

## ALLELOPATHY OF DIFFERENT AQUEOUS LEAF EXTRACTS ON THE SEED GERMINATION AND SEEDLING GROWTH OF *PENNISETUM ALOPECUROIDES* (L.) SPRENG.

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### Abstract

Allelopathic chemicals are different in coniferous and broadleaved plants, so the impacts on other plants are also different. In this paper, the effect of aqueous leaf extracts from *Metasequoia glytostroboides* Hu et Cheng, *Cedrus deodara* (Roxb.) G. Don 'Aurea', *Liquidambar formosana* Hance, *Platanus acerifolia* (Aiton) Willd. and a mixture of coniferous and broadleaf trees on seed germination and seedling growth of *Pennisetum alopecuroides* (L.) Spreng. At the same concentration (50 gDW/L) was investigated using an indoor filter paper culture dish method. The results were evaluated using the Response Index (RI) and the Synthesis Effect Index (SE). The results showed that: (1) The leaf extract of *P. acerifolia* (Aiton) Willd. promoted the germination of *P. alopecuroides* (L.) Spreng. seeds, and inhibited the growth of *P. alopecuroides* (L.) Spreng. seedlings. The metabolism of *M. glytostroboides* Hu et Cheng, *C. deodara* (Roxb.) G. Don 'Aurea' and *L. formosana* Hance leaf extracts inhibited the seed germination and seedling growth of *P. alopecuroides* (L.) Spreng. The allelopathic inhibitory effects were: *M. glytostroboides* Hu et Cheng > *C. deodara* (Roxb.) G. Don 'Aurea' > *L. formosana* Hance. (2) In this study, the single leaf extract of the coniferous trees (*M. glytostroboides* Hu et Cheng and *C. deodara* (Roxb.) G. Don 'Aurea') and the broadleaved trees (*L. formosana* Hance and *P. acerifolia* (Aiton) Willd.) did not show significant differences in the allelopathic effect on *P. alopecuroides* (L.) Spreng. Seeds. (3) Compared with their corresponding single leaf extracts, all of the coniferous-broadleaved mixed extract treatments showed neutralization effects on the allelopathic effects of *P. alopecuroides* (L.) Spreng. Namely, one single leaf extract > coniferous-broadleaved mixed extract > another single leaf extract. The results could provide a scientific basis for the management of urban forests and ecosystems.

**Key words:** Leaf aqueous extract, *Pennisetum alopecuroides* (L.) Spreng., Germination, Seedling growth, Allelopathy.

### Introduction

Allelopathy was proposed by Molisch in 1937 (Weston *et al.*, 2013), and the interpretation of this concept by different scholars has varied according to the field of study and focus (Wang *et al.*, 2024). Rice's definition of allelopathy is currently widely used; Rice defined allelopathy as a chemical ecological phenomenon through which the donor plant releases a number of specific chemical substances to the external environment, so that the nearby receptor flora, fauna, microorganisms and their own growth and development experience beneficial or harmful effects (Rice, 1979; Callaway *et al.*, 2003; Abenavoli *et al.*, 2006; García-Romeral *et al.*, 2024).

The way plants perform allelopathy is to release allelochemicals around them (Tesio *et al.*, 2012; Yang *et al.*, 2024). The chemical substances they release often exist in the plant-soil system are polymer secondary compounds (such as tannins, terpenes and steroid compounds, etc.), which can affect the growth and development of other plants by volatilization, leaching, or other means (Jabran *et al.*, 2015; Xu *et al.*, 2023).

Many plant and tree species practice allelopathy, which causes different ecological compositions under different tree species (Valera-Burgos *et al.*, 2012; Afridi & Khan, 2015; Plaza *et al.*, 2022). *Cedrus deodara* (Roxb.) G. Don 'Aurea' is a common green tree species in Southern China, and its planting area is very wide. It has been found that there is less species diversity, or even bare surface and no plant settlement under *C. deodara* (Roxb.) G. Don

'Aurea', compared with other afforestation trees (Li *et al.*, 2015). Some studies have found that the allelopathic substances released from the leaves of *Larix principis-rupprechtii* Mayr have a poisonous effect on their seed germination and can also produce allelopathy to other plants that grow together (Han *et al.*, 2008a). *Metasequoia glytostroboides* Hu et Cheng litter has a certain allelopathic inhibitory effect on its own seed germination and growth (Matuda *et al.*, 2021). Both, *Cunninghamia lanceolata* (Lamb.) Hook. and *C. deodara* (Roxb.) G. Don 'Aurea' are coniferous gymnosperm. Some studies have found that *C. lanceolata* (Lamb.) Hook. also produces a certain degree of allelopathy inhibition to other plants and a self-toxicity phenomenon (Cao *et al.*, 2008; Zhang *et al.*, 2021). The extracts from the organs of a broadleaved birch (*Betula platyphylla* Sukaczew) showed the effect of promoting germination of larch (*Larix olgensis* A. Henry), but extracts of larch leaves had an inhibitory effect on the seeds of *B. platyphylla* Sukaczew (Liu *et al.*, 2011). There is sparse vegetation under Japanese pine (*Pinus densiflora* Sieb. et Zucc.) forests. Two kinds of growth inhibitors, namely, 15-hydroxy-7-oxygen dehydrogenation rosin acid ester and 7-oxygen dehydrogenation rosin acid, were isolated from the soil under the forest, and the effects of the allelopathic substances on 6 plant species, including ryegrass (*Lolium multiflorum* Lam.) were studied (Kato-Noguchi & Macías, 2005, 2008; Kato-Noguchi *et al.*, 2017). *Eucalyptus robusta* Smith is a strongly competitive tree species, and the secretion of chemical substances that strongly inhibit other species is also an important reason

for its competitive success. Under natural conditions, the vegetation of *E. robusta* Smith under the canopy is relatively scarce, and soil erosion is severe. *Eucalyptus citriodora* Hook. f. and *Litchi chinensis* Sonn. are planted in mixed forests, and a large number of *L. chinensis* Sonn. will die out 3 years later. *E. citriodora* Hook. f. essential oil expresses a strong negative allelopathic effect against some weeds (Zhang *et al.*, 2009; Benchaa *et al.*, 2018).

