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Effect of extracts of Chinese pine on its own seed germination and seedling growth

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Abstract The allelopathic potential of Chinese pine (*Pinus tabulaeformis* Carr.) against its own seed germination and seedling growth was tested with aqueous extracts (0.01, 0.02, 0.05, and 0.10 g·mL⁻¹) obtained from different organs (roots and litter needles) at different individual ages (12, 52, and 110 years old). The results showed that root and litter extracts had different effects on seed germination and seedling growth, and the effects varied with the concentrations, the organs, and the tree age of extracts. The strongest stimulatory effect on seed germination of Chinese pine was exposed to 0.02 g·mL⁻¹ root extract from the 110 years old Chinese pine trees and exposed to 0.02 g·mL⁻¹ litter extract from the 12 years old Chinese pine trees. Meanwhile, the strongest stimulatory effect on growth of Chinese pine seedlings was exposed not only to 0.01 g·mL⁻¹ root extracts from the 110 years old Chinese pine but also to 0.01 g·mL⁻¹ litter extract from the 12 years old Chinese pine. The promoting effect of the extracts of root on seed germination and seedling growth increased in the order of 12, 52, and 110 years old. The promoting effect of the extracts of litter on seed germination and seedling growth increased in the order of 110, 52, and 12 years old. Our results suggested that litter leachates or root exudates of Chinese pine may influence the natural regeneration within Chinese pine stands via the release of allelochemicals into the environment.

Keywords Chinese pine (*Pinus tabulaeformis* Carr.), allelopathy, age, root, litter, aqueous extracts

1 Introduction

Allelopathy is a topic of great interest in the field of chemical ecology. Rice (1984) defined allelopathy as the effect of one plant (including microorganisms) on the growth and development of another plant through actions of chemical compounds, which are released into the environment via volatilization, leaf leaching, root exudation, and decomposition from the residual body. Both positive and negative effects are included in this definition. Allelopathy plays an important role in chemical interactions in natural plant communities, especially in forest ecosystems (Ma et al., 2002; Chen and Wang, 2003; Hu, 2007). It determines vegetation recovery and succession and is important in forest production and management (Lin et al., 2002; Li et al., 2006). To some extent, it also affects the regeneration process of forest (Zackrisson and Nilsson, 1992). A variety of plants exudate allelochemical through roots, under natural conditions, such as *Oryza sativa*, *Triticum aestivum*, *Glycine max*, *Ambrosia artemisiifolia*, *Populus* spp., *Malus pumila*, and so on (Chen and Wang, 2003). Most studies on allelopathy have focused on the effects of extracts from plant organs on seed germination and seedling growth, because it is difficult to identify the effects of allelochemicals from the effects of competition among plants (Ridenour et al., 2001).

Chinese pine is a special tree species in China and is one of the main afforestation tree species. It is widely distributed in the mountainous regions in Northern China. The pine is tolerant to low temperatures and drought and shows strong adaptability to the environment. Long-term observations indicate that Chinese pine can naturally regenerate very well (Yuan et al., 1999; Zhang et al., 2001), and it is assumed that the results from reasonable conditions with respect to site, density, rotation, and harvesting methods (Sun et al., 2005). However, there is little information available on the role of allelopathy in renewal processes within Chinese pine stands. In addition, woody plants have longer life cycles than herb plants, and

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therefore, it is necessary to study the effects of plant age on allelopathy.

Accordingly, we conducted the experiment to confirm the effect of aqueous extracts of different organs from Chinese pine at different ages upon its own seed germination and seedling growth to discuss the function of allelopathy in the process of spontaneous regeneration of the pine, and to provide a theoretical basis for forestry production.

2 Materials and methods

2.1 Plant materials and aqueous extracts

Chinese pine seeds were obtained from the Jindao Co., Yangling city, Shaanxi Province, and were kept in closed jars at 0–3°C before use.

