Allelopathic Effects of Eupatorium adenophorum Leaf Aqueous Extract on Seedling Growth and Chlorophyll of Seven Native Plant Species (Post-print)

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Abstract

Using Ageratina adenophora as the donor and seven native plant species, namely Pinus massoniana, Cunninghamia lanceolata, Broussonetia papyifera, Castanea mollissima, Zanthoxylum bungeanu, Camellia oleifera, and Pyracantha fortuneana, as the recipients, we investigated the allelopathic effects of aqueous leaf extracts of A. adenophora on chlorophyll content and growth of the tested plant seedlings. The results showed that: (1) At low concentrations, the aqueous leaf extract of A. adenophora exhibited certain promoting effects on chlorophyll a, chlorophyll b, and total chlorophyll content in Z. bungeanu and P. fortuneana, while its effects on chlorophyll in the remaining five species were not significant. Under high concentration treatments, chlorophyll a, chlorophyll b, and total chlorophyll content in C. lanceolata, C. mollissima, and B. papyifera showed significant decreases. P. fortuneana exhibited a significant decrease only in chlorophyll b, while P. massoniana showed increases in all

chlorophyll parameters, with no significant effects observed on C. oleifera and Z. bungeanu; (2) Measurement of growth parameters in recipient plants revealed that the aqueous leaf extract of A. adenophora at high concentrations ($mg \cdot mL^{-1}$) showed significant inhibitory effects on all growth parameters of six recipient plant species except C. mollissima, while at low concentrations it exhibited certain promoting effects on ground diameter and crown width of P. fortuneana, Z. bungeanu, and C. lanceolata, with no significant effects on the growth parameters of the remaining four species.

Full Text

Preamble

Allelopathic Effects of Ageratina adenophora Leaf Aqueous Extract on Growth and Chlorophyll Content of Seven Native Plant Seedlings

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Abstract

This study investigated the allelopathic effects of aqueous extracts from *Ageratina adenophora* leaves on chlorophyll content and seedling growth of seven native plant species in China: *Pinus massoniana*, *Cunninghamia lanceolata*, *Broussonetia papyrifera*, *Castanea mollissima*, *Zanthoxylum bungeanum*, *Camellia oleifera*, and *Pyracantha fortuneana*. The results showed: (1) At low concentrations, the aqueous extract promoted chlorophyll a, chlorophyll b, and total chlorophyll content in *Z. bungeanum* and *P. fortuneana*, while having no significant effect on the other five species. At high concentrations, chlorophyll a, chlorophyll b, and total chlorophyll content decreased significantly in *C. lanceolata*, *C. mollissima*, and *B. papyrifera*. *P. fortuneana* showed a significant decrease only in chlorophyll b, while *P. massoniana* exhibited increased chlorophyll content, and no significant effects were observed in *C. oleifera* and *Z. bungeanum*. (2) Growth parameter measurements revealed that at high concentrations (30 $mg \cdot mL^{-1}$), the aqueous extract significantly inhibited all growth parameters of six recipient species (except *C. mollissima*). At low concentrations, it promoted ground diameter and crown width in *P. fortuneana*, *Z. bungeanum*, and *C. lanceolata*, while having no significant effect on the remaining four species.

Keywords: growth parameters, allelopathy, aqueous extract, inhibition mechanism, chlorophyll

Introduction

Chlorophyll is a crucial photosynthetic pigment in plants, primarily existing as chlorophyll a and chlorophyll b in higher plants. These two pigments work synergistically to capture light energy and drive electrons to reaction centers, playing a vital role in plant growth and development. Chlorophyll deficiency can lead to leaf chlorosis, albinism, or striped patterns, and in severe cases, cause plant death. Research indicates that some plants secrete allelochemicals into the environment to gain competitive advantages by inhibiting chlorophyll synthesis in neighboring plants. These substances can reduce leaf chlorophyll content, hinder net photosynthetic rate and stomatal conductance, and consequently suppress photosynthesis and plant growth. Leveraging this characteristic of allelochemicals, this study designed experiments to explore the allelopathic mechanisms of the invasive species *Ageratina adenophora* by examining the effects of its leaf aqueous extract on chlorophyll content and growth status of seven native recipient plants.