We speculate that different plant leaves release different types or contents of allelopathic substances. The differences in the composition of allelopathic substances of coniferous plants such as *C. deodara* (Roxb.) G. Don 'Aurea' and broadleaved plants such as *B. platyphylla* Sukaczew may lead them to participate in different directions of allelopathy, which are manifested as promotion or inhibition (Han *et al.*, 2008b; Xu *et al.*, 2023).

In this paper, based on different kinds and contents of allelopathic substances that can be released from different plant leaves, which affect the germination of seeds. A single leaf extract of the same concentration from 4 species of plants (*C. deodara* (Roxb.) G. Don 'Aurea', *M. glyptostroboides* Hu et Cheng, *L. formosana* Hance, and *P. acerifolia* (Aiton) Willd.) is used to treat *P. alopecuroides* (L.) Spreng. Seeds. And the effects of different leaf extracts on seed germination and seedling growth of *P. alopecuroides* (L.) Spreng. are discussed according to germination rate, germination index, seedling height, root length, fresh weight, etc. Furthermore, this study includes two kinds of coniferous species and two kinds of broadleaved species. So, the allelopathy among tree species could be compared to the effect of mixed coniferous-broadleaved leaf extracts.

The interspecific relationship of the mixed forest is comprehensively embodied by many functions, and its mechanism is more complex, especially biochemically (Dorning & Cipollini, 2006; Chen *et al.*, 2014; El-Maarouf-Bouteau *et al.*, 2015). Allelopathy is prevalent among tree species and is one of the main methods of their interaction, so it is an important direction to study interspecific relationships (Liu & Herbert, 2002; Khanh *et al.*, 2005; Huo *et al.*, 2019). In this experiment, leaf extracts of 4 species of plants and mixed coniferous-broadleaved extracts were used to treat *P. alopecuroides* (L.) Spreng. seeds and compared with the corresponding leaf extracts to predict the seed germination and the corresponding physiological index of seedling growth. Through simulating the allelopathy of the coniferous-broadleaved mixed forest. In this paper, the difference in allelopathy between mixed forests and single species forests is discussed, which has some significance in the study of interspecific relationships and ecosystem structure.

## Materials and Methods

**Target plants:** *P. alopecuroides* (L.) Spreng. is selected as the receptor plant. *P. alopecuroides* (L.) Spreng. is a perennial clump-like herb with a stout stem, which is distributed widely around the world, including Asia, Oceania and Africa where the altitude ranges from 50 m to 3200 m. *P. alopecuroides* (L.) Spreng. prefers an adequate light environment. *P. alopecuroides* (L.) Spreng. has strong ecological adaptability, resistance to drought and

excessive moisture, strong resistance to cold and shelter, and almost no pests and diseases. This species competes in the field with crops for light, water and nutrients. *P. alopecuroides* (L.) Spreng. has developed a root system that is difficult to eradicate. *P. alopecuroides* (L.) Spreng. has a growth advantage with water and fertilizer consumption, and it can absorb soil moisture and nutrients, which often exceeds the consumption of crop growth. It is a typical harmful weed and is the main object of biological control. *P. alopecuroides* (L.) Spreng. tastes sweet, has abundant coarse fat, crude protein, crude fiber in fresh grass, rich in nutrients and is a high-grade and favorite forage grass. The cultivation of *P. alopecuroides* (L.) Spreng. has been promoted in the south of China. As a good feed source of herbivores and fishes, *P. alopecuroides* (L.) Spreng. has high value in forage utilization. In the autumn of 2017, *P. alopecuroides* (L.) Spreng. seeds were collected and put in a cool dry place, the seed was peeled from the glume and put in an envelope for storage.

**Facilities:** Twenty-seven petri dishes (d: 90 mm), 54 pieces of filter paper, nine 500 ml cone bottles, a blade grinder, medical scissors, electronic scales, cylinders, glass rods and so on, were used.

**Allelopathic sources - preparation for leaf aqueous extract:** *C. deodara* (Roxb.) G. Don 'Aurea', *M. glyptostroboides* Hu et Cheng, *L. formosana* Hance and *P. acerifolia* (Aiton) Willd. were selected as allelopathic sources. In the autumn of 2017, the leaves of the 4 plants that had just fallen off were collected, spread out at room temperature in the shade, and packed in a woven bag stored in a ventilated dry place. Before the experiment, plant blades were cut with medical scissors, crushed into powder with a blade grinder, and 37.5 g of each were weighed and divided into the following kinds of treatments: the single leaf extract and the coniferous-broadleaved mixed leaf extract.