In July 2007, 20 healthy Chinese pine trees were randomly sampled in an artificial plantation in the Qing Xiling Urban Forest Demonstration of Agricultural University of Hebei, Baoding, China. We selected 12-, 52-, and 110-year-old trees (hereinafter the “12 a,” “52 a,” and “110 a,” respectively). The climate in the area is semiarid with an annual average precipitation of 760 mm and an annual accumulated temperature of 4021°C. The soil type is of cinnamon soil. After collecting the previous-year litter, lateral roots were carefully removed from the soil, and then washed with tap water and dried at room temperature. Dried roots and litter were cut into approximately 1-cm long pieces and macerated in distilled water (10 g plant material per 100 mL water), and then kept at room temperature for 48 h. These extracts were used as stock solutions. The solutions were sterilized by filtration through a 0.45- μm pore size membrane under a laminar flow hood. Three concentrations (0.01, 0.02, and 0.05 $\text{g}\cdot\text{mL}^{-1}$) were obtained by diluting the stock solutions with distilled water. Moreover, the solutions were stored at 4°C until use.

2.2 Bioassay

Quartz sand (0.4 mm particle size) was sterilized by autoclaving 20 min at 1.4 $\text{kg}\cdot\text{cm}^{-3}$, and then, sand was aseptically added to 9-cm-diameter Petri dishes (50 g per dish) (Jobidon and Thibault, 1981). Each Petri dish was saturated with 15 mL of solution, or 15 mL distilled water as a control. Seeds were transferred aseptically into the dish and were arranged evenly on the sand medium. Three replicates were prepared for each treatment, giving a total of 150 seeds per treatment. The Petri dishes were placed in a growth chamber under the following growth conditions: night temperature (20°C), day temperature (25°C), 16/8 light/dark photoperiod, 15000 lx illumination, and 80% relative humidity. Seeds were considered to have germinated when the radicle was half the length of the seed.

Germinated seeds were counted and recorded each day during the experimental period. The length of the radicle and hypocotyls were measured on the final day.

2.3 Statistic analyses

We used the national standard method GB2772-1999 to calculate the seed germination rate and germination index. Seed germination rate $G(\%) = (\text{number of germinated seeds over entire experimental period}) / \text{total seed number} \times 100$, and germination index $GI = \sum (Gt/Dt)$, where Gt is the germination number on day t , and Dt is day t .

All data were analyzed using SPSS12.0 software.

3 Results and analysis

3.1 Effect of root aqueous extracts on seed germination and seedling growth of Chinese pine

3.1.1 Effect of root aqueous extracts on seed germination of Chinese pine

Root aqueous extracts from trees of three ages (12 a, 52 a, 110 a) affected seed germination of Chinese pine differently (Table 1). Compared with the control, the germination rate was increased by 37.8%, 38.6%, and 55.7% treated with 0.02 $\text{g}\cdot\text{mL}^{-1}$ root extracts of 12 a, 52 a, and 110 a, respectively. The stimulatory effect on germination gradually decreased as the extract concentration increased. Seed germination rate was reduced at the highest concentration of extracts from 12 a and 52 a trees, with 38.6% and 12.9% less, respectively, than that of the control. Moreover, differences were significant between the 12 a treatment and the control.

The germination index can reflect the speed of seed germination, and a higher germination index indicates that seeds germinate more rapidly (Yan and Sun, 2000). Root extracts from the trees of three different ages had similar effects on the germination index to the germination rate. The highest stimulatory effect was observed in the 0.02 $\text{g}\cdot\text{mL}^{-1}$ root extract, and the germination index was increased by 65.0%, 77.4%, and 123.2% treated with extracts from the 12 a, 52 a, and 110 a trees, respectively. At higher concentrations, the root extracts reduced germination index. The 110 a extract retained its promoting effect at 0.1 $\text{g}\cdot\text{mL}^{-1}$, whereas 0.1 $\text{g}\cdot\text{mL}^{-1}$ root aqueous extracts of 12 a and 52 a trees inhibited the germination index.

Within each concentration, the root aqueous extracts of 110 a showed the strongest stimulatory effect on seed germination rate and germination index, followed by root aqueous extracts from 52 a and root aqueous extracts of 12 a. At higher concentrations (0.05 and 0.1 $\text{g}\cdot\text{mL}^{-1}$), the differences were significant among the three ages of source trees. At lower concentrations (0.01 and 0.02 $\text{g}\cdot\text{mL}^{-1}$),

