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Responses of *Sesamum indicum* to Allelopathy of Coniferous and Broadleaved Trees

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Abstract: The relationships among species and the mechanics of those relationships are very complicated in mixed forests, and allelopathy is one of the most important mediators of these relationships. The types and quantities of allelopathic chemicals are different in coniferous and broadleaved trees; studying the responses of some sensitive plants, such as certain crops, to allelopathy mediated by the leaf extracts of coniferous and broadleaved trees would be an effective technique to evaluate the relationships among species in a mixed forest. In this paper, the effects of leaf extracts from *Metasequoia glyptostroboides*, *Cedrus deodara*, *Liquidambar formosana*, *Platanus acerifolia* and a mixture of of coniferous and broadleaved trees on seed germination and seedling growth of *Sesamum indicum* at a constant concentration (50 gDW/L) were investigated using an indoor filter paper culture dish method. The test results were evaluated using the response index (RI) and the synthesis allelopathic effect index (SE). The results showed that: (1) Four kinds of leaf extracts inhibited the germination and seedling growth of *S. indicum*. The order of the allelopathic inhibitory effects were as follows: *L. formosana* > *M. glyptostroboides* > *C. deodara* > *P. acerifolia*; (2) In this experiment, the single leaf extract of the coniferous species (*M. glyptostroboides* and *C. deodara*) and the single leaf extract of the broadleaved species (*L. formosana* and *P. acerifolia*) did not exhibit significant differences in the direction of the allelopathic effect on *S. indicum*; (3) Compared to their corresponding single leaf extracts, *M. glyptostroboides* + *P. acerifolia* and *C. deodara* + *P. acerifolia* mixed leaf extracts promoted the allelopathic effects of *S. indicum*, namely, the coniferous-broadleaved mixed leaf extract > one single leaf extract > another single leaf extract. The rest of the coniferous-broadleaved mixed leaf extract treatment groups neutralized the allelopathic effects of *S. indicum*, namely, one single leaf extract > coniferous leaf mixed extract > another single leaf extract. The conclusions could provide a scientific basis for managing forests, especially mixed forest ecosystems.

Keywords: leaf aqueous extract; coniferous; broadleaved; *Sesamum indicum*; germination; seedling growth; allelopathy

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1. Introduction

The types of forest ecosystems are complex and diverse, with evergreen forests, deciduous forests, coniferous forests, broadleaved forests, pure forests and mixed forests [1–3]. The study of the interspecific connectivity of plant communities in forests is helpful to understand the structure, function and status of populations in habitats, and to predict

the dynamic changes of population numbers and community succession [4]. Interspecific relationships are a comprehensive embodiment of various functions, and the mechanism mediating these relationships is complex, especially in regard to the biochemical aspects. Allelopathy is prevalent among tree species and is one of the main methods of interaction among species in a forest, it is an important direction for studying interspecific relationships [5–7]. Allelopathy was presented by Molisch in 1937, and different scholars have explained the concept differently. The definition proposed by Rice is currently widely used: the donor plant releases specific chemical substances to the external environment so that the nearby receptor flora and fauna, microorganisms and their own growth and development may be affected by the beneficial or harmful effects [8–11]. This is a very important chemical ecological phenomenon [12–15]. According to a newer definition, allelopathy encompasses any process in which plants produce secondary metabolites that can affect the growth and development of agricultural and biological systems, including microorganisms, viruses and fungi [16]. The negative relationship between phylogenetic distance and allelopathy indicates that allelopathy might contribute to coexistence of closely related species dominance of single species [17]. Plant allelopathy is one of the modes of interaction between receptor and donor plants and may exert either positive effects (e.g., for agricultural management, such as weed control, crop protection, or crop reestablishment) or negative effects (e.g., autotoxicity, soil sickness, or biological invasion) [18].

Plants biosynthesize a wide variety of allelochemicals including phenolics (simple phenolic acids, flavonoids, coumarins, and quinones), terpenoids (monoterpenes, sesquiterpenes, diterpenes, and steroids), nitrogen-containing chemicals (alkaloids, benzoxazinoids, and cyanogenic glycosides), and many other chemical families [19,20]. Plants achieve allelopathy by releasing allelopathic substances into the environment. Four ecological processes, volatilization, leaching, litter decomposition and root exudation, can bring allelochemicals into air or soil [21]. When allelochemicals contact or approach the associated plants, they directly demonstrate allelopathic action by disturbing the systems of photosynthesis, respiration, and metabolism, or indirectly affect target species by altering environmental conditions, particularly for soil physicochemical properties and microbial communities [22–24]. They cause the species composition of different trees to display certain differences [25]. Many coniferous trees have such a function; Cedar (*Cedrus deodara*) is a common green tree species in southern China that has a very wide plant area compared to other green tree species, and there are fewer types of trees, or even bare surfaces with no plants settled, in *C. deodara* forests [26,27]. The allelopathic substances released from the leaves and branches of *Larix principis-rupprechtii* are toxic to their own seed germination and they can also produce allelopathic effects in other plants that grow together [28]. Some experiments showed that the leaves and the leaf extracts of *C. obtusa* have growth inhibition, the leaves and the leaf extracts of *C. obtusa* have growth-inhibitory activity against several plant species, and the leaves potentially contain allelochemicals [29]. The Chinese fir (*Cunninghamia lanceolata*) is a subtropical coniferous tree species that has a considerable litterfall and fine roots. This species is grown widely in the subtropical areas in China and covers around a quarter of the plantation area. However, there is a regeneration failure and productivity decline in successive plantations [30,31]. It has been reported that the roots of Chinese fir release into the soil environment the allelochemical cyclic dipeptide 31, (6-hydroxy-1,3-dimethyl-8-nonadecyl-[1,4]-diazocane-2,5-diketone), which causes self-growth inhibition and thereby regeneration failure and productivity decline. This cyclic dipeptide has also been found in leaf litter and soils, in which its concentration was higher in replanted Chinese fir plantations. A substantial number of secondary metabolites such as terpenoids, phenolics, cinnamic acids, carboxylic acids, fatty acids, and flavonoids have been identified in pine needles and roots, litter and soil