The single leaf extract treatment included 25 g of leaf powders of *C. deodara* (Roxb.) G. Don 'Aurea', *M. glyptostroboides* Hu et Cheng, *L. formosana* Hance, *P. acerifolia* (Aiton) Willd. into conical bottles of 500 mL. The coniferous-broadleaved mixed leaf extract treatments included leaf powders of *M. glyptostroboides* Hu et Cheng (12.5 g) + *L. formosana* Hance (12.5 g), *M. glyptostroboides* Hu et Cheng (12.5 g) + *P. acerifolia* (Aiton) Willd. (12.5 g), *C. deodara* (Roxb.) G. Don 'Aurea' (12.5 g) + *L. formosana* Hance (12.5 g), and *C. deodara* (Roxb.) G. Don 'Aurea' (12.5 g) + *P. acerifolia* (Aiton) Willd. (12.5 g). These extracts were put in 500 mL conical bottles. Then, 500 ml distilled water was added, and the treatments were soaked for 48 hrs with vibration 1 time every 6 hrs. Then, the samples were kept static for 3 hrs, and a coarse filter using two-layer gauze was used, and then the mixture was filtered. Five hundred mL flasks containing 50 g dry matter content per liter (50 gDW/L) of 4 different proportions of leaf extracts were prepared. Then, the contents were sealed and refrigerated at 4°C until use.

**Facilities preparation:** At the bottom of the 90 mm-diameter petri dish, two pieces of filter paper of appropriate size were padded. Then, *P. alopecuroides* (L.) Spreng.

seeds, for which the grain was plump, and the size was essentially consistent and soaked into solution of 0.02%  $\text{KMnO}_4$  for 10 min for disinfection, the seeds were cleaned with distilled water 3 times.

**Seed germination:** Sixty-five *P. alopecuroides* (L.) Spreng. seeds were evenly placed in each petri dish, and the 8 groups of leaf extracts were fetched from the refrigerator and warmed to room temperature. In each petri dish, 10 milliliters of the leaf extract from each respective group were sequentially introduced. 10 mL distilled water was added into the control group; each treatment had 3 replications.

The seeds were placed in the natural environment (Greenhouse of Biological Experiment Station of East China Normal University), and a shading cloth was used for shading treatment. During the experiment, distilled water was added to keep the filter paper moist.

**Measurement of germination rate and germination index:** The number of germinated seeds was counted daily from the date of immersion to the time when the seeds were no longer germinated. The germination rate (GR) (formula 1) and germination index (Gi) (formula 2) were calculated, and germination curves were drawn. Here, the embryo root was considered the mark of seed germination at 1 mm outside the seed coat and the seed was no longer germinating for 5 consecutive days.

$$\text{GR} = (n/N) \times 100\% \quad (1)$$

where n is the number of germinated seeds and N is the total number of seeds.

$$G_i = \sum (G_t/D_t) \quad (2)$$

where  $G_t$  are the germinated seeds at the  $t$ th day, and  $D_t$  is the days of the germination experiment.

Measurement of root length, height, and fresh weight of seedling: When the seeds were no longer sprouting, they were taken out, and the water was drained by absorbent paper; the root length and seedling height of 6 seedlings were randomly sampled in each group. The fresh weight of each group of seedlings was weighed by an electronic balance and recorded.

**Allelopathic response index and synthesis effect index:** An allelopathic response index (RI) was adopted to measure the type and intensity of allelopathy, as in formula 3 and formula 4 (Williamson & Richardson, 1988).

$$\text{RI} = 1 - C/T \quad (T \geq C) \quad (3)$$

$$\text{RI} = T/C - 1 \quad (T < C) \quad (4)$$

where C is the control value, and T is the treatment value.  $\text{RI} < 0$  represents inhibition,  $\text{RI} > 0$  represents promotion, and the absolute value of RI is related to the intensity of allelopathy.

The effects of allelopathy on different parts of plants are different; some allelopathic substances inhibit root growth, but they may not affect height or may even promote seedling growth. Therefore, the allelopathic synthesis effect index (SE) reflects the synthetic effects of allelochemicals on plants, as shown in formula 5 (Yang *et al.*, 2015).

$$\text{SE} = (\text{RI}_{\text{Gr}} + \text{RI}_{\text{Gi}} + \text{RI}_{\text{Hs}} + \text{RI}_{\text{Rl}} + \text{RI}_{\text{Fw}}) / 5 \quad (5)$$

where  $\text{RI}_{\text{Gr}}$  is the RI for the germination rate,  $\text{RI}_{\text{Gi}}$  is the RI for the germination index,  $\text{RI}_{\text{Hs}}$  is the RI for the seedling height,  $\text{RI}_{\text{Rl}}$  is the RI for the root length,  $\text{RI}_{\text{Fw}}$  is the RI for the fresh weight,  $\text{SE} > 0$  represents promotion,  $\text{SE} < 0$  represents inhibition, and the absolute value is related with the intensity of allelopathy.

**Statistical analysis:** The average value of the measured index and the standard deviation were calculated by Microsoft Excel 2010. Single factor variance analysis (one-way ANOVA) and Tukey's HSD test were conducted by SPSS 17.0, the difference between each treatment group was analyzed, and the significance level was  $\alpha = 0.05$ . The fitting of the logistic curve was carried out by Origin 8.0.

## Results

### Allelopathy of different leaf extracts on the germination of *P. alopecuroides* (L.) Spreng. seeds

**Germination rate:** The order of germination rate of *P. alopecuroides* (L.) Spreng. seeds after treatment with 4 kinds of single leaf extract solutions was as follows: *P. acerifolia* (Aiton) Willd. (86.15%) > CK (84.62%) > *M. glyptostroboides* Hu et Cheng (72.82%) > *L. formosana* Hance (72.31%) > *C. deodara* (Roxb.) G. Don 'Aurea' (67.69%) (Table 1). Compared with the control group, the treatments showed inhibition on the germination rate of *P. alopecuroides* (L.) Spreng. seeds, except for the *P. acerifolia* (Aiton) Willd. leaf extract (promotion), and the order was as follows: *C. deodara* (Roxb.) G. Don 'Aurea' > *L. formosana* Hance > *M. glyptostroboides* Hu et Cheng.

