STUDY ON ALLELOPATHY OF THREE SPECIES OF *PINUS* IN NORTH CHINA

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Abstract. Two-year-old seedlings of *Pinus* species typical in North China (*Pinus thunbergii, Pinus tabuliformis* and *Pinus koraiensis*) were selected as the research object. The water extracted from the litter of *P. thunbergii*, *P. tabuliformis* and *P. koraiensis* were used to test the changes of photosynthetic pigment Chl. a, Chl. b and allelochemicals. The results were as follows: aqueous extract of *P. thunbergii* needle litter had significantly low inhibition on *P. thunbergii* while strongly inhibited *P. tabuliformis* and *P. koraiensis*. It also showed a markedly low inhibition on *P. thunbergii*, a moderate inhibition on *P. tabuliformis*, and a greatly strong inhibition on *P. koraiensis*. Obviously strong inhibition on *P. thunbergii*, *P. tabuliformis* and *P. koraiensis* were observed as well. Our study showed that pine needle water leaching solutions, such as *P. tabuliformis*, *P. thunbergii* and *P. koraiensis*, significantly inhibit their Chl. a and Chl. b, and will be a norm, which provides a new explanation for the decline in the coniferous forests of northern China.
Keywords: *Pinus thunbergii, Pinus tabuliformis, Pinus koraiensis, allelopathy, Chl. a, Chl. b*

Introduction

Allelopathy refers to the process that those chemical substances produced by plants, which can have beneficial or detrimental effects on the growth of the plants themselves or other plants around, are released into the environment through leaching, volatilization, stubble degradation and root excretion, (Peng and Shao, 2001; Kong et al., 2002; Zhou et al., 2004). Studies on allelopathy have dramatically increased since the 1970s and have undergone rapid development since the 1990s. In recent years, the research has become a hot topic in the fields of botany, ecology, agronomy and soil science (Albuquerque et al., 2011; Cheng and Cheng, 2015). At present, allelochemicals mean basically secondary metabolites. The most common allelochemicals are organic acids, phenols and terpenoids (Yan et al., 2000; Chen et al., 2015).

Allelopathy exists widely in nature and significantly affects forest community succession, vegetation restoration and forestry production (Li et al., 2010; Albuquerque et al., 2011; Cheng and Cheng, 2015). Conifers are important timber and afforestation species in China. In recent years, many coniferous plantations have faced such problems as productivity decline, soil degradation and natural regeneration obstacles. The traditional view is that this phenomenon is caused by rotation, inappropriate harvesting

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methods, harvesting residues, tillage and land preparation (Chen et al., 2003; Wang et al., 2007). However, in recent years, convincing evidence has showed that allelopathy may be the key factor affecting the natural regeneration of coniferous forests. Allelochemicals can be transported to the ground through pine needles and litters (Kimura et al., 2015), which will limit the growth of seedlings and herbaceous plants (Kil and Yang, 1983; Kato-Noguchi et al., 2011) and will impede the regeneration of the stands (Pan et al., 2009; Wang et al., 2007). Researches on the allelopathy of the main afforestation species in northern China can provide important theoretical and practical guidance for the management and survival of regional man-made forests.

The type and dose of allelochemicals released by plants to the environment are decided by combined effects, namely the plant itself and environmental factors. Environmental stress can increase the release of allelochemicals (Albuquerque et al., 2011). Therefore, both the environment and the plant itself must be taken into consideration in the study of allelopathy. *P. thunbergii*, *P. tabuliformis* and *P. koraiensis* are the main afforestation species in northern China (Wei et al., 2017; Yin et al., 2018). At present, only few studies focus on the allelopathic effects of *P. thunbergii* tissue and understory extracts on Brassica rapa pekinensis (Zhang et al., 2012), litter leaf and soil extract on seed germination and seedling growth of *P. koraiensis* (Chen et al., 2016), Allelopathic effects on the growth and photosynthesis of *P. tabuliformis* seedlings (Jia et al., 2003), the autotoxicity of *P. tabuliformis* (Li et al., 2010), the allelopathic effect of *P. tabuliformis* soil in the Loess Plateau (Zhu et al., 2014), and the allelopathic effect of *P. tabuliformis* root in Hilly Loess Plateau (Wang et al., 2015), etc. There is no study focusing on the interaction between *P. thunbergii*, *P. tabuliformis* and *P. koraiensis*. In this research, 2-year-old seedlings of *P. thunbergii*, *P. tabuliformis* and *P. koraiensis* were selected for the pot experiment to study the effects of water and aqueous solution of *P. thunbergii* pine needles, *P. tabuliformis* pine needles and *P. koraiensis* pine needles. Changes of Chl. a, Ch. b, allelochemicals, also total triterpenes in seedlings were examined after 30 days.

