Postprint: Allelopathic Effects of Aqueous Leaf Extracts from Six Broad-Leaved Tree Species on Seed Germination and Growth of Pinus yunnanensis

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Abstract

To select tree species for establishing Yunnan pine insect-resistant mixed forests, this study examined the allelopathic effects of aqueous leaf extracts at various concentrations from six broad-leaved tree species that are non-hosts of Tomicus yunnanensis—Alnus ferdinandi-coburgii, Michelia champaca, Celtis yunnanensis, Cinnamomum camphora, Quercus acutissima, and Camellia japonica—on Yunnan pine seed germination and seedling growth. The results demonstrated that leaf extracts from Alnus ferdinandi-coburgii, Celtis yunnanensis, Quercus acutissima, and Camellia japonica exhibited stimulatory effects at low concentrations and inhibitory effects at high concentrations on Yunnan pine seed germination and seedling growth. Within the tested concentration range, leaf extracts of Cinnamomum camphora and Michelia champaca consistently inhibited Yunnan pine seed germination and seedling growth. In summary, the six broad-leaved species exhibited differential allelopathic sensitivities toward Yunnan pine; aqueous leaf extracts of Alnus ferdinandi-coburgii, Celtis yunnanensis, Quercus acutissima, and Camellia japonica at low concentrations promoted Yunnan pine growth, whereas those of Cinnamomum camphora and Michelia champaca were inhibitory. Therefore, integrated with appropriate silvicultural techniques, broad-leaved species such as Alnus ferdinandi-coburgii, Celtis yunnanensis, and Quercus acutissima may be selected for interplanting with Yunnan pine to establish insect-resistant mixed forests.

Full Text

Allelopathic Effects of Leaf Aqueous Extracts from Six Broad-Leaved Tree Species on Seed Germination and Seedling Growth of *Pinus yunnanensis*

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Abstract: To screen suitable tree species for constructing insect-resistant mixed forests of *Pinus yunnanensis*, this study investigated the allelopathic effects of aqueous leaf extracts from six broad-leaved non-host species of *Tomicus yunnanensis*—*Alnus ferdinandi-coburgii*, *Michelia alba*, *Celtis kunmingensis*, *Cinnamomum camphora*, *Quercus acutissima*, and *Camellia japonica*—on seed germination and seedling growth of *P. yunnanensis* at different concentrations. The results demonstrated that extracts from *A. ferdinandi-coburgii*, *C. kunmingensis*, *Q. acutissima*, and *C. japonica* exhibited a "low-concentration promotion, high-concentration inhibition" effect on both seed germination and seedling growth. Within the tested concentration range, extracts from *C. camphora* and *M. alba* consistently showed inhibitory effects. In conclusion, the six broad-leaved species displayed varying sensitivities in their allelopathic effects on *P. yunnanensis*. At low concentrations, aqueous extracts from *A. ferdinandi-coburgii*, *C. kunmingensis*, *Q. acutissima*, and *C. japonica* promoted *P. yunnanensis* growth, whereas extracts from *C. camphora* and *M. alba* were inhibitory. Therefore, integrated with silvicultural techniques, species such as *A. ferdinandi-coburgii*, *C. kunmingensis*, and *Q. acutissima* are recommended for establishing insect-resistant mixed forests with *P. yunnanensis*.

Keywords: aqueous extract; allelopathic effect; *Pinus yunnanensis*; seed germination; seedling growth

Introduction

Pinus yunnanensis is an endemic species distributed across Yunnan, Sichuan, Tibet, and Guangxi provinces in China, characterized by drought tolerance, broad ecological adaptability, and strong natural regeneration capacity (Yang et al., 2009; Chen et al., 2012). As a pioneer species for afforestation in Yunnan Province, it accounts for 52% of the province's forested area and 32% of forest stock volume, playing an irreplaceable role in maintaining regional ecological security (Wang et al., 2018). However, *P. yunnanensis* forests are predominantly pure stands with extensive planting areas and single species composition, making them vulnerable to pest infestations (Chai et al., 2002; Wang et al., 2013; Liu et al., 2014). Among these pests, the Yunnan pine shoot beetle (*Tomicus yunnanensis*) is particularly destructive, causing the death of approximately 75,000 hectares of healthy *P. yunnanensis* stands annually (Ma et al., 2016). Current control measures primarily include manual removal of infested trees, chemical pesticide application, and biological control, but none have proven effective in mitigating the beetle's damage (Lu, 2008). Research by Li et al. (2005) indicated that establishing mixed forests could help resist pest damage, while Yue (2011, 2013) demonstrated that mixed forests effectively control *T. yunnanensis* infestations. Zhou (2012) identified six broad-leaved species—*Alnus ferdinandi-coburgii*, *Michelia alba*, *Celtis kunmingensis*, *Cinnamomum camphora*, *Quercus acutissima*, and *Camellia japonica*—that exhibit repellent effects against *T. yunnanensis* and could be used to construct insect-resistant mixed forests.