Table 1 Effect of root aqueous extracts from three ages of Chinese pine trees on their seed germination and seedling growth

concentration/(g·mL ⁻¹)	tree age/a	germination rate/%	germination index	radicle length/cm	hypocotyl length/cm
0.01	CK	46.67±1.45c	1.77±0.15c	2.54±0.14c	3.47±0.22c
	12	52.67±2.40b	2.27±0.09b	2.86±0.08bc	4.50±0.18b
	52	55.00±0.58b	2.32±0.12b	3.15±0.12ab	4.72±0.16b
	110	67.67±1.45a	3.67±0.15a	3.45±0.16a	5.69±0.32a
0.02	CK	46.67±1.45c	1.77±0.15c	2.54±0.14b	3.47±0.22b
	12	64.33±1.20b	2.92±0.12b	2.53±0.14b	3.65±0.21b
	52	64.67±0.88b	3.14±0.19b	3.08±0.17a	3.55±0.12b
	110	72.67±1.76a	3.95±0.17a	3.27±0.12a	4.34±0.15a
0.05	CK	46.6±1.45c	1.77±0.15bc	2.54±0.14a	3.47±0.22b
	12	47.33±2.38c	1.58±0.18c	1.80±0.15b	3.16±0.14b
	52	53.33±0.67b	2.18±0.13b	2.49±0.10a	3.32±0.08b
	110	59.33±1.56a	3.16±0.04a	2.67±0.09a	4.12±0.17a
0.10	CK	46.67±1.45a	1.77±0.15a	2.54±0.14a	3.47±0.22a
	12	28.67±4.05c	0.70±0.10b	1.39±0.12c	2.53±0.20b
	52	40.67±2.91b	1.15±0.10c	2.26±0.10ab	2.62±0.19b
	110	52.00±1.15a	2.18±0.03a	2.08±0.11b	3.27±0.08a

Note: Different letters indicate significant difference at the same concentration among different ages (including control) ($P < 0.05$). The results are mean±SE, which is the same for Table 2.

there were no differences between the 12 a and 52 a treatments.

3.1.2 Effect of root aqueous extracts on radical and hypocotyl of Chinese pine

The extract of 110 a Chinese pine reduced the radicle length of seedlings at the 0.1 g·mL⁻¹ concentration but increased radicle length significantly in the other three concentration treatments. The highest stimulatory effect (35.8% of increase) was observed at the 0.01 g·mL⁻¹ concentration. The root extract of 52 a Chinese pine markedly promoted radicle growth at the 0.01 and 0.02 g·mL⁻¹ concentrations (24.02% and 21.25% of increases, respectively) but reduced radicle length at 0.05 and 0.1 g·mL⁻¹ concentrations, and there was a significant difference between the 0.1 g·mL⁻¹ treatment and the control. The 12 a Chinese pine extract slightly increased radicle length at 0.01 g·mL⁻¹ but showed inhibitory effects at other concentrations.

Hypocotyl growth was increased in all 110 a treatments, and the difference was significant between treatments and control, except for the treatments of 0.1 g·mL⁻¹ and control. The 0.01 g·mL⁻¹ extracts from 12 a and 52 a trees obviously increased the hypocotyl growth, whereas with the concentration increasing, positive effects on the hypocotyl growth declined. The 0.05 g·mL⁻¹ treatment had no significant effects, while the 0.1 g·mL⁻¹ treatments of both the 12 a and 52 a extracts had inhibitory effects.

At the same concentration, the greatest promoting effect in radicle and hypocotyl growth was observed in the oldest trees. That is, the order of promoting effects was

110 a > 52 a > 12 a. Moreover, the differences were significant between 12 a and 110 a treatments.

3.2 Effect of litter aqueous extracts on seed germination and seedling growth of Chinese pine

3.2.1 Effect of litter aqueous extracts on seed germination of Chinese pine

The 12 a litter extracts obviously increased the pine's seed germination rate and germination index. The 0.02 g·mL⁻¹ concentration had the greatest stimulatory effect with an increased germination rate by 45.70% and an increased germination index by 58.76% compared with the control. Extracts from the 52 a trees had similar promoting effects. The highest stimulatory effect was in the 0.02 g·mL⁻¹ treatment, which increased seed germination rate by 35.70% and germination index by 41.24%, compared with controls. The stimulating effects decreased or even disappeared with the concentration increasing. The 110 a litter extracts had the greatest stimulatory effects at 0.01 g·mL⁻¹ with an increased seed germination rate by 21.43% and an increased germination index by 39.28% compared with controls. The promoting effects decreased or disappeared at higher concentrations.