Materials and methods

Testing materials

The pine needle litter of *P. thunbergii* and *P. tabuliformis* was collected from Tianmeng area of Mountain Meng, China. All target trees grew in a natural environment, with a height of about 10 m, and a diameter of about 15 cm. The pine needle litter of *P. koraiensis* was purchased from Hunchun seedling breeding base in Jilin, China. *P. thunbergii* and *P. tabuliformis* seedlings were purchased from the seedling breeding base of Junan County in Linyi city, China, the seedlings were about 0.5 m in height and 1.5 cm in diameter. *P. koraiensis* seedlings were purchased from Hunchun seedling breeding base in Jilin, China, the seedlings were about 0.5 m in height and 1.5 cm in diameter. The pot experiment was conducted at the science exploration laboratory base in Linyi, China.

Testing method

1) Five 600 m^2 *P. thunbergii* and *P. tabuliformis* sample plots were set up, and a 1 m^2 litter box was placed in each plot; 2) From January 2017 to July 2017, pine needles in the collection boxes of *P. thunbergii* and *P. tabuliformis* were packaged and transferred to the laboratory and summarized for natural drying; 3) From July 25 to August 25, 2017, pine needle litters of *P. thunbergii*, *P. tabuliformis* and *P. koraiensis* were soaked; 4) From July

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20 to August 25, 2017, *P. thunbergii*, *P. tabuliformis* and *P. koraiensis* seedlings were potted; 5) From August 25 to September 25, 2017, the seedlings of *P. thunbergii*, *P. tabuliformis* and *P. koraiensis* were inoculated in pots. Four treatments (clean water control group, *P. thunbergii* needle water leaching solution treatment group, *P. tabuliformis* needle water leaching solution treatment group and *P. koraiensis* needle water leaching solution treatment group) were set for each of the three young pine trees. Five plants were cultivated in parallel. 100 mL water or pine needles water leachate was added to the young pine trees every 5 days for 6 times, a total volume of 600 mL was used; 5) On September 25, all pine needles were sampled and sealed in bags for inspection.

Determination method

Determination of Chl. a and Chl. b content

0.1 g pine needles were weighed, cut and placed into the mortar. More than 15 mL absolute ethanol was added to the mortar until the total volume of the liquid was 25 mL. These solutions were stored in the dark for 24 h and then were filtered. The pigment's optical density was measured by a spectrophotometer 721 at a wavelength of 665 nm and 649 nm, and the content Chl. a and Chl. b were calculated.

Determination of total triterpene content

After extraction and centrifugation of the pine needles the sample solution was filtered through a 0.3 μm membrane filter. The residue was separated on a C_{18} column by high performance liquid chromatography. The mobile phase was $(NH_4)_2HPO_4$-H_3PO_4 buffer solution (pH = 2.7). For detection a UV detector at 210 nm was used. External standard method was performed for the determination of total triterpene content. The test was completed in the Environment Laboratory of Scistd Testing Qingdao Branch, China.

Data processing

Excel 2003 and SPSS 17.0 Chinese version were used for processing, and one-way analysis of variance was performed.

Results

Effects of 4 kinds of treatment on the Chl. a content in 3 kinds of seedlings

Groups of *P. thunbergii* seedlings for Chl. a content test: clean water control group> *P. thunbergii* needle water leaching solution treatment group> *P. tabuliformis* needle water leaching solution treatment group> *P. koraiensis* needle water leaching solution treatment group. *P. thunbergii* needle water leaching solution treatment group, *P. tabuliformis* needle water leaching solution treatment group and *P. koraiensis* needle water leaching solution treatment group showed significantly lower results than clean water control group ($P < 0.01$, $P < 0.01$, $P < 0.01$), and *P. koraiensis* needle water leaching solution treatment group showed significantly lower results than *P. thunbergii* needle water leaching solution treatment group and *P. tabuliformis* needle water leaching solution treatment group ($P < 0.01$, $P < 0.01$) (Fig. 1A).

Groups of *P. tabuliformis* seedlings for Chl. a content test: clean water control group> *P. thunbergii* needle water leaching solution treatment group> *P. tabuliformis*

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needle water leaching solution treatment group> *P. koraiensis* needle water leaching solution treatment group. *P. thunbergii* needle water leaching solution treatment group, *P. tabuliformis* needle water leaching solution treatment group and *P. koraiensis* needle water leaching solution treatment group showed significantly lower results than clean water control group ($P < 0.05$, $P < 0.01$, $P < 0.01$), and *P. koraiensis* needle water leaching solution treatment group showed significantly lower results than *P. thunbergii* needle water leaching solution treatment group and *P. tabuliformis* needle water leaching solution treatment group ($P < 0.01$, $P < 0.01$) (Fig. 1A).