The establishment of insect-resistant mixed forests is a complex system regulated by multiple factors including species selection, mixing patterns, ratios, and cultivation measures. Species selection is a critical step that must consider both afforestation objectives and interspecific interactions, particularly allelopathic effects. Allelopathy, the chemical interaction whereby plants release autogenic compounds into the environment, is widespread in nature and holds significant importance for weed control, pest management, vegetation restoration, and mixed forest species selection (Bais et al., 2003; Li et al., 2006; Li et al., 2017; Qian et al., 2019). Numerous studies on plant aqueous extracts have demonstrated that allelopathic effects on seed germination and seedling growth typically follow a "low-promotion, high-inhibition" pattern, with low-concentration stimulation often exceeding high-concentration inhibition in magnitude (Young & Bush, 2009; Guo et al., 2018; Puig et al., 2018). However, few reports have addressed allelopathic effects on *P. yunnanensis* seed germination and seedling growth. Zhu et al. (2009) found that low-concentration salicylic acid treatment improved germination rates and promoted seedling growth, while Cao et al. (2012) reported that gaseous volatiles from *Eupatorium adenophorum* significantly reduced *P. yunnanensis* seed germination and seedling growth at high concentrations, with inhibitory effects diminishing as concentration decreased. No studies have investigated the allelopathic effects of broad-leaved species used in mixed forest establishment on *P. yunnanensis*. Therefore, this study selected leaves from six previously screened non-host species of *T. yunnanensis* to examine their effects on *P. yunnanensis* seed germination and seedling growth, aiming to identify broad-leaved species that promote *P. yunnanensis* growth and provide a theoretical basis for constructing and transforming insect-resistant mixed forests.

Materials and Methods

1.1 Experimental Materials

Leaves of the six broad-leaved species (*A. ferdinandi-coburgii*, *M. alba*, *C. kunmingensis*, *C. camphora*, *Q. acutissima*, and *C. japonica*) were collected from ten-year-old trees in the arboretum of Southwest Forestry University. Trees with consistent height and diameter at breast height were randomly selected, and branches were cut at the same height using pole pruners. Healthy leaves were collected and transported to the laboratory in insulated containers. *Pinus yunnanensis* seeds were purchased from the Yunnan Provincial Forest Seed and Seedling Station.

1.2 Experimental Instruments

The study utilized temperature-controlled electric heating mantles, autoclaves, rotary evaporators, and artificial climate chambers.

1.3 Experimental Methods

1.3.1 Preparation of Leaf Aqueous Extracts Extract preparation followed the method of Zhang et al. (2015). Fresh leaves of the six broad-leaved species were washed with tap water and rinsed three times with distilled water. After air-drying, the leaves were crushed and passed through a 60-mesh sieve. For each species, 100 g of leaf powder was placed in a round-bottom flask and soaked in distilled water at a 1:3 mass-to-volume ratio for 48 hours. The mixture was then vacuum-filtered three times, and the filtrates were combined and diluted to 1,000 mL to obtain a stock solution with a concentration of 0.1 $g \cdot mL^{-1}$. The stock solution was further diluted to concentrations of 0.02, 0.04, 0.06, and 0.08 $g \cdot mL^{-1}$. All extracts were stored at 4 °C until use.

1.3.2 Seed Pre-treatment Healthy, uniformly sized *P. yunnanensis* seeds were selected and disinfected with 0.5% $KMnO_4$ solution for 10 minutes, then rinsed three times with sterile distilled water. The seeds were soaked in sterile distilled water for 4 hours before use.

1.3.3 Effects of Extracts on Seed Germination Petri dishes (12 cm diameter) were lined with two layers of filter paper, and 50 treated seeds were evenly placed in each dish. Six milliliters of plant extract of different species and concentrations were added to each dish, with three replicates per treatment. The control group received sterile distilled water. Dishes were incubated in an artificial climate chamber at 25 °C, 75% relative humidity, and a 12-hour photoperiod. Every three days, 2 mL of sterile distilled water or extract was added to each dish. Germination was recorded daily starting from the day of sowing. Germination energy was calculated on day 15, and the germination experiment was terminated on day 21 for final germination rate calculation.