There was no significant difference between coniferous trees (*M. glyptostroboides* Hu et Cheng and *C. deodara* (Roxb.) G. Don 'Aurea') and broadleaved trees (*L. formosana* Hance and *P. acerifolia* (Aiton) Willd.) for allelopathy on the germination rate of *P. alopecuroides* (L.) Spreng. seeds.

In the treatment of mixed coniferous-broadleaved leaf extracts, the intensity of allelopathy of *M. glyptostroboides* Hu et Cheng + *L. formosana* Hance mixed extracts on the germination rate RI of *P. alopecuroides* (L.) Spreng. showed promotion, namely, coniferous-broadleaved mixed extract > one single leaf extract > another single leaf extract. The intensity of allelopathy of all other mixed coniferous-broadleaved leaf extracts on the germination rate RI of *P. alopecuroides* (L.) Spreng. showed a neutral effect, namely, one single leaf extract > coniferous-broadleaved mixed extracts > another single leaf extract (Table 1).

**Table 1. Effect of aqueous extracts on germination of *Pennisetum alopecuroides* (L.) Spreng. (mean ± SD, n=3).**

Treatment	Germination rate (%)	Germination index
Control	84.62 ± 4.62 bc	33.17 ± 3.00 ab
<i>Metasequoia glyptostroboides</i> Hu et Cheng (coniferous)	72.82 ± 5.40 ab	27.52 ± 3.51 a
<i>Cedrus deodara</i> (Roxb.) G. Don 'Aurea' (coniferous)	67.69 ± 4.07 a	27.89 ± 2.65 ab
<i>Liquidambar formosana</i> Hance (broadleaved)	72.31 ± 6.71 ab	32.43 ± 4.36 ab
<i>Platanus acerifolia</i> (Aiton) Willd. (broadleaved)	86.15 ± 6.10 c	34.80 ± 4.00 b
<i>Metasequoia glyptostroboides</i> Hu et Cheng + <i>Liquidambar formosana</i> Hance	71.28 ± 4.95 a	28.42 ± 3.21 ab
<i>Metasequoia glyptostroboides</i> Hu et Cheng + <i>Platanus acerifolia</i> (Aiton) Willd.	72.82 ± 3.87 ab	27.81 ± 2.52 ab
<i>Cedrus deodara</i> (Roxb.) G. Don 'Aurea' + <i>Liquidambar formosana</i> Hance	70.77 ± 1.54 a	31.37 ± 1.00 ab
<i>Cedrus deodara</i> (Roxb.) G. Don 'Aurea' + <i>Platanus acerifolia</i> (Aiton) Willd.	71.79 ± 0.89 a	27.30 ± 0.58 ab

Note: The data with different letters in the same column represent significant differences at p<0.05. Data in the table are reserved after two decimal places

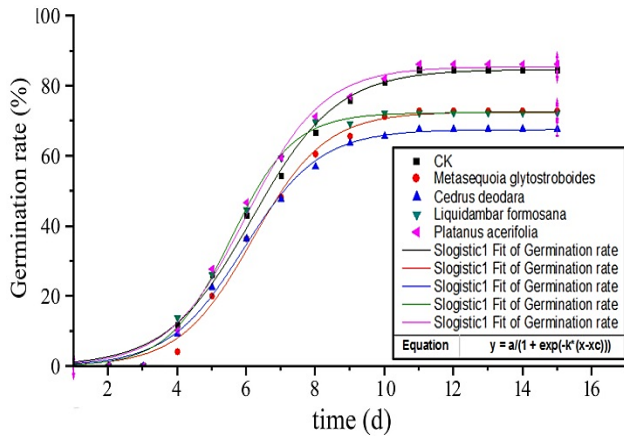


Fig. 1. Effect of leaf extracts on the germination process of *Pennisetum alopecuroides* (L.) Spreng.

**Germination index:** The order of the germination indices of *P. alopecuroides* (L.) Spreng. seeds after treatment with 4 kinds of single leaf extract solutions was as follows: *P. acerifolia* (Aiton) Willd. (34.80) > CK (33.17) > *L. formosana* Hance (32.43) > *C. deodara* (Roxb.) G. Don 'Aurea' (27.89) > *M. glyptostroboides* Hu et Cheng (27.52) (Table 1). Compared with the control group, the treatments showed inhibition on the germination index of *P. alopecuroides* (L.) Spreng. seeds, except for the *P. acerifolia* (Aiton) Willd. leaf extract, and the order was: *M. glyptostroboides* Hu et Cheng > *C. deodara* (Roxb.) G. Don 'Aurea' > *L. formosana* Hance.

There was no significant difference between coniferous trees (*M. glyptostroboides* Hu et Cheng and *C. deodara* (Roxb.) G. Don 'Aurea') and broadleaved trees (*L. formosana* Hance and *P. acerifolia* (Aiton) Willd.) for allelopathy to germination index of *P. alopecuroides* (L.) Spreng. seeds.

The intensity of allelopathy of all mixed coniferous-broadleaved leaf extracts on the germination index RI of *P. alopecuroides* (L.) Spreng. showed the neutral effect (Table 1).

**Germination process:** The process of seed germination, similar to other biological processes, is a logarithmic growth process followed by a logistic curve (Fig. 1). The effect of different single leaf extracts on the *P. alopecuroides* (L.) Spreng. seed germination process was as follows: The seeds of the control group and the experimental group began to germinate on the 3rd day after sowing and continued till the 8th day; the germination rate gradually slowed after the 9th day.