Groups of *P. koraiensis* seedlings for Chl. a content test: clean water control group> *P. thunbergii* needle water leaching solution treatment group> *P. tabuliformis* needle water leaching solution treatment group> *P. koraiensis* needle water leaching solution treatment group. *P. thunbergii* needle water leaching solution treatment group, *P. tabuliformis* needle water leaching solution treatment group and *P. koraiensis* needle water leaching solution treatment group showed significantly lower results than clean water control group ($P < 0.05$, $P < 0.01$, $P < 0.01$), and *P. koraiensis* needle water leaching solution treatment group showed significantly lower results than *P. thunbergii* needle water leaching solution treatment group ($P < 0.01$, $P < 0.05$) and *P. tabuliformis* needle water leaching solution treatment group, *P. tabuliformis* needle water leaching solution treatment group showed significantly lower results than *P. thunbergii* needle water leaching solution treatment group ($P < 0.01$) (Fig. 1A).

Effects of 4 kinds of treatment on the Chl. b content in 3 kinds of seedlings

Groups of *P. thunbergii* seedlings for Chl. b content test: clean water control group> *P. thunbergii* needle water leaching solution treatment group> *P. tabuliformis* needle water leaching solution treatment group> *P. koraiensis* needle water leaching solution treatment group. *P. koraiensis* needle water leaching solution treatment group showed significantly lower results than clean water control group, *P. thunbergii* needle water leaching solution treatment group and *P. tabuliformis* needle water leaching solution treatment group ($P < 0.01$, $P < 0.01$, $P < 0.05$) (Fig. 1B).

Groups of *P. tabuliformis* seedlings for Chl. b content test: clean water control group> *P. thunbergii* needle water leaching solution treatment group> *P. tabuliformis* needle water leaching solution treatment group> *P. koraiensis* needle water leaching solution treatment group. *P. thunbergii* needle water leaching solution treatment group, *P. tabuliformis* needle water leaching solution treatment group and *P. koraiensis* needle water leaching solution treatment group showed significantly lower results than clean water control group ($P < 0.01$, $P < 0.01$, $P < 0.01$), and *P. koraiensis* needle water leaching solution treatment group showed significantly lower results than *P. thunbergii* needle water leaching solution treatment group and *P. tabuliformis* needle water leaching solution treatment group ($P < 0.01$, $P < 0.01$) (Fig. 1B).

Groups of *P. koraiensis* seedlings for Chl. b content test: clean water control group> *P. thunbergii* needle water leaching solution treatment group> *P. tabuliformis* needle water leaching solution treatment group> *P. koraiensis* needle water leaching solution treatment group. *P. thunbergii* needle water leaching solution treatment group, *P. tabuliformis* needle water leaching solution treatment group and *P. koraiensis* needle water leaching solution treatment group showed significantly lower results than clean water control group ($P < 0.01$, $P < 0.01$, $P < 0.01$), and *P. koraiensis* needle water leaching solution treatment group showed significantly lower results than *P. thunbergii* needle water leaching solution treatment group and *P. tabuliformis* needle water

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leaching solution treatment group ($P < 0.01$, $P < 0.05$), *P. tabuliformis* needle water leaching solution treatment group was significantly lower than *P. thunbergii* needle water leaching solution treatment group ($P < 0.01$) (Fig. 1B).