At a given concentration, litter extracts from 12 a trees had the greatest stimulatory effect on germination, while extracts from 110 a trees had the least. Differences among the three ages of source trees were significant at the 0.02 g·mL⁻¹ concentration, and there were often no significant differences among the tree ages at the other concentrations.

Table 2 Effect of litter aqueous extracts from three ages of Chinese pine trees on their own seeds germination and seedling growth

concentration/(g·mL ⁻¹)	tree age/a	germination rate/%	germination index	radicle/cm	hypocotyl/cm
0.01	CK	46.67±1.45c	1.77±0.15c	2.54±0.14bc	3.47±0.22c
	12	52.00±1.56b	2.35±0.12b	3.45±0.12a	4.67±0.10a
	52	56.67±1.72b	2.21±0.08b	3.15±0.08a	4.25±0.15b
	110	65.00±1.25a	2.52±0.04a	2.90±0.17b	4.17±0.13b
0.02	CK	46.67±1.45d	1.77±0.15c	2.54±0.14b	3.47±0.22c
	12	68.00±1.23a	2.81±0.16a	2.93±0.13a	5.22±0.08a
	52	63.33±0.97b	2.50±0.19ab	2.75±0.17ab	3.83±0.12b
	110	55.67±1.52c	2.16±0.12b	2.45±0.12b	3.42±0.22c
0.05	CK	46.67±1.45c	1.77±0.15b	2.54±0.14a	3.47±0.22bc
	12	56.33±1.72a	2.48±0.09a	2.72±0.09a	4.183±0.16a
	52	51.67±1.56a	2.33±0.05a	2.43±0.18a	3.42±0.17b
	110	48.67±0.67c	1.89±0.13b	1.93±0.13b	3.02±0.09c
0.10	CK	46.67±1.45a	1.77±0.15b	2.54±0.14a	3.47±0.22a
	12	48.33±2.38a	2.19±0.12a	2.12±0.15ab	3.52±0.08b
	52	42.67±1.72ab	1.84±0.10b	1.95±0.12bc	2.63±0.12c
	110	39.33±1.20bc	1.57±0.13b	1.73±0.10c	2.57±0.10c

3.2.2 Effect of litter aqueous extracts on the growth of Chinese pine radicles and hypocotyls

The litter aqueous extracts from 12 a trees increased the growth of radicles and hypocotyls at all concentrations. Moreover, the greatest promoting effects were observed at 0.01 g·mL⁻¹, and the radicle and hypocotyl increased by 35.83% and 34.58%. The differences were significant compared with controls in radical at 0.01 and 0.02 g·mL⁻¹. The effects of 52 a and 110 a litter aqueous extracts on the growth of radicles and hypocotyls were similar. The 0.01 g·mL⁻¹ concentration of both kind extracts markedly increased the growth of hypocotyls and radicles, whereas higher concentrations had lower promoting effects or even inhibitory effects.

At a given concentration, the greatest stimulatory effects were observed in the extracts from 12 a trees, that is, the order of growth promotion among the extracts was 12 a > 52 a > 110 a, and the differences in radical were significant between the treatments of 12 a and 110 a at all concentrations. There were significant differences among the three ages at both 0.02 and 0.05 g·mL⁻¹ concentrations in the hypocotyl growth.

4 Discussion

4.1 Allelopathic effects of Chinese pine on seed germination and seedling growth

Allelochemicals have been suggested to affect the synthetic and application of hormones to alter the cell division, elongation, and ultrastructure and to influence the