under pine trees. The evidence also suggests that some of these compounds are probably released into the soil through the decomposition of the plant litter, and into the surrounding environment as volatiles. The most active compounds found in the pine soil were methyl 15-hydroxy-7-oxodehydroabietate and 7-oxodehydroabietic acid; both compounds may also be formed through the degradation of resin acids, which were found abundantly in pine trees. Bioactive compounds released into the soil and surrounding environment possibly act as allelochemicals and suppress the invasion of undergrowth plants into the forests, resulting in the establishment of the sparse understory vegetation [32].

Broadleaved trees can also release allelopathic substances into the surrounding environment and affect the growth and development of other plants to improve their own ecological suitability. Liu [33] found that the inhibitor is a low molecular weight phenolic compound through the analysis of the inhibitory effects of a shrub (*Cistus ladanifer*) on the germination of herbaceous plants. When the *Ailanthus altissima* was introduced to the United States, it was found to have a strong inhibitory effect on other plants [34]. *Eucalyptus robusta* is a strong competitive tree species, and the secretion of these chemical substances also exhibit a strong inhibition on other species [35]. Under natural conditions, the vegetation of eucalyptus under the canopy is relatively scarce and soil erosion is severe [36]. For example, when Citriodora (*Eucalyptus citriodora*) and Litchi (*Litchi chinensis*) are planted in mixed forests, a large number of litchis will die out 3 years later. Volatile substances from Citriodora leaves can strongly inhibit the germination of radish (*Raphanus sativus*) seeds [35,37].

Forests in nature are dominated by mixed forests, and the allelopathy of tree species in mixed forests is more complex [38]. The allelochemicals released from mixed plant root exudates serve as crucial regulators of facilitate interspecific interactions [39]. First, there are many sources of allelopathy, which may be from both coniferous and broadleaved trees, such as the broadleaved Birch (*Betula platyphylla*) and some extracts of the leaves of coniferous species of Larch (*Larix gmelinii*), appear to promote germination. However, some extracts of larch leaves showed inhibitory effects on the seeds of *B. platyphylla* [40]. Due to the different leaf falling times, the proportion of deciduous leaves often varies with the seasons, so it is more difficult to study. Wu [40] studied the effects of larch litter on the growth of *Fraxinus mandshurica* in mixed forests of *L. gmelinii—F. mandshurica*. Yang [41] studied the allelopathic effect between *L. gmelinii* and *Juglans mandshurica*. The contents of allelopathic substances released by the leaves of trees of different species are different, and coniferous trees are similar to *C. deodara* while the broadleaved trees are similar to Maples (*Liquidambar formosana*); these differences in chemical composition cause them to manifest allelopathy differently, as either promotion or inhibition [42,43]. *M. glyptostroboides* has survived under conditions of tremendous geological, climate, and ecological changes, and countless attacks of pathogens and herbivores from generation to generation [44]. It may be possible *M.glyptostroboides* possesses allelopathic activity and substances because of the longevity of the species. In the present research, we have found that the extract of fallen leaves of *M. glyptostroboides* showed allelopathic activity [45]. Besides, research shows that *Platanus acerifolia* has a Strong growth inhibition of lettuce seedlings [46].

In summary, these 4 kinds of plants (*C. deodara*, *Metasequoia glyptostroboides*, *L. formosana*, and *Platanus acerifolia*) are suitable for studying the interactions between coniferous forests, broad-leaved forests, and mixed forests.

This study evaluates the effects of allelopathic substances from different species on germination and seedling growth in relatively sensitive plant seeds. A single leaf extract of the same concentration from 4 kinds of plants (*C. deodara*, *Metasequoia glyptostroboides*, *L. formosana*, and *Platanus acerifolia*) was used to treat *Sesamum indicum* seeds and to evaluate the effects of different leaf extracts on *S. indicum* seed germination and seedling growth

according to the germination rate, germination index, seedling height, root length, and fresh weight, etc. Furthermore, there are two kinds of coniferous species and two kinds of broadleaved species, so the allelopathy among tree species could be compared by the effect of mixed coniferous-broadleaved leaf extracts. At the same time, the mixed coniferous-broadleaved extracts of 4 species of trees of the same concentration were used to treat *S. indicum* seeds and were compared to the corresponding single leaf extraction treatment group by the seed germination and the corresponding physiological index of seedling growth. We first hypothesized that four leaf extracts inhibited rice germination and seedling growth and compared to single leaf extracts, mixed leaf extracts promotes *S. indicum* allelopathic effect more. Through simulation of the allelopathy of coniferous-broadleaved mixed forests, this paper discusses the difference of allelopathy between mixed forests and pure forests and has some significance in studying interspecific relationships and ecosystem structure.