1.3.4 Effects of Extracts on Seedling Growth Uniformly sized, plump *P. yunnanensis* seeds were selected for germination induction. Once radicles emerged, the seedling growth experiment was conducted using a pot culture method (Li & Yang, 2019). Five seedlings were planted per pot (44 cm × 29 cm) containing sterilized vermiculite as substrate. Twenty milliliters of extract at different concentrations were added to each pot, with distilled water as the control. Each treatment had three replicates. Pots were placed in an artificial climate chamber, and 15 mL of extract or distilled water was added every three days. Seedling height and biomass were measured after 28 days.

1.4 Data Processing

Seed germination indices were calculated following Shen et al. (2017). Germination energy (GE) = (number of normally germinated seeds on day 15 / total seeds tested) × 100%; Germination percentage (GP) = (total germinated seeds / total seeds tested) × 100%. The allelopathic response index (RI) was used to quantify allelopathic intensity: RI = 1 - C/T when $T \geq C$, and RI = T/C - 1 when $T < C$, where C is the control value and T is the treatment value. $RI > 0$ indicates promotion, $RI < 0$ indicates inhibition, with absolute values representing effect magnitude. Statistical analysis and significance testing were performed using SPSS 19.0.

Results

2.1 Effects of Extracts on Seed Germination

As shown in and , aqueous extracts from all six broad-leaved species affected both germination rate and germination energy of *P. yunnanensis* seeds, with both parameters decreasing as extract concentration increased. At 0.02 $g \cdot mL^{-1}$, extracts from *A. ferdinandi-coburgii*, *Q. acutissima*, *C. kunmingensis*, and *C. japonica* produced positive RI values for both germination rate and energy, indicating promotional effects. *Celtis kunmingensis* extract yielded the highest germination rate (95.70%) and germination energy (90.30%), representing increases of 12.06% and 14.02% over the control, respectively. In contrast, *C. camphora* and *M. alba* extracts showed inhibitory effects across all tested concentrations. At 0.10 $g \cdot mL^{-1}$, all treatments exhibited extremely significant inhibition compared to the control ($P < 0.01$), demonstrating that extracts from all six species suppressed *P. yunnanensis* seed germination to varying degrees at high concentrations.

2.2 Effects of Extracts on Seedling Growth

reveals that at concentrations of 0.02 and 0.04 $g \cdot mL^{-1}$, extracts from *A. ferdinandi-coburgii*, *Q. acutissima*, *C. kunmingensis*, and *C. japonica* promoted seedling growth, while *C. camphora* and *M. alba* extracts were inhibitory. At concentrations of 0.06–0.10 $g \cdot mL^{-1}$, all six species produced negative RI values for seedling height, with absolute values increasing as concentration rose, indicating progressively stronger growth inhibition. At the lowest concentration (0.02 g·mL^{-1}), *C. kunmingensis* showed the strongest promotional effect, increasing seedling height by 1.60 cm compared to the control.

2.3 Effects of Extracts on Seedling Biomass

demonstrates that *C. camphora* and *M. alba* extracts inhibited seedling biomass accumulation across all tested concentrations, with inhibition intensifying as concentration increased. At 0.02 g·mL^{-1}, extracts from *A. ferdinandi-coburgii*, *Q. acutissima*, *C. kunmingensis*, and *C. japonica* promoted biomass growth, but this effect shifted to inhibition at concentrations above 0.06 g·mL^{-1}. At 0.10 g·mL^{-1}, all six species produced extremely significant reductions in seedling biomass compared to the control ($P < 0.01$), indicating strong inhibitory effects.

Effects of Different Concentrations of Six Broad-Leaved Tree Extracts on Germination Rate of *Pinus yunnanensis* Seeds

Water extract concentration (g·mL^{-1})	*Alnus ferdinandi-coburgii*	*Quercus acutissima*	*Celtis yunnanensis*	*Cinnamomum camphora*	*Michelia alba*	*Camellia japonica*
0.02	(90.1$±2.31)*bcdAB*	(87.8±3.53)*bcAB*	(95.7±1.16)*aA*	(83.2±1.86)*cdB*	(83.1±1.73)*dB*	(92.3±1.73)*b*

Note: Data are mean ± standard error. Different lowercase letters in the same row indicate significant differences from the control (P < 0.05), while different uppercase letters indicate extremely significant differences (P < 0.01). The same below.