During the process of seed germination (Fig. 1), the treatment of *P. acerifolia* (Aiton) Willd. leaf extracts on *P. alopecuroides* (L.) Spreng. seeds showed a little promotion in the germination rate and speed. The treatments of *L. formosana* Hance, *M. glyptostroboides* Hu et Cheng and *C. deodara* (Roxb.) G. Don 'Aurea' on *P. alopecuroides* (L.) Spreng. seeds showed significant inhibition.

$$\text{(Control: } y = \frac{84.67}{1+e^{-0.81(x-6.17)}} \text{ (R}^2=0.9948\text{). } M. \text{ glyptostroboides Hu et Cheng: } y = \frac{72.57}{1+e^{-0.95(x-6.18)}} \text{ (R}^2=0.9953\text{). } C. \text{ deodara (Roxb.) G. Don 'Aurea': } y = \frac{67.53}{1+e^{-0.92(x-5.93)}} \text{ (R}^2=0.9962\text{). } L. \text{ formosana Hance: } y = \frac{72.33}{1+e^{-1.11(x-5.54)}} \text{ (R}^2=0.9970\text{). } P. \text{ acerifolia (Aiton) Willd.: } y = \frac{85.45}{1+e^{-0.89(x-5.98)}} \text{ (R}^2=0.9946\text{)}$$

The effects of the extract of single kind of leaf and its conifer-broadleaved mixed extract on *P. alopecuroides* (L.) Spreng. were obviously different (Fig. 2). Overall, the effect of coniferous-broad-leaved mixed extracts on *P. alopecuroides* (L.) Spreng. were neutral.

$$\text{(A: } M. \text{ glyptostroboides Hu et Cheng } y = \frac{72.57}{1+e^{-0.95(x-6.18)}} \text{ (R}^2=0.9953\text{). } L. \text{ formosana Hance } y = \frac{72.33}{1+e^{-1.11(x-5.54)}} \text{ (R}^2=0.9970\text{). } M. \text{ glyptostroboides Hu et Cheng + } L. \text{ formosana Hance } y = \frac{70.61}{1+e^{-0.99(x-6.02)}} \text{ (R}^2=0.9966\text{). B: } M. \text{ glyptostroboides Hu et Cheng } y = \frac{72.57}{1+e^{-0.95(x-6.18)}} \text{ (R}^2=0.9953\text{). } P. \text{ acerifolia (Aiton) Willd. } y = \frac{85.45}{1+e^{-0.89(x-5.98)}} \text{ (R}^2=0.9946\text{). } M. \text{ glyptostroboides Hu et Cheng + } P. \text{ acerifolia (Aiton) Willd. } y = \frac{73.12}{1+e^{-0.86(x-6.26)}} \text{ (R}^2=0.9940\text{). C: } C. \text{ deodara (Roxb.) G. Don 'Aurea' } y = \frac{67.53}{1+e^{-0.92(x-5.93)}} \text{ (R}^2=0.9962\text{). } L. \text{ formosana Hance } y = \frac{72.33}{1+e^{-1.11(x-5.54)}} \text{ (R}^2=0.9970\text{). } C. \text{ deodara (Roxb.) G. Don 'Aurea' + } L. \text{ formosana Hance } y = \frac{69.71}{1+e^{-1.07(x-5.43)}} \text{ (R}^2=0.9941\text{). D: } C. \text{ deodara (Roxb.) G. Don 'Aurea' } y = \frac{67.53}{1+e^{-0.92(x-5.93)}} \text{ (R}^2=0.9962\text{). } P. \text{ acerifolia (Aiton) Willd. } y = \frac{85.45}{1+e^{-0.89(x-5.98)}} \text{ (R}^2=0.9946\text{). } C. \text{ deodara (Roxb.) G. Don 'Aurea' + } P. \text{ acerifolia (Aiton) Willd. } y = \frac{76.61}{1+e^{-1.04(x-6.27)}} \text{ (R}^2=0.9960\text{). Control: } y = \frac{84.67}{1+e^{-0.81(x-6.17)}} \text{ (R}^2=0.9948\text{).)}$$