2. Materials and Methods

2.1. Target Plant

S. indicum was selected as the target plant. *S. indicum* belongs to the genus Sesamum, family Pedaliaceae, which is high-quality crop and is often planted in China. *S. indicum* contains a large amount of fat and protein and is rich in vitamin A, vitamin E, lecithin, calcium, iron, chromium and other nutrients. Functions of *S. indicum* could include protecting the stomach and liver, promoting the growth of red blood cell, and increasing systemic melanin, among other functions. Due to the small seeds and shallow roots, it is suitable for loose and weakly acidic to neutral soils (pH 6.5–7.5). *S. indicum* seeds have strict requirements for temperature, moisture, light, and soil conditions, and are sensitive to changes in the growth environment, making them excellent experimental materials. The loose soil can coordinate water, fertilizer, and air supply to support the extension of the root system. *S. indicum* is an environmentally sensitive plant, so it is a good material for the study of allelopathy, especially the effects of coniferous, broadleaved and mixed aqueous extracts. *S. indicum* is a widely cultivated economic crop, and research on its growing environment has agricultural application value. Although *S. indicum* does not naturally grow in forests, in some agroforestry systems, it may be planted in mixed cultivation (or adjacent planting) with trees. Research on the allelopathic effects on coniferous and broad-leaved trees on sesame can provide guidance for agricultural planting practices.

2.2. Methods

2.2.1. Allelopathic Sources—Preparation of Leaf Aqueous Extracts

C. deodara, *M. glyptostroboides*, *L. formosana* and *P. acerifolia* were selected as the 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 under shade, and were packed in a woven bag placed in a dry and ventilated place to save for later use. Before the experiment, the plant blades were cut with medical scissors, and a blades grinder was used to crush the leaves into powder, each weighing 37.5 g, that was divided into the following two kinds of treatment: a single leaf extract and a coniferous-broadleaved mixed leaf extract.

For the single leaf extract treatment, leaf powder was weighed out as follows: *M. glyptostroboides* 25 g, *C. deodara* 25 g, *L. formosana* 25 g, *P. acerifolia* 25 g. For the coniferous-broadleaved mixed leaf extract treatment, leaf powder was weighed out as follows: *M. glyptostroboides* 12.5 g + *L. formosana* 12.5 g, *M. glyptostroboides* 12.5 g + *P. acerifolia* 12.5 g, *C. deodara* 12.5 g + *L. formosana* 12.5 g, *C. deodara* 12.5 g + *P. acerifolia* 12.5 g. After weighing, each treatment was put into a 500 mL conical bottle to which 500 mL distilled water was added, and the samples were soaked for 48 h (vibrating once every 6 h during

this period). The extracts were kept static for 3 h then were coarse filtered using two-layer gauze prior to filtering with filter paper. A volume of 500 mL containing 25 g dry matter content per liter (50 gDW/L) for the 4 different proportion of leaf extracts was prepared. The treatments were sealed and kept at 4 °C until use.

2.2.2. Facility Preparation

Necessary equipment included: Petri dish (d: 90 mm), 27; filter paper, 54; 500 mL conical bottle, 9; blade grinder; medical scissors; electronic scales; cylinder; glass rods; and so on.

At the bottom of the 90 mm-diameter petri dish, two pieces of filter paper of appropriate size were placed as padding. 1755 *S. indicum* seeds with plump grain and consistent size were soaked in a solution of 0.02% $KMnO_4$ for 10 min for disinfection, then the seeds were cleaned with distilled water 3 times.

2.2.3. Seed Germination

65 *S. indicum* seeds were evenly placed in each petri dish, and the 8 groups of leaf extracts were removed from the refrigerator and brought to room temperature before adding 10 mL to each petri dish; 10 mL distilled water was added to the control group (CK). Each treatment was replicated 3 times.

In this experiment, the indoor filter paper culture dish method was used to study the specific concentration of leaf extract, so as to control other environmental variables and reduce the impact of competition effect.

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

2.2.4. Measurement of Germination Rate and Germination Index

The number of seeds germinated was counted daily from the date of immersion to the time when the seeds no longer germinated. The germination rate (GR) (Formula (1)) and germination index (Gi) (Formula (2)) were calculated and the germination curves were plotted. Here, seed germination is considered as the embryo root reaching 1 mm outside the seed coat; the seed is no longer germinating if there is no embryo root change for 5 consecutive days.

$$GR = (n/N) \times 100\%, \quad (1)$$

where n is the number of seeds geminated, and N is the total number of seeds to be tested.

$$Gi = \Sigma(Gt/Dt), \quad (2)$$

where Gt is number of germinated seeds at the t-th day, and Dt is the number of days from the germination experiment.

2.2.5. Measurement of the Root Length, Height, and Fresh Weight of Seedling

When the seeds were no longer sprouting, they were taken out of the petri dish, the water was drained by absorbent paper, and 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 electronic balance and recorded.

2.2.6. Allelopathic Response Index and Synthesis Effect Index

The allelopathic response index (RI) is adopted to measure the type and intensity of allelopathy, as described by Formulae (3) and (4) (Williamson and Richardson, 1988).

$$RI = 1 - C/T \ (T \geq C), \quad (3)$$

$$RI = T/C - 1 \ (T < C), \quad (4)$$

where C is the control value and T is the treatment value. RI < 0 represents inhibition, 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 the plants are different, with some allelopathic substances inhibiting root growth but not height, and they may even promote seedling growth. Therefore, the allelopathic synthesis effect index (SE) reflects synthetic effects of allelochemicals on plants as displayed by Formula (5) (Yang et al., 2010).

$$SE = (RI_{Gr} + RI_{Gi} + RI_{Hs} + RI_{Rl} + RI_{Fw})/5, \quad (5)$$

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

2.3. 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 the TukeyHSD test methods were conducted by SPSS 17.0, the difference between each treatment group was analyzed, the significance level was $\alpha = 0.05$ and the statistical significance was evaluated at $p < 0.05$ level. According to the data analysis results, there is a significant difference between the group. The fitting of logistic curves was carried out using Origin 8.0.