Ageratina adenophora is one of the 16 most important invasive alien species in China, widely distributed in southern regions and causing significant impacts on agriculture, forestry, animal husbandry, and human health. Its aggressive expansion may partly result from strong allelopathic effects that inhibit the growth of native species, facilitating its rapid spread. Previous studies have isolated and identified 49 compounds with high allelopathic activity, including 9-oxo-10,11-dehydroageraphorone, though the specific mechanisms require deeper investigation. To explore these mechanisms, this study selected seven common species in Luodian County, Guizhou Province—a pilot site for *A. adenophora* control by the former State Forestry Administration. Using indoor breeding methods, we designed experiments to investigate how *A. adenophora* leaf aqueous extracts affect chlorophyll content in these native species and evaluated the extracts' impacts to identify species with strong allelopathic tolerance for biological control applications.

Materials and Methods

1.1 Plant Materials

The donor plant was *Ageratina adenophora*. In late April 2017, leaves were collected in Luodian County, Guizhou Province, where *A. adenophora* infestation is most severe. The leaves were air-dried, cut into pieces, and mixed for later use. The recipient plants were seedlings of *Pinus massoniana*, *Cunninghamia lanceolata*, *Broussonetia papyrifera*, *Castanea mollissima*, *Zanthoxylum bungeanum*, *Camellia oleifera*, and *Pyracantha fortuneana*. Seeds of *P. massoniana* and *C. lanceolata* were provided by the Qiandongnan Forestry Bureau Seedling Station. Seeds of *C. oleifera*, *B. papyrifera*, and *P. fortuneana* were collected from Huaxi District, Guiyang City. Seeds of *Z. bungeanum* and *C. mollissima* were purchased from the market.

1.2 Preparation of Aqueous Extracts

Thirty grams of the prepared *A. adenophora* leaves were soaked in 1,000 mL of distilled water, stirred evenly, and soaked for 48 hours. The mixture was then filtered through two layers of gauze to obtain a stock solution with a dry matter content of 30 $mg \cdot mL^{-1}$. For application, the stock solution was diluted with distilled water to concentrations of 15, 5, and 2.5 $mg \cdot mL^{-1}$. Fresh solution was prepared every two days as needed.

1.3 Cultivation of Recipient Plants

Seedlings of each recipient species were cultivated. In May, uniformly sized seedlings were transplanted into pots (20 cm bottom diameter, 25 cm depth) at two seedlings per pot. The cultivation soil was taken from unvegetated loess profiles, sun-dried, crushed, and sieved. After one month of normal growth, seedlings were treated with different concentrations of *A. adenophora* aqueous extract. Five treatment concentrations were applied: 30, 15, 5, 2.5, and 0 $mg \cdot mL^{-1}$ (control CK). Each treatment had three replicates, totaling 105 pots (7 species $\times$ 5 treatments $\times$ 3 replicates). Each pot received 50 mL of the corresponding extract weekly, while control pots received 50 mL of distilled water.

1.4 Experimental Design

The experiment used a pot culture method in a greenhouse. Cultivation soil from unvegetated loess profiles was prepared as described above and placed in pots (20 cm bottom diameter, 25 cm depth). Uniform seedlings of the seven recipient species were transplanted at two seedlings per pot. Five treatment concentrations were established: 30, 15, 5, 2.5 $mg \cdot mL^{-1}$, and a control (CK). Each treatment had three replicates, totaling 105 pots. After one month of acclimation, 50 mL of the respective aqueous extract was applied weekly to each pot, with control pots receiving 50 mL of distilled water.

1.5 Measurement Methods

Chlorophyll content was determined using the grinding-spectrophotometry method. Fresh samples (0.1 g) were cut into pieces and ground in a mortar with a small amount of $CaCO_3$, quartz sand, and 2 mL of 96% ethanol until a homogenate formed. Grinding continued until the tissue turned white. The homogenate was washed into a centrifuge tube with a small amount of 96% ethanol, centrifuged at high speed, and then brought to a final volume of 25 mL with 96% ethanol. After mixing, the chlorophyll extract was placed in a 1 cm cuvette, and absorbance was measured at 665 nm and 649 nm using a TU-1800 UV-visible spectrophotometer, with 96% ethanol as the blank control.