Effects of Different Concentrations of Six Broad-Leaved Tree Extracts on Germination Potential of *Pinus yunnanensis* Seeds

Water extract concentration (g·mL^{-1})	*Alnus ferdinandi-coburgii*	*Quercus acutissima*	*Celtis yunnanensis*	*Cinnamomum camphora*	*Michelia alba*	*Camellia japonica*
0.02	(82.3$±1.53)*cdBCD*	(80.5±2.08)*bcdB*	(90.3±1.00)*aA*	(78.6±2.08)*cdBC*	(74.4±3.51)*dC*	(85.1±1.73

Effects of Different Concentrations of Six Broad-Leaved Tree Extracts on Seedling Growth of *Pinus yunnanensis*

Water extract concentration (g · mL^{-1})	*Alnus ferdinandi-coburgii*	*Quercus acutissima*	*Celtis yunnanensis*	*Cinnamomum camphora*	*Michelia alba*	*Camellia japonica*
0.02	(8.6$±0.17)bAB	(8.5±0.35)bABC	(9.3±0.12)aA	(7.1±0.14)cD	(7.3±0.23)cD	(8.3±0.26)bBC
0.04						

Effects of Different Concentrations of Six Broad-Leaved Tree Extracts on Fresh Weight of *Pinus yunnanensis* Seedlings

Water extract concentration (g · mL^{-1})	*Alnus ferdinandi-coburgii*	*Quercus acutissima*	*Celtis yunnanensis*	*Cinnamomum camphora*	*Michelia alba*	*Camellia japonica*
0.02	(7.1$±0.31)abAB	(6.6±0.20)bcABC	(7.7±0.44)aA	(4.8±0.10)dD	(4.9±0.16)dD	(6.3±0.10)abAB
0						

Discussion and Conclusion

In natural ecosystems, allelochemicals enter the soil environment through rainwater leaching and fog droplets, where they inhibit or promote seed germination and growth of neighboring plants (Shen et al., 2018; Young & Bush, 2009; Yang et al., 2000). Previous research has shown that low concentrations of allelochemicals can promote plant germination and growth, but accumulation beyond a critical threshold inhibits plant development (Chen et al., 2017; Alam et al., 2004), demonstrating that allelopathy plays a regulatory role in forest community succession. Therefore, allelopathic interactions must be considered when establishing mixed forests.

Our results indicate that the six broad-leaved species exerted either promotional or inhibitory effects on *P. yunnanensis* seed germination and seedling growth. At low concentrations, extracts from *A. ferdinandi-coburgii*, *Q. acutissima*, *C. kunmingensis*, and *C. japonica* promoted germination and seedling growth, but these effects shifted to inhibition as concentrations increased. This pattern may occur because low concentrations enhanced cell membrane permeability in receptor plants, facilitating nutrient and water uptake while promoting growth hormone expression. At higher concentrations, however, cell division became suppressed, potentially triggering apoptosis and resulting in stunted growth. In contrast, *C. camphora* and *M. alba* extracts consistently inhibited germination and seedling growth across all tested concentrations. These differences likely reflect variations in allelochemical composition among species and distinct tolerance mechanisms in receptor plants. The allelochemicals from *C. camphora* and

M. alba may have suppressed cell division and membrane permeability in *P. yunnanensis*, impairing nutrient absorption and photosynthesis, thereby inhibiting growth. These findings align with those of Yang (2020) and Liu (2019).

For practical forestry applications, when *A. ferdinandi-coburgii*, *Q. acutissima*, *C. kunmingensis*, and *C. japonica* are initially mixed with *P. yunnanensis*, the low concentrations of allelochemicals released into the environment will promote seed germination and growth. However, as stands develop and population density increases, allelochemicals may accumulate beyond critical thresholds, potentially inhibiting regeneration. Therefore, during stand management, population densities of these four species should be appropriately controlled and leaf litter should be promptly removed to facilitate natural regeneration and healthy growth of *P. yunnanensis*.

Species selection for insect-resistant mixed forests must consider both pest resistance and allelopathic interactions. Zhou (2012) identified the six broad-leaved species used in this study as repellent to *T. yunnanensis* and suitable for mixed forests, but did not evaluate their allelopathic effects. Allelopathy is a complex process influenced by multiple factors including receptor species, rainfall, temperature, and metabolic pathways in receptor plants, which can directly or indirectly affect seed germination and seedling growth (Yan et al., 2000; Anaya, 1999; Chen, 2009). Using aqueous extraction, our study screened species with promotional effects on *P. yunnanensis* growth, providing a theoretical basis for establishing and transforming *T. yunnanensis*-resistant mixed forests. However, since these species were pre-selected based on field surveys, the results may not fully represent their complete allelopathic potential. Further research should examine rhizosphere soils and community soils from stands of these six species to determine their actual effects on *P. yunnanensis* seed germination and seedling growth under natural conditions.

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Note: Figure translations are in progress. See original paper for figures.