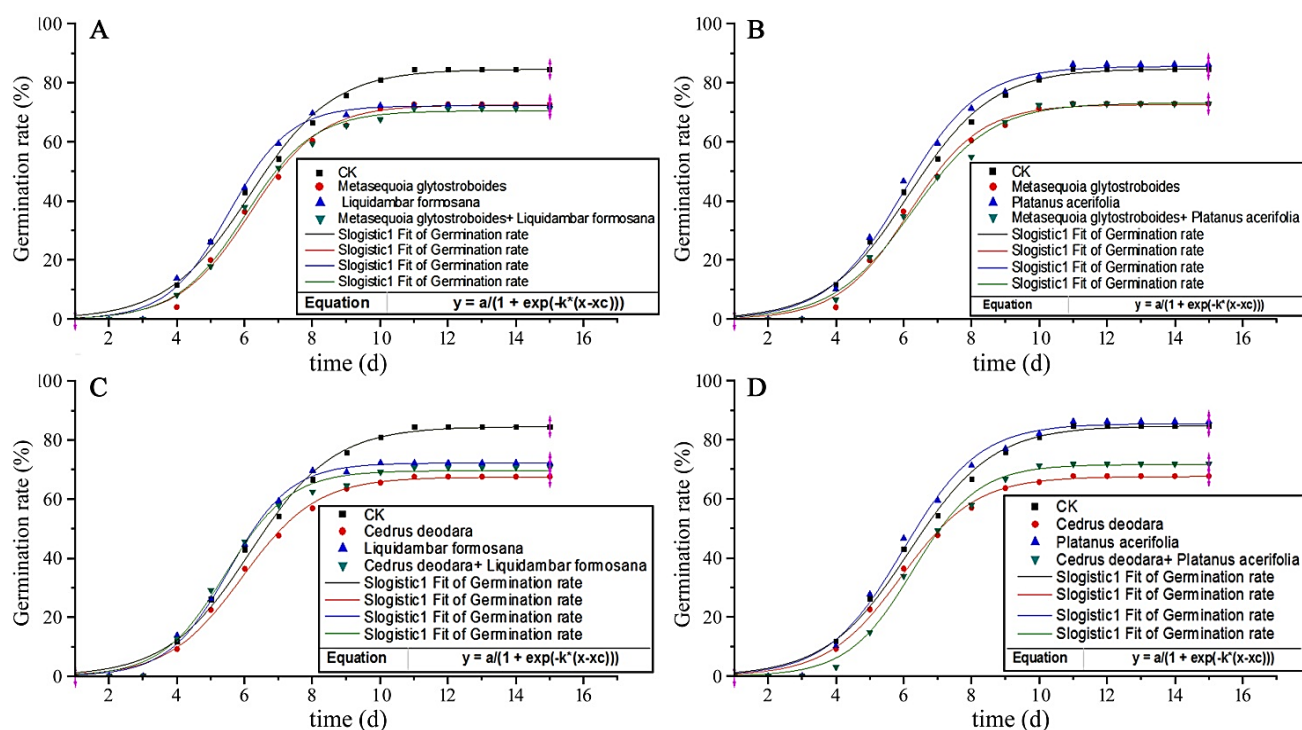


Fig. 2. Effect of the single leaf extract and the coniferous-broadleaf mixed extracts on the germination process of *Pennisetum alopecuroides* (L.) Spreng.

### The effects of different leaf extracts on seedling growth of *P. alopecuroides* (L.) Spreng.

**Seedling height:** The order of height of *P. alopecuroides* (L.) Spreng. seedlings after treatment with 4 kinds of single leaf extract solutions was as follows: CK (2.13cm) > *P. acerifolia* (Aiton) Willd. (1.97cm) > *M. glytostroboides* Hu et Cheng (1.82cm) > *C. deodara* (Roxb.) G. Don 'Aurea' (1.61cm) > *L. formosana* Hance (1.35cm) (Table 2). Compared with the control group, all treatment groups showed a decrease in seedling height, of which the group treated with *L. formosana* Hance decreased most, and the effect was significant. The effect on seedling height of *P. alopecuroides* (L.) Spreng. showed inhibition, except for the *P. acerifolia* (Aiton) Willd. leaf extract, and the order is: *L. formosana* Hance > *C. deodara* (Roxb.) G. Don 'Aurea' > *M. glytostroboides* Hu et Cheng.

There was no significant difference in the effect of allelopathy on the height of *P. alopecuroides* (L.) Spreng. seedlings between coniferous trees (*M. glytostroboides* Hu et Cheng and *C. deodara* (Roxb.) G. Don 'Aurea') and broadleaved trees (*L. formosana* Hance and *P. acerifolia* (Aiton) Willd.).

The intensity of allelopathy of all mixed coniferous-broadleaved leaf extracts to seedling height of *P. alopecuroides* (L.) Spreng. showed a neutral effect.

**Root length:** The order of root length of *P. alopecuroides* (L.) Spreng. seedlings after treatment with 4 kinds of single leaf extract solutions was as follows: CK (2.16cm) > *P. acerifolia* (Aiton) Willd. (2.00cm) > *C. deodara* (Roxb.) G. Don 'Aurea' (1.98cm) > *M. glytostroboides* Hu et Cheng (1.65cm) > *L. formosana* Hance (1.34cm) (Table 2). The treatments showed inhibition of the root length of *P. alopecuroides* (L.) Spreng. seedlings, except for *P.*

*acerifolia* (Aiton) Willd. leaf extracts, and the order was: *L. formosana* Hance > *C. deodara* (Roxb.) G. Don 'Aurea' > *M. glytostroboides* Hu et Cheng.

There was no significant difference between coniferous trees (*M. glytostroboides* Hu et Cheng and *C. deodara* (Roxb.) G. Don 'Aurea') and broadleaved trees (*L. formosana* Hance and *P. acerifolia* (Aiton) Willd.) in the allelopathic effects on the root length of *P. alopecuroides* (L.) Spreng. seedlings.

The intensity of allelopathy of *M. glytostroboides* Hu et Cheng + *L. formosana* Hance, *M. glytostroboides* Hu et Cheng + *P. acerifolia* (Aiton) Willd., and *C. deodara* (Roxb.) G. Don 'Aurea' + *L. formosana* Hance mixed leaf extracts on the root length of *P. alopecuroides* (L.) Spreng. showed a neutral effect. The intensity of allelopathy of *C. deodara* (Roxb.) G. Don 'Aurea' + *P. acerifolia* (Aiton) Willd. extracts on the root length of *P. alopecuroides* (L.) Spreng. showed promotion.

**Fresh weight:** The order of fresh weight of *P. alopecuroides* (L.) Spreng. seedlings after treatment with 4 kinds of single leaf extract solutions was as follows: *P. acerifolia* (Aiton) Willd. (1.19g) > CK (1.06g) > *M. glytostroboides* Hu et Cheng (0.88g) = *C. deodara* (Roxb.) G. Don 'Aurea' (0.88g) > *L. formosana* Hance (0.83g) (Table 2). The treatments inhibited the fresh weight of *P. alopecuroides* (L.) Spreng. seedlings, except for the *P. acerifolia* (Aiton) Willd. leaf extract, and the order was: *L. formosana* Hance > *C. deodara* (Roxb.) G. Don 'Aurea' > *M. glytostroboides* Hu et Cheng.