3. Results

3.1. Allelopathy of Different Leaf Extracts to Germination of S. indicum Seeds

3.1.1. Seed Germination Rate

The order of germination rates of *S. indicum* seeds after treatment with the 4 kinds of single leaf extract solutions was as follows: *P. acerifolia* (81.03%) > CK (78.97%) > *C. deodara* (67.69%) > *M. glyptostroboides* (60.51%) > *L. formosana* (34.36%) (Table 1). Compared to the control group, the treatments showed inhibition of the on germination rate of the *S. indicum* seeds, except for the *P. acerifolia* leaf extract (promotion); the order is as follows: *M. glyptostroboides* > *C. deodara* > *L. formosana*.

There is no significant difference between coniferous trees (*M. glyptostroboides* and *C. deodara*) and broadleaved trees (*L. formosana* and *P. acerifolia*) for allelopathy to germination rate of *S. indicum* seeds.

The intensity of allelopathy of all other mixed coniferous-broadleaved leaf extracts on the germination rate RI of *P. alopecuroides* displayed neutralization, namely, one single leaf extract > coniferous-broadleaved mixed extracts > another single leaf extract (Table 1).

3.1.2. Seed Germination Index

The order of the germination index of *S. indicum* seeds after treatment with the 4 kinds of single leaf extract solutions was as follows: CK (31.66) > *P. acerifolia* (20.88) > *C. deodara* (18.83) > *M. glyptostroboides* (13.65) > *L. formosana* (6.90) (Table 1). Compared to the control

group, the treatment of *S. indicum* seeds produced an inhibition of the germination index, and the order is: *L. formosana* > *M. glyptostroboides* > *C. deodara* > *P. acerifolia*.

The intensity of allelopathy of all mixed coniferous-broadleaved leaf extracts on the germination index RI of *S. indicum* are displayed neutralization (Table 1).

Table 1. Effects of the aqueous extracts on the germination of *Sesamum indicum* (mean ± SD, n = 3).

Treatment	Germination Rate (%)	Germination Index
Control	78.97 ± 3.20 de	31.66 ± 2.08 g
Metasequoia glyptostroboides (coniferous)	60.51 ± 1.78 bc	13.65 ± 1.15 bc
Cedrus deodara (coniferous)	67.69 ± 6.71 cd	18.83 ± 4.36 def
Liquidambar formosana (broadleaved)	34.36 ± 2.35 a	6.90 ± 1.53 a
Platanus acerifolia (broadleaved)	81.03 ± 3.20 e	20.88 ± 2.08 f
Metasequoia glyptostroboides + *Liquidambar formosana*	52.31 ± 4.07 b	10.56 ± 2.83 ab
Metasequoia glyptostroboides + *Platanus acerifolia*	68.21 ± 2.35 cde	15.56 ± 1.53 cde
Cedrus deodara + *Liquidambar formosana*	55.90 ± 6.94 bc	15.46 ± 4.51 cd
Cedrus deodara + *Platanus acerifolia*	76.92 ± 5.33 de	19.46 ± 3.46 ef

Note: The data with different letters in the same column represent significant differences at $p < 0.05$.

3.1.3. Seed Germination Process

The process of seed germination, such as other biological processes, is a logarithmic growth process illustrated by a logistic curve (Figure 1). The effects of different single leaf extracts on the *S. indicum* seed germination process are as follows: the seeds of the control group and the experimental group began to germinate on the 3rd day after sowing; after the 15th day of the monitoring period, the germination rate was gradually slowed.

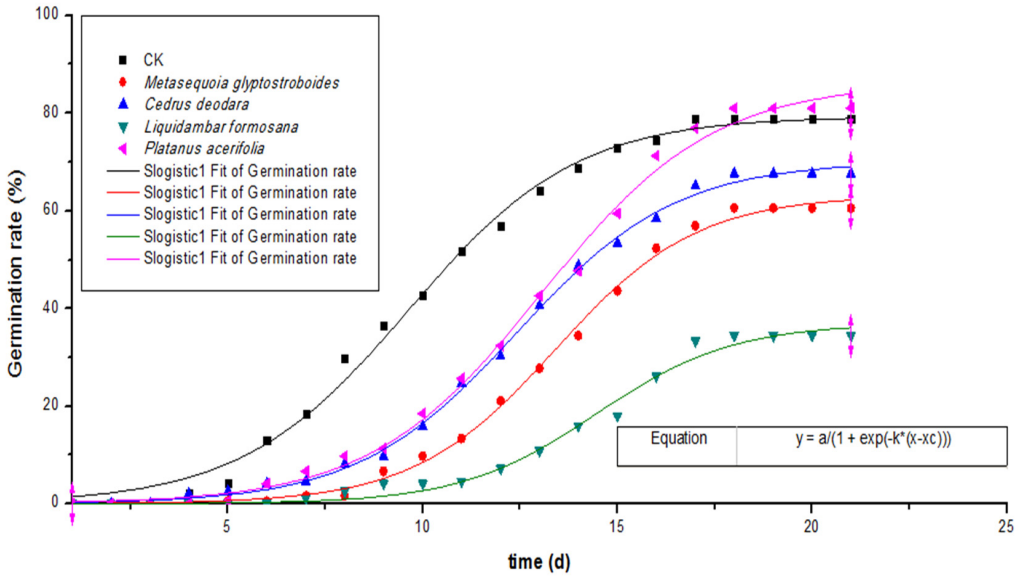


Figure 1. Effects of leaf extracts from different leaves on the germination process of *Sesamum indicum*.