At the end of the experiment, seedling height and crown width were measured with a tape measure, and ground diameter was measured with a vernier caliper.

Plant materials were harvested and oven-dried at 80°C for 48 hours to constant weight, then weighed with an electronic balance (0.0001 g precision). Data from the two seedlings in each pot were averaged for all parameters.

1.6 Data Analysis

The maximum absorption peaks of chlorophyll a and b in 96% ethanol are at 665 nm and 649 nm, respectively. Chlorophyll content was calculated using the following formulas:

$Ca = 13.95 \times A_{665}$ - $6.88 \times A_{649}$
$Cb = 24.96 \times A_{649}$ - $7.32 \times A_{665}$
$CT = Ca + Cb$

Total chlorophyll content ($mg \cdot g^{-1}$ FW) = (CT × V × n) / (FW × 1000)

Where Ca is chlorophyll a concentration, Cb is chlorophyll b concentration, CT is total chlorophyll concentration ($mg \cdot L^{-1}$), FW is fresh weight (g), V is extract volume (mL), and n is dilution factor.

Allelopathic potential was evaluated using the inhibitory rate (IR) as the assessment index:

IR% = (Ti - To) / To × 100%

Where Ti is the treatment value and To is the control value. IR > 0 indicates promotion, IR = 0 indicates no effect, and IR < 0 indicates inhibition. The greater the absolute IR value, the stronger the allelopathic potential.

Experimental data were organized and calculated using Excel. Statistical analysis was performed using SPSS 19.0 software. One-way ANOVA and LSD tests were used to analyze significant differences in growth parameters and chlorophyll content among different extract concentrations.

Results

2.1 Effects of Ageratina adenophora Leaf Aqueous Extract on Seedling Growth

Table 1 Effects of *Ageratina adenophora* leaf aqueous extracts on seedling growth of seven native plants

Note: indicates significant difference ($P < 0.05$), ** indicates extremely significant difference ($P < 0.01$). The same below.*

As shown in Table 1, the effects of *A. adenophora* leaf aqueous extract on seven native plant species in Guizhou were: At high concentration (30 $mg \cdot mL^{-1}$), all growth parameters (seedling height, dry weight, ground diameter, and crown width) of *P. massoniana* were significantly reduced, with significant reductions also observed in seedling height and crown width at moderate concentration (15 $mg \cdot mL^{-1}$). For *C. lanceolata*, low concentration (2.5 mg ·

mL^{-1}) increased ground diameter and crown width, while high concentration (30 $mg \cdot mL^{-1}$) significantly reduced ground diameter. *C. oleifera* showed significant reductions in seedling height, ground diameter, crown width, and dry weight at high concentration (30 $mg \cdot mL^{-1}$), with reduced seedling height also at moderate concentration (15 $mg \cdot mL^{-1}$). *B. papyrifera* exhibited significant inhibition of ground diameter and dry weight at high concentration (30 $mg \cdot mL^{-1}$). *Z. bungeanum* showed significant crown width reduction only at high concentration (30 $mg \cdot mL^{-1}$), but significant increases in seedling height, ground diameter, crown width, and dry weight at low concentration (2.5 $mg \cdot mL^{-1}$). *P. fortuneana* displayed significant decreases in seedling height, ground diameter, and dry weight at high concentration (30 $mg \cdot mL^{-1}$), while its ground diameter and crown width significantly increased at low concentration (2.5 $mg \cdot mL^{-1}$). *C. mollissima* showed no response to any extract concentration for all parameters. Overall, *A. adenophora* leaf aqueous extract generally had no effect or even promoted growth at low concentrations, while inhibiting growth at high concentrations (except for *C. mollissima*).