membrane permeability, nucleic acid and protein metabolism (Rice, 1984). Aqueous extracts from both roots and litter obtained from trees of different ages are as effective as allelochemicals in plant growth, and they promoted the seed germination and seedling growth probably because of the stimulation for cell division and elongation. In nature, the process of seed germination is a crucial stage in plant growth. Allelochemicals can affect the establishment or regeneration of populations by affecting seed germination (Yang et al., 2005). In fact, a number of plants negatively affect growth and development of neighboring or succession plants by releasing allelopathic substances into the soil. Deng et al. (1996) reported that *Casuarina equisetifolia* inhibited the natural self-renewal when its own allelochemicals accumulated to a certain concentration in the soil. Similar situation also happened in populations of *Cunninghamia lanceolata* (Lin et al., 1999). Phenolic compounds strongly influenced the regeneration of spruce within a subalpine bilberry-spruce forest (Gallet, 1994; Mallik and Pellissier, 2000). In areas dominated by the dwarf shrub *Empetrum hermaphroditum*, its allelopathic effects inhibit the regeneration of *Pinus sylvestris*, *Populus tremuloides*, *Betula pendula* (Nisson, 1994; Keech et al., 2005). In addition, allelochemicals in the leaf litter in a mixed stand of *Picea koraiensis* and *Pinus abies* inhibit the seed germination and seedling growth of *P. abies* and prevent its succession (Jon et al., 1984; Richard et al., 1991). The results of our study differed from those of the previous published studies. We found that root and litter aqueous extracts of *P. tabulaeformis* from 12 a, 52 a, and 110 a trees significantly promoted the seed germination and seedling growth at lower concentrations, which indicated that there are indeed some allelochemicals in

root and litter extracts of the pine and that these compounds can stimulate seed germination and seedling growth of pine to some extent. An increased germination rate can result in more individuals within a community. Increasing germination index can enhance the competitive ability of plants for aboveground and underground resources. The promoting effects on growth of radicles and hypocotyls can enhance the absorption of water and fertilizers, light interception, and promote rapid plant growth. The effect on seed germination and seedling growth can also directly affect plant growth and development and contribute to species dominance within the plant community (Ross and Harper, 1972; Fowler, 1986; Weiner et al., 1997). Consequently, allelopathy of *P. tabulaeformis* may be one of the most important factors in successful regeneration of this tree species.

4.2 Differences in allelopathic effects of root and litter extracts from trees of different ages

It was suggested that litter extracts from *P. tabulaeformis* had no effect on seedling growth, while occasionally slightly stimulated at lower concentrations, but inhibited at higher concentrations in reach of Jia et al. (2003). The similar effects were observed in the root extracts from 12 a and the litter aqueous extracts from 110 a but not in other treatments in our research. Allelochemicals are secondary metabolites that are released by plants. Their type and quantity may vary with different developmental stages of plants (Huang et al., 2002; Peng et al., 2004; Fernandez et al., 2006). For example, the batatasin-III concentration was the highest in the first and second year shoots, while the concentration in the third year shoots was approximately half of the highest concentration (Wallstedt et al., 1997). The inhibitory effects of *Eupatorium adenophorum* leaf extracts on native plants gradually increased with plant age (Han and Feng, 2007), and the inhibitory effects of *Ageratum conyzoides* on other plants were stronger in the extracts taken during the reproductive period than in the vegetative period (Hu and Kong, 1997). The inhibitory effects of grafted eggplant root exudates against eggplant seedlings were greater at a later growth stage (Zhang et al., 2005). Sharma showed that older *Populus deloides* plants had greater effects on *Phalaris minor* (Sharma et al., 2000).

In our study, the root extracts from the 110 a trees showed the strongest stimulating effect on the seed germination and seedling growth, followed by the 52 a extracts, and then by the 12 a extracts. That is, the positive effects tended to increase with increasing tree age. It is interesting to note that the stimulatory effect of litter extracts on the seed germination and seedling growth of Chinese pine decreased with tree age. This was contrary to the trend observed in root extracts. Moreover, we obtained roots and litter from differently aged trees in adjacent

forests; thus, the effects of geographical location were minor. The differences observed in the allelopathic effects could be attributed mainly to the different types and concentrations of allelochemicals in each organ tested (Fernandez et al., 2006). Fernandez showed that the needle extracts of young *Pinus halepensis* trees (± 10 years old) had the strongest inhibitory effect on the germination and growth of *Linum strictum*. Root extracts of older *P. halepensis* (> 30 years old) had similar effects.

Chinese pine is one of the most important forest tree species in the mountainous area in Northern China. At present, most Chinese pine stands in this area are reaching their natural climax in the succession and are facing regeneration. The stimulatory effect of allelopathy of the pine is important in the regeneration of its stands. We found that the extracts from organs of different tree ages had various effects on seedling growth and seed germination. Our results may provide an important theoretical basis for sustainable management of Chinese pine stands. Future researches are suggested to be aimed at identifying the compounds in these extracts with stimulatory effects on seed germination and growth and at determining how these allelochemicals change with age.

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