During the process of seed germination (Figure 1), the treatment of *P. acerifolia* leaf extracts on *P. alopecuroides* seeds showed little promotion of germination rate and germination speed. The treatment of *S. indicum* seeds with *L. formosana* and *M. glyptostroboides* produced significant inhibition.

Logistic curves of germination rate are as follows:

$$\text{Control}: \ y = \frac{79.33}{1 + e^{-0.46(x-9.64)}} \quad (R^2 = 0.9952)$$

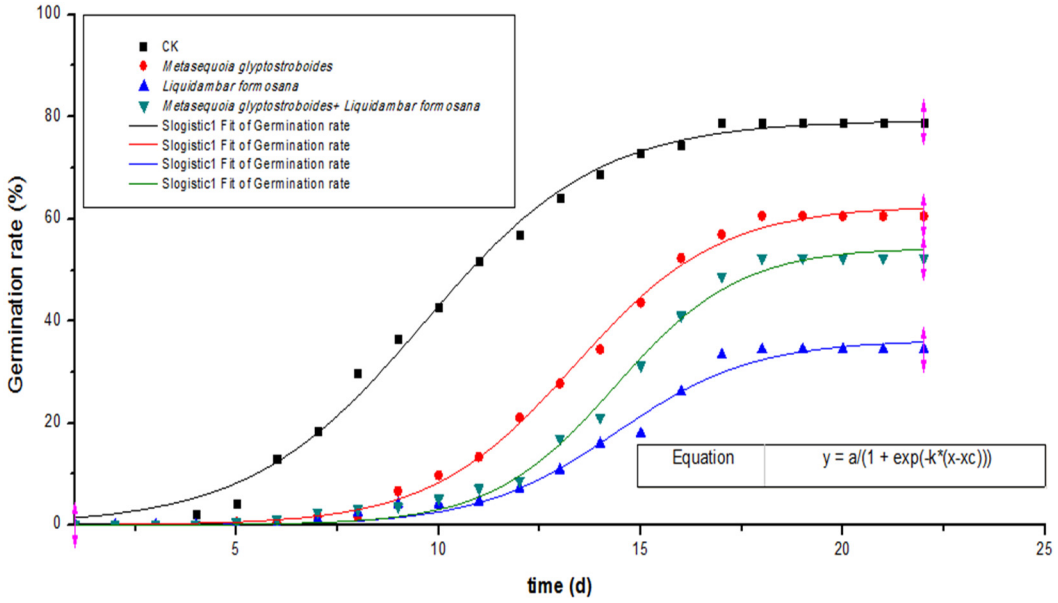
$$M.\ glyptostroboides:\ y = \frac{62.58}{1 + e^{-0.56(x-13.36)}}\ (R^2 = 0.9976)$$

$$C.\ deodara:\ y = \frac{69.62}{1 + e^{-0.49(x-12.38)}}\ (R^2 = 0.9983)$$

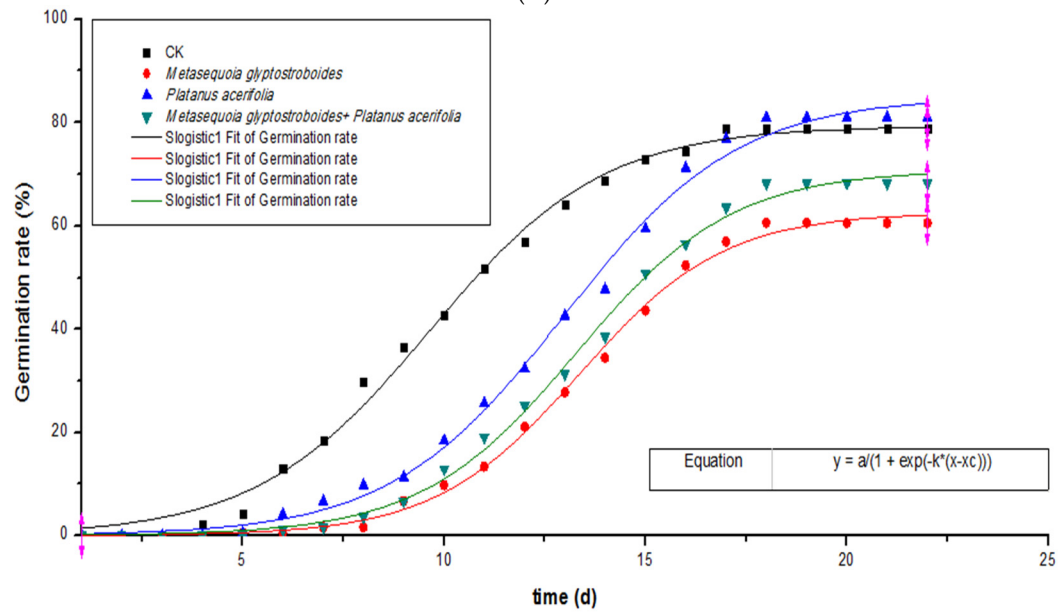
$$L.\ formosana:\ y = \frac{36.24}{1 + e^{-0.59(x-14.38)}}\ (R^2 = 0.9980)$$

$$P.\ acerifolia:\ y = \frac{85.02}{1 + e^{-0.46(x-12.97)}}\ (R^2 = 0.9959)$$

The effects of the extract on a single kind of leaf and its conifer-broadleaved mixed extract on *S. indicum* were obviously different (Figure 2). On the whole, the effect of coniferous-broadleaved mixed extract on *P. alopecuroides* was neutralization.