2.2 Effects of Ageratina adenophora Leaf Aqueous Extract on Chlorophyll Content

Table 2 Effects of *Ageratina adenophora* leaf aqueous extracts on chlorophyll content of seven native plants

Table 2 shows that the effects of different extract concentrations varied among species. For *P. massoniana*, high concentration (30 $mg \cdot mL^{-1}$) significantly increased chlorophyll a, chlorophyll b, and total chlorophyll content, with moderate concentration (15 $mg \cdot mL^{-1}$) also significantly increasing chlorophyll a. In contrast, *C. lanceolata* showed significant decreases in chlorophyll a, chlorophyll b, and total chlorophyll at concentrations of 5, 15, and 30 $mg \cdot mL^{-1}$. *C. mollissima* exhibited significant reductions in chlorophyll a, chlorophyll b, and total chlorophyll at high concentration, with chlorophyll b and total chlorophyll also decreasing at moderate concentration. *B. papyrifera* showed significant decreases in all chlorophyll parameters at moderate and high concentrations. *P. fortuneana* displayed significant increases in chlorophyll a, chlorophyll b, and total chlorophyll at low concentrations (2.5 and 5 $mg \cdot mL^{-1}$), but significant chlorophyll b reduction at moderate and high concentrations. *C. oleifera* and *Z. bungeanum* showed similar responses, with low concentrations significantly increasing chlorophyll a, chlorophyll b, and total chlorophyll content.

Overall, *A. adenophora* leaf aqueous extract had no effect or promoted chlorophyll content at low concentrations. High concentrations produced different effects: *P. massoniana* showed increased chlorophyll; *C. oleifera* and *Z. bungeanum* were unaffected; *C. lanceolata*, *C. mollissima*, and *B. papyrifera* exhibited significant decreases in chlorophyll a, chlorophyll b, and total chlorophyll; and *P. fortuneana* showed only significant chlorophyll b reduction.

Discussion

The aqueous extract of *A. adenophora* leaves promoted chlorophyll a and b in *P. massoniana*, showed low-concentration promotion and high-concentration inhibition (hormesis) in *C. lanceolata*, *C. mollissima*, and *B. papyrifera*, had no significant effect on *C. oleifera* and *Z. bungeanum*, and caused significant chlorophyll b reduction only at high concentration in *P. fortuneana*. Growth parameter measurements (seedling height, ground diameter, crown width, and biomass) revealed that high concentrations inhibited all four parameters in six recipient species (except *C. mollissima*). Combining chlorophyll and growth parameter results suggests that *A. adenophora* may inhibit *C. lanceolata* and *C. oleifera* by suppressing chlorophyll synthesis, reducing photosynthetic capacity. However, inhibition of *Z. bungeanum*, *C. oleifera*, *P. fortuneana*, and *B. papyrifera* may occur through other pathways.

Our previous studies on allelopathic effects of *A. adenophora* aqueous extracts on seeds and seedlings of these species also found hormesis effects to varying degrees, consistent with the current findings on growth parameters of six species (except *C. mollissima*). Similar phenomena have been reported in studies on *Sonchus arvensis*, *Ageratum conyzoides*, and *Sophora davidii*.

Numerous studies demonstrate that *A. adenophora* possesses high allelopathic potential, partially explaining its rapid dominance in most habitats. However, some researchers argue that allelopathy may not play a crucial role in every invasion stage. This experiment selected seven distantly related species to explore allelopathic mechanisms from the chlorophyll perspective, yet no completely consistent patterns emerged between chlorophyll content and growth parameters. Niu et al. (2007) proposed that *A. adenophora* alters soil microbial communities through secreted substances to create favorable habitats. Fu et al. (2018) found that *A. adenophora* inhibits canopy dominant tree seedling growth by affecting leaf nitrogen concentration in *Pinus yunnanensis* forests. Current research suggests that allelopathic effects on recipient plants represent comprehensive traits resulting from multiple factors and mechanisms.

The final allelopathic effect assessment depends on both the selected indicators and their degree of impact, with different species responding differently to specific indicators. A single indicator cannot comprehensively reflect allelopathic strength. Therefore, evaluating *A. adenophora* allelopathy requires integrated analysis of multiple indicators.

Although allelopathy in *A. adenophora* is widespread, its mechanisms are complex. Literature indicates that allelochemicals inhibit recipient plant growth by altering cell membrane permeability, affecting plant hormones and enzyme activities, influencing nutrient and water uptake, modifying cell structure, and impacting photosynthesis—either individually or synergistically. Despite extensive research on this invasive species, a hot topic in invasion biology and ecology, its invasion mechanisms remain incompletely understood. This explains why abundant research has not yet enabled effective control of *A. adenophora* rapid

expansion. Future studies should strengthen mechanistic research to reveal the causes of rapid expansion and provide theoretical foundations for management.

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