There was no significant difference between the effects of allelopathy from coniferous trees (*M. glytostroboides* Hu et Cheng and *C. deodara* (Roxb.) G. Don 'Aurea') and broadleaved trees (*L. formosana* Hance and *P. acerifolia* (Aiton) Willd.) on the fresh weight of *P. alopecuroides* (L.) Spreng. seedlings.

The intensity of allelopathy of *C. deodara* (Roxb.) G. Don 'Aurea' + *P. acerifolia* (Aiton) Willd. mixed leaf extracts increased the fresh weight of *P. alopecuroides* (L.) Spreng., and the intensity of allelopathy of the other mixed coniferous-broadleaved leaf extracts had neutral effects on the fresh weight of *P. alopecuroides* (L.) Spreng.

**Synthesis effect index of *P. alopecuroides* (L.) Spreng. after treatment of leaf extracts:** The RI of the germination rate and germination index of *P. acerifolia* (Aiton) Willd. leaf extracts on *P. alopecuroides* (L.) Spreng. was greater than zero, and the RI of seedling height, root length and fresh weight were less than zero (Table 3). However, the SE of *P. acerifolia* (Aiton) Willd. leaf extract is greater than zero, which indicates its allelopathic effect on *P. alopecuroides* (L.) Spreng. is promotion. Five RI of *M. glyptostroboides* Hu et

Cheng, *C. deodara* (Roxb.) G. Don 'Aurea', and *L. formosana* Hance leaf extracts to on *P. alopecuroides* (L.) Spreng. were less than zero, and the SE was also less than zero which indicating the allelopathic effect on *P. alopecuroides* (L.) Spreng. is inhibition, and the order was: *L. formosana* Hance > *M. glyptostroboides* Hu et Cheng > *C. deodara* (Roxb.) G. Don 'Aurea'.

There is no significant difference between the allelopathy of coniferous trees (*M. glyptostroboides* Hu et Cheng and *C. deodara* (Roxb.) G. Don 'Aurea') and broadleaved trees (*L. formosana* Hance and *P. acerifolia* (Aiton) Willd.) on *P. alopecuroides* (L.) Spreng. seed germination and seedling growth.

The intensity of allelopathy of all mixed coniferous-broadleaved extracts to *P. alopecuroides* (L.) Spreng. showed the neutralization.

**Table 2. Effect of aqueous extracts on seedlings of *Pennisetum alopecuroides* (L.) Spreng. (mean  $\pm$  SD, n=6).**

Treatment	Seedling height (cm)	Root length (cm)	Fresh weight (g)
Control	2.13 $\pm$ 0.64 b	2.16 $\pm$ 0.28 c	1.06 $\pm$ 0.14 a
<i>Metasequoia glyptostroboides</i> Hu et Cheng	1.82 $\pm$ 0.15 ab	1.65 $\pm$ 0.30 abc	0.88 $\pm$ 0.37 a
<i>Cedrus deodara</i> (Roxb.) G. Don 'Aurea'	1.61 $\pm$ 0.13 ab	1.98 $\pm$ 0.25 bc	0.88 $\pm$ 0.10 a
<i>Liquidambar formosana</i> Hance	1.35 $\pm$ 0.46 a	1.34 $\pm$ 0.24 a	0.83 $\pm$ 0.09 a
<i>Platanus acerifolia</i> (Aiton) Willd.	1.97 $\pm$ 0.25 ab	2.00 $\pm$ 0.34 bc	1.19 $\pm$ 0.23 a
<i>Metasequoia glyptostroboides</i> Hu et Cheng + <i>Liquidambar formosana</i> Hance	1.67 $\pm$ 0.17 ab	1.64 $\pm$ 0.12 abc	0.84 $\pm$ 0.07 a
<i>Metasequoia glyptostroboides</i> Hu et Cheng + <i>Platanus acerifolia</i> (Aiton) Willd.	1.75 $\pm$ 0.32 ab	1.99 $\pm$ 0.49 bc	0.69 $\pm$ 0.29 a
<i>Cedrus deodara</i> (Roxb.) G. Don 'Aurea' + <i>Liquidambar formosana</i> Hance	1.57 $\pm$ 0.27 ab	1.52 $\pm$ 0.30 ab	0.88 $\pm$ 0.09 a
<i>Cedrus deodara</i> (Roxb.) G. Don 'Aurea' + <i>Platanus acerifolia</i> (Aiton) Willd.	1.62 $\pm$ 0.32 ab	1.41 $\pm$ 0.25 a	0.81 $\pm$ 0.23 a

Note: The data with different letters in the same column represent significant differences at  $p < 0.05$ . Data in the table is reserved after two decimal places

**Table 3. Allelopathic response index of *Pennisetum alopecuroides* (L.) Spreng.**

Treatment	Allelopathic Response Index (RI)					Synthesis Effect (SE)
	Germination rate	Germination index	Seedling height	Root length	Fresh weight	
Control	-	-	-	-	-	-
<i>Metasequoia glyptostroboides</i> Hu et Cheng	-0.16	-0.21	-0.17	-0.31	-0.20	-0.21
<i>Cedrus deodara</i> (Roxb.) G. Don 'Aurea'	-0.25	-0.19	-0.32	-0.09	-0.20	-0.21
<i>Liquidambar formosana</i> Hance	-0.17	-0.02	-0.58	-0.61	-0.28	-0.33
<i>Platanus acerifolia</i> (Aiton) Willd.	0.02	0.05	-0.08	-0.08	0.13	0.01
<i>Metasequoia glyptostroboides</i> Hu et Cheng + <i>Liquidambar formosana</i> Hance	-0.19	-0.17	-0.28	-0.32	-0.27	-0.24
<i>Metasequoia glyptostroboides</i> Hu et Cheng + <i>Platanus acerifolia</i> (Aiton) Willd.	-0.16	-0.19	-0.22	-0.09	-0.21	-0.17
<i>Cedrus deodara</i> (Roxb.) G. Don 'Aurea' + <i>Liquidambar formosana</i> Hance	-0.20	-0.06	-0.36	-0.42	-0.21	-0.25
<i>Cedrus deodara</i> (Roxb.) G. Don 'Aurea' + <i>Platanus acerifolia</i> (Aiton) Willd.	-0.18	-0.22	0.03	-0.54	-0.31	-0.24