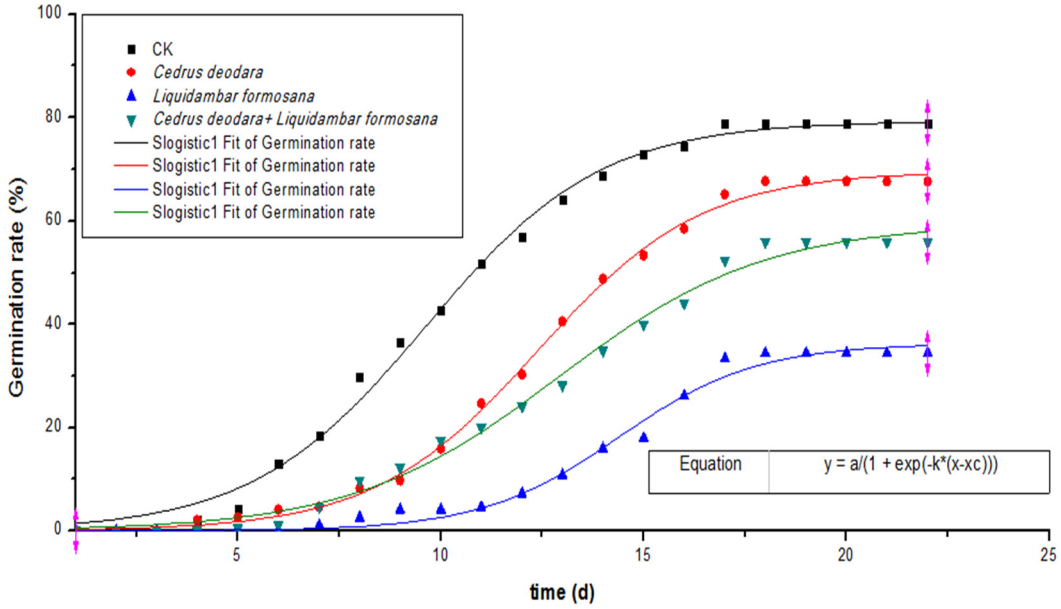


(**A**)

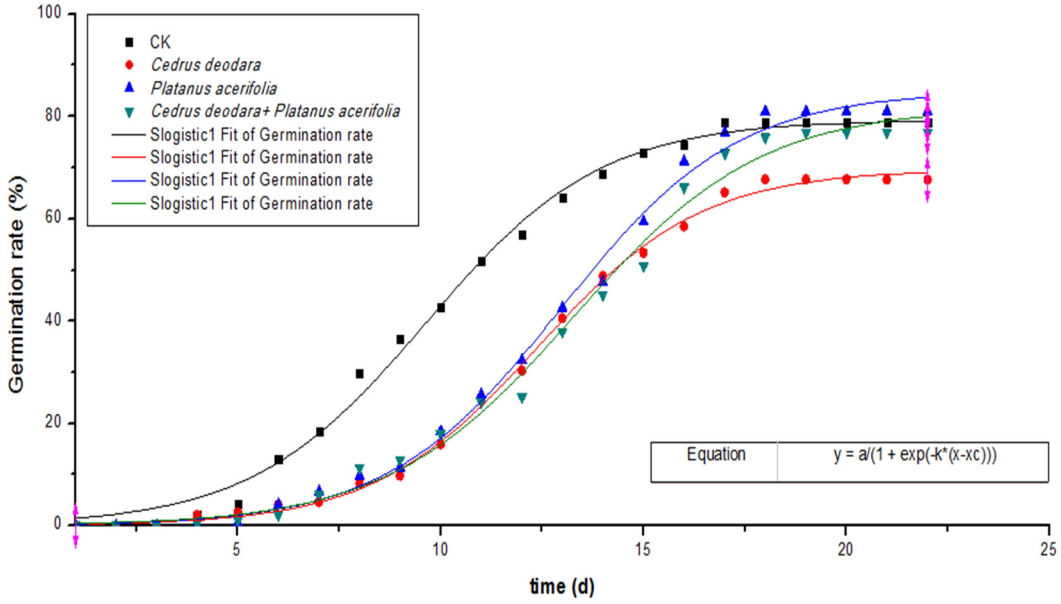


(**B**)

Figure 2. *Cont.*



(**C**)



(**D**)

Figure 2. (**A**–**D**) Effects of single leaf extracts and coniferous-broadleaved mixed leaf extracts on the germination process of *Sesamum indicum*.

3.2. The Effects of Different Leaf Extracts on Seedling Growth of S. indicum

3.2.1. Seedling Height

The order of height of *S. indicum* seedlings after treatment with 4 kinds of single leaf extract solutions was as follows: CK (1.70) > *P. acerifolia* (1.65) > *M. glyptostroboides* (1.45) > *L. formosana* (1.35) > *C. deodara* (1.21) (Table 2). Compared to the control group, all of the treatment groups showed inhibition, and the order of inhibition intensity is: *C. deodara* > *L. formosana* > *M. glyptostroboides* > *P. acerifolia*.

The intensity of allelopathy of all mixed coniferous-broadleaved leaf extracts on seedling height of *S. indicum* exhibited neutralization.

