusions

In the present study, the tested plant was treated with the N-solution under a laboratory environment to understand the effect of exotic plant allelochemicals and nitrogen on native plants. N-deposition facilitated the three invasive compositae species by enhancing their allelopathy. In addition, allelochemicals of three invasive compositae species decreased the growth of the seedlings of native Lettuce under N-deposition. However, the laboratory environment is different from the natural ecosystem, where native and invasive species coexist and nutrient competition occurs. Therefore, more research would be required concerning nutrient competition to gain an insight into the effects of phytochemicals on plant invasion.

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## **Conflict of Interest**

The authors declare no conflict of interest.

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