## Discussion

It was found that single leaf extracts of *M. glyptostroboides* Hu et Cheng and *C. deodara* (Roxb.) G. Don 'Aurea' had inhibitory effects on the growth of other plants (Li *et al.*, 2015; Hisashi *et al.*, 2023). In this experiment, the effect of single leaf extracts of 50 gDW/L of *M. glyptostroboides* Hu et Cheng, *C. deodara* (Roxb.) G. Don 'Aurea', *L. formosana* Hance and *P. acerifolia* (Aiton) Willd. on *P. alopecuroides* (L.) Spreng. were examined, and the corresponding physiological indices of seed germination and seedling growth were analyzed. It was found that at this concentration, 4 kinds of plant leaf extracts had different allelopathic effects on *P. alopecuroides* (L.) Spreng. *P. acerifolia* (Aiton) Willd. showed a certain degree of allelopathic promotion; *M. glyptostroboides* Hu et Cheng, *C. deodara* (Roxb.) G. Don 'Aurea', and *L. formosana* Hance showed allelopathic inhibition of *P. alopecuroides* (L.) Spreng. The order of the

intensity of the inhibitory effect was: *L. formosana* Hance > *M. glyptostroboides* Hu et Cheng > *C. deodara* (Roxb.) G. Don 'Aurea'; four leaf extract solutions showed different intensities of allelopathy to *P. alopecuroides* (L.) Spreng. The absolute value of the synthesis effect index (SE) of *P. alopecuroides* (L.) Spreng. was less than zero (Table 3). *P. acerifolia* (Aiton) Willd. showed allelopathic promotion on *P. alopecuroides* (L.) Spreng.

The allelopathy of 4-leaf extracts on *P. alopecuroides* (L.) Spreng. was not correlated with the leaf type (coniferous or broadleaved). The effects of plant allelopathic substances on seeds were divided into two aspects. On one hand, most allelopathic substances can inhibit seed germination and seedling growth (Dias *et al.*, 2016; Sher *et al.*, 2023), while on the other hand, partial allelopathy can also promote seed germination and seedling growth (Chu *et al.*, 2014; Xiao *et al.*, 2020). In addition, allelopathic substances often reflect the phenomenon of "low promotion and high inhibition", that

is, a low concentration promotes germination and growth, and high concentrations inhibit germination and growth (Rejila & Vijayakumar, 2011; Schittko & Wurst, 2014; Shu *et al.*, 2015; Seifu *et al.*, 2023). At the same time, there is a great relationship with different types of allelopathic substances and the species of receptor plants (Kalinová, 2008; Bauer *et al.*, 2012; Chen *et al.*, 2017). In this experiment, the coniferous trees *M. glyptostroboides* Hu et Cheng and *C. deodara* (Roxb.) G. Don 'Aurea' and the broadleaved tree *L. formosana* Hance showed inhibitory effects on *P. alopecuroides* (L.) Spreng., while the broadleaved tree *P. acerifolia* (Aiton) Willd. showed a promotional effect on *P. alopecuroides* (L.) Spreng. In this concentration, the effects 4 kinds of leaf extracts on *P. alopecuroides* (L.) Spreng. were not correlated with respect of coniferous and broadleaved.

The allelopathy of the mixture of different substances to the receptor can be divided into three types: synergistic, antagonistic, and neutral (Weitbrecht *et al.*, 2011; Mbombo & Ngobese, 2024). However, there were few studies on the classification of the allelopathy of receptor plants to different leaf extracts (Shi *et al.*, 2020). In this experiment, mixed coniferous-broadleaved leaf extracts of 50 gDW/L were used to treat *P. alopecuroides* (L.) Spreng. and compared with the corresponding single leaf extracts according to the seed germination and the corresponding physiological index of seedling growth. The results showed that the effect of the treatment group of coniferous-broadleaved mixed extraction solutions was neutral on *P. alopecuroides* (L.) Spreng., namely, a single leaf extract > a coniferous-broadleaved mixed extract > another single leaf extract. However, whether this phenomenon represents allelopathic synergism or allelopathic antagonism of different leaf extracts has yet to be determined (Blanco, 2007; Yang *et al.*, 2015).

In this experiment, the difference of allelopathy between mixed forest and single forest can be compared to agriculture. If some allelopathic growth promoting effect of monoculture forest is prominent, it can provide experience for monoculture cultivation. If the mixed forest is stronger than the monocultural forest in inhibiting weeds, it can be considered to construct a mixed crop planting mode to suppress weeds in the field and reduce the use of herbicides (Al-Qthanin *et al.*, 2024). If the allelopathy of mixed forest is more conducive to soil microbial activity and nutrient cycling, it can be applied to improve farmland soil, maintain fertility, reduce fertilizer dependence, and promote sustainable agricultural development. The value of allelopathy for human beings is also reflected in many aspects. In forestry, a mixed forest is constructed to promote tree growth through allelopathy. The extraction of active substances from allelopathic plants is used in medicine and other fields (Chemlali *et al.*, 2024). In short, allelopathy will continue to contribute its important value to mankind in the future.

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