3.2.2. Root Length

The order of root lengths of *S. indicum* seedlings after treatment with 4 kinds of single leaf extract solutions was as follows: CK (1.99) > *P. acerifolia* (1.44) > *M. glyptostroboides* (1.38) > *C. deodara* (1.30) > *L. formosana* (1.25) (Table 2). The treatments showed inhibition on the root length of *P. alopecuroides* seedlings, and the order is: *L. formosana* > *C. deodara* > *M. glyptostroboides* > *P. acerifolia*.

There is a significant difference between *L. formosana* and the other treatments. The intensity of allelopathy of mixed leaf extracts on root length of *S. indicum* showed promotion.

3.2.3. Fresh Weight

The order of fresh weights of *S. indicum* seedlings after treatment with 4 kinds of single leaf extract solutions was as follows: CK (1.46) > *P. acerifolia* (1.38) > *M. glyptostroboides* (1.13) = *C. deodara* (1.13) > *L. formosana* (0.77) (Table 2). The treatments showed inhibition on the fresh weight of *S. indicum* seedlings, and the order is: *L. formosana* > *C. deodara* = *M. glyptostroboides* > *P. acerifolia*. The intensity of allelopathy of *C. deodara* + *P. acerifolia* mixed leaf extracts on the fresh weight of *S. indicum* showed promotion. In addition, the intensity of allelopathy of the other mixed coniferous-broadleaved leaf extracts on the fresh weight of *S. indicum* showed neutralization.

Table 2. Response of growth indexes of *Sesamum indicum* seedlings to different aqueous extracts (mean ± SD, n = 6).

Treatment	Seedling Height (cm)	Root Length (cm)	Fresh Weight (g)
Control	1.70 ± 0.65 a	1.99 ± 0.35 b	1.46 ± 0.06 d
Metasequoia glyptostroboides	1.45 ± 0.08 a	1.38 ± 0.31 ab	1.13 ± 0.07 bcd
Cedrus deodara	1.21 ± 0.56 a	1.30 ± 0.77 ab	1.13 ± 0.14 bcd
Liquidambar formosana	1.35 ± 0.31 a	1.25 ± 1.30 ab	0.77 ± 0.22 a
Platanus acerifolia	1.65 ± 0.26 a	1.44 ± 2.05 ab	1.38 ± 0.14 cd
Metasequoia glyptostroboides + *Liquidambar formosana*	1.36 ± 0.15 a	1.19 ± 0.12 b	1.06 ± 0.05 abc
Metasequoia glyptostroboides + *Platanus acerifolia*	1.11 ± 0.30 a	0.99 ± 0.44 b	0.81 ± 0.07 ab
Cedrus deodara + *Liquidambar formosana*	1.22 ± 0.47 a	1.00 ± 0.31 b	0.95 ± 0.11 ab
Cedrus deodara + *Platanus acerifolia*	1.36 ± 0.43 a	1.02 ± 0.20 b	0.92 ± 0.14 ab

Note: The data with different letters in the same column represent significant differences at $p < 0.05$.

3.3. Synthesis Effect Index of S. indicum After Treatment of Leaf Extracts

The RI and SE of germination rates and germination indexes of *S. indicum* treated by *M. glyptostroboides*, *C. deodara* and *L. formosana* leaf extracts are less than zero, which indicate that their allelopathy to *S. indicum* is inhibitory, and the order of inhibition is *L. formosana* > *M. glyptostroboides* > *C. deodara* > *P. acerifolia* (Table 3).

There is no significant difference between coniferous trees (*M. glyptostroboides* and *C. deodara*) and broadleaved trees (*L. formosana* and *P. acerifolia*) for allelopathy to *S. indicum* seed germination and seedling growth.

The intensity of allelopathy of mixed extracts of *M. glyptostroboides* + *P. acerifolia*, *C. deodara* + *P. acerifolia* to *S. indicum* exhibited promotion and that of *M. glyptostroboides* + *L. formosana*, *C. deodara* + *L. formosana* to *S. indicum* exhibited neutralization.

Table 3. Allelopathic response index (RI) and allelopathic synthesis effect index (SE) of *Sesamum indicum*.

Treatment	Allelopathic Response Index (RI)					Synthesis Effect (SE)
	Germination Rate	Germination Index	Seedling Height	Root Length	Fresh Weight	
Control	-	-	-	-	-	-
Metasequoia glyptostroboides	−0.31	−1.32	−0.17	−0.44	−0.29	−0.51
Cedrus deodara	−0.17	−0.68	−0.41	−0.52	−0.29	−0.41
Liquidambar formosana	−1.30	−3.59	−0.26	−0.60	−0.89	−1.33
Platanus acerifolia	0.03	−0.52	−0.03	−0.38	−0.06	−0.19
Metasequoia glyptostroboides + *Liquidambar formosana*	−0.51	−2.00	−0.25	−0.67	−0.38	−0.76
Metasequoia glyptostroboides + *Platanus acerifolia*	−0.16	−1.03	−0.53	−1.01	−0.81	−0.71
Cedrus deodara + *Liquidambar formosana*	−0.41	−1.05	−0.39	−0.99	−0.53	−0.67
Cedrus deodara + *Platanus acerifolia*	−0.03	−0.63	−0.25	−0.95	−0.58	−0.49

4. Discussion

It was found that single leaf extracts of *M. glyptostroboides* and *C. deodara* have inhibitory effects on the growth of other plants [47]. In this experiment, the effects of single leaf extracts of 50 gDW/L *M. glyptostroboides*, *C. deodara*, *L. formosana* and *S. indicum* on *P. alopecuroides* were processed, and the corresponding physiological indices of seed germination and seedling growth were analyzed. It was found that under this concentration, the 4 kinds of plant leaf extracts have different allelopathy on *S. indicum*.

The allelopathy of 4 leaf extracts on *S. indicum* was not correlated with the leaf type (coniferous or broadleaved). The effect of plant allelopathic can be either positive or negative on the growth of the surrounding plants [48]. The effects of plant allelopathic substances on seeds can also divided into these two aspects. On one hand, most allelopathic substances can inhibit seed germination and seedling growth [49], and on the other hand, partial allelopathy can also promote seed germination and seedling growth [50]. In addition, allelopathic substances often reflect the phenomenon of "low promotion and high inhibition", that is, allelochemicals released by donor plants exerted positive effects on recipient plants at low concentrations, but were phytotoxic above certain threshold doses [51,52]. At the same time, there is a great relationship with different types of allelopathy substances and the species of receptor plants [35,53]. In this experiment, the coniferous trees *M. glyptostroboides* and *C. deodara* and the broadleaved tree *L. formosana* showed an inhibitory effect on *S. indicum*, while the broadleaved tree *P. acerifolia* showed a promoting effect on *S. indicum*. At this concentration, the effects of the 4 kinds of leaf extracts on *S. indicum* were not correlated with the classifications of coniferous and broadleaved.

Leaves differ in nature, as seen with conifers and broadleaved trees, and they contain different chemical substances. The allelopathy of the mixture of different substances on the receptors can be divided into three types: synergism, antagonism, and neutralism [28]. However, there have been only a few studies on the classification of the allelopathy of receptor plants in different leaf extracts. In this experiment, mixed coniferous-broadleaved leaf aqueous extracts of 50 gDW/L were used to treat *S. indicum* and were compared to the corresponding single leaf extract according to seed germination and the corresponding physiological index of seedling growth. The results showed that the effects of the treatment group of the coniferous-broadleaved mixed extraction solutions of *M. glyptostroboides* + *P. acerifolia* and *C. deodara* + *P. acerifolia* promoted *S. indicum*, namely, a coniferous-broadleaved mixed extract > a single leaf extract > and another single leaf extract. In addition, the effects of the treatment group of coniferous-broadleaved mixed extraction solutions of *M. glyptostroboides* + *L. formosana* and *C. deodara* + *L. formosana* were neutral to *S. indicum*, namely, a single leaf extract > a coniferous-broadleaved mixed extract > another single leaf extract. However,

whether this phenomenon is a result of allelopathic synergism, or allelopathic antagonism of different leaf extracts has yet to be validated [54].

The concentrations of allelopathic chemicals are different, and the intensities of allelopathy are different. In this study, in both single leaf extracts and coniferous-broadleaved mixed leaf extracts, the concentration was 50 gDW/L. At this concentration, the allelopathy of the various leaf extracts of the solution on *S. indicum* was different, revealing the phenomenon of "low promotion and high inhibition". It is necessary to select different concentrations of allelopathy sources, or different proportions of the mixed extracts of coniferous and broadleaved leaves as a source of allelopathy, this is our follow-up research direction.

5. Conclusions

In this paper, the interactions between coniferous forests, broad-leaved forests and mixed forests were explored from the perspective of allelopathy. In terms of agricultural production, the normal germination and emergence of seeds have a great impact on crop yield. In addition, the effects of leaf extracts from *Metasequoia glyptostroboides*, *Cedrus deodara*, *Liquidambar formosana*, *Platanus acerifolia* and a mixture of coniferous and broadleaved trees on seed germination and seedling growth of *Sesamum indicum* at a constant concentration (50 gDW/L) were investigated using an indoor filter paper culture dish method.

The test results were evaluated using the response index (RI) and the synthesis allelopathic effect index (SE). The results showed that: (1) Four kinds of leaf extracts inhibited the germination and seedling growth of *S. indicum*. The order of the allelopathic inhibitory effects were as follows: *L. formosana* > *M. glyptostroboides* > *C. deodara* > *P. acerifolia*; (2) In this experiment, the single leaf extract of the coniferous species (*M. glyptostroboides* and *C. deodara*) and the single leaf extract of the broadleaved species (*L. formosana* and *P. acerifolia*) did not exhibit significant differences in the direction of the allelopathic effect on *S. indicum*; (3) Compared to their corresponding single leaf extracts, *M. glyptostroboides* + *P. acerifolia* and *C. deodara* + *P. acerifolia* mixed leaf extracts promoted the allelopathic effects on *S. indicum*, namely, the coniferous-broadleaved mixed leaf extract > one single leaf extract > another single leaf extract. The rest of the coniferous-broadleaved mixed leaf extract treatment groups neutralized the allelopathic effects on *S. indicum*, namely, one single leaf extract > coniferous leaf mixed extract > another single leaf extract. The conclusions could provide a scientific basis for managing forests, especially mixed forest ecosystems.

According to the experimental results, allelopathy can promote species competition and coexistence. And the results of this experiment can be applied to the construction of mixed forests. When selecting tree species for creating artificial mixed forests, special attention should be paid to the influence of allelopathy among tree species.

Different leaf extracts had different allelopathic effects on *Artemisia annua*, showing the phenomenon of "low promotion and high inhibition". This phenomenon can be used to improve the germination rate and seedling formation rate of crops. This result can also be used to inhibit the growth of weed seeds. To ensure sustainable agricultural development, it is important to exploit cultivation systems that take advantage of the stimulatory/inhibitory influence of allelopathic plants to regulate plant growth and development and to avoid allelopathic autotoxicity.

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