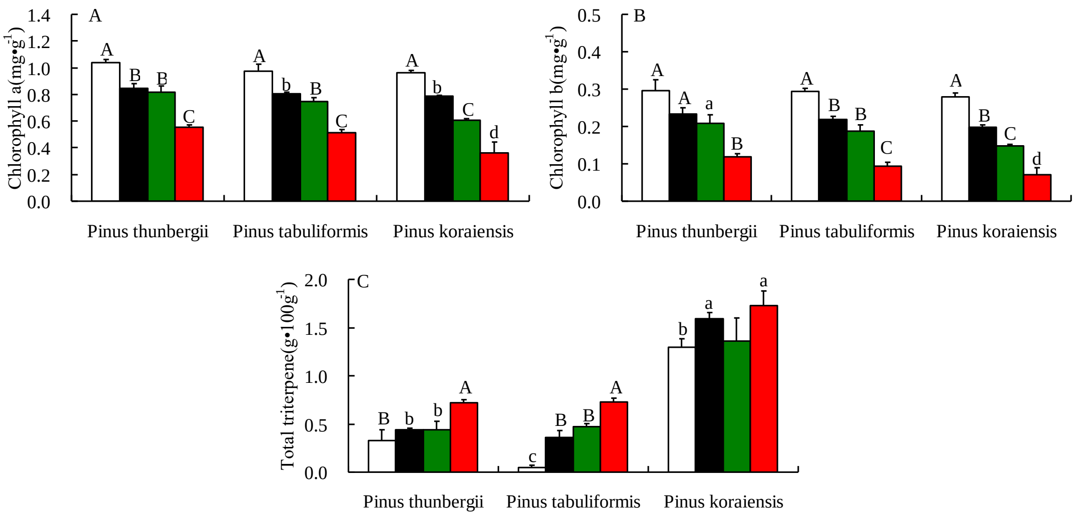

Figure 1. *Effects of 4 kinds of treatment on Chl. a (A), Chl. b (B) and total triterpene (C) in 3 kinds of seedlings (average + standard error). □ Clean water control group; ■ P. thunbergii needle water leaching solution treatment group; ■ P. tabuliformis needle water leaching solution treatment group; ■ P. koraiensis needle water leaching solution treatment group The difference between the adjacent letters is significant, capitalized letters P < 0.01, and lowercase letters P < 0.05*

Effects of 4 kinds of treatment on the total triterpene content in 3 kinds of seedlings

Groups of *P. thunbergii* seedlings for total triterpene content test: *P. koraiensis* needle water leaching solution treatment group> *P. thunbergii* needle water leaching solution treatment group> *P. tabuliformis* needle water leaching solution treatment group>clean water control group. *P. koraiensis* needle water leaching solution treatment group showed significantly higher results than *P. thunbergii* needle water leaching solution treatment group, *P. tabuliformis* needle water leaching solution treatment group and clean water control group, while *P. thunbergii* needle water leaching solution treatment group, *P. tabuliformis* needle water leaching solution treatment group and clean water control group had no significant difference ($P < 0.01$, $P < 0.01$, $P < 0.05$) (Fig. 1C). *P. koraiensis* needle water leaching solution had a significant allelopathic effect on *P. thunbergii* seedlings.

Groups of *P. tabuliformis* seedlings for total triterpene content test: *P. koraiensis* needle water leaching solution treatment group> *P. tabuliformis* needle water leaching solution treatment group> *P. thunbergii* needle water leaching solution treatment group>clean water control group. *P. koraiensis* needle water leaching solution treatment group, *P. tabuliformis* needle water leaching solution treatment group and *P. thunbergii* needle water leaching solution treatment group showed significantly higher results than clean water control group ($P < 0.01$, $P < 0.01$, $P < 0.05$), and *P. koraiensis* needle water leaching solution treatment group showed significantly higher results than *P. tabuliformis* needle water leaching solution treatment group and *P. thunbergii* needle water leaching solution treatment group ($P < 0.01$, $P < 0.01$), while *P. tabuliformis*

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needle water leaching solution treatment group and *P. thunbergii* needle water leaching solution treatment group had no significant difference (Fig. 1C). *P. koraiensis* needle water leaching solution, *P. tabuliformis* needle water leaching solution and *P. thunbergii* needle water leaching solution had a significant allelopathic effect on *P. tabuliformis* seedlings.

Groups of *P. koraiensis* seedlings for total triterpene content test: *P. koraiensis* needle water leaching solution treatment group> *P. thunbergii* needle water leaching solution treatment group> *P. tabuliformis* needle water leaching solution treatment group>clean water control group. *P. koraiensis* needle water leaching solution treatment group and *P. thunbergii* needle water leaching solution treatment group showed significantly higher results than clean water control group ($P < 0.05$, $P < 0.05$), while *P. tabuliformis* needle water leaching solution treatment group and clean water control group had no significant difference (Fig. 1C). *P. koraiensis* needle water leaching solution and *P. thunbergii* needle water leaching solution had a significant allelopathic effect on *P. koraiensis* seedlings.

Relevance of Chl. a, Chl. b and total triterpene

Analysis indicated significant positive linear correlation between Chl. a and Chl. b in 3 kinds of seedlings, such as *P. tabuliformis*, *P. thunbergii* and *P. koraiensis* ($y = 0.3976\ x - 0.1056$, $R^2 = 0.9435$, $P < 0.01$, $n = 58$) (Fig. 2A). Relevance of Chl. a and total triterpene is significant in 3 kinds of seedlings ($y = -0.1303\ x^3 + 0.4627\ x^2 - 0.4896\ x + 0.3312$, $R^2 = 0.37$, $P < 0.01$, $n = 58$) (Fig. 2B). Relevance of Chl. b and total triterpene is significant in 3 kinds of seedlings ($y = -0.3161\ x^3 + 1.1339\ x^2 - 1.2147\ x + 1.0971$, $R^2 = 0.3815$, $P < 0.01$, $n = 58$) (Fig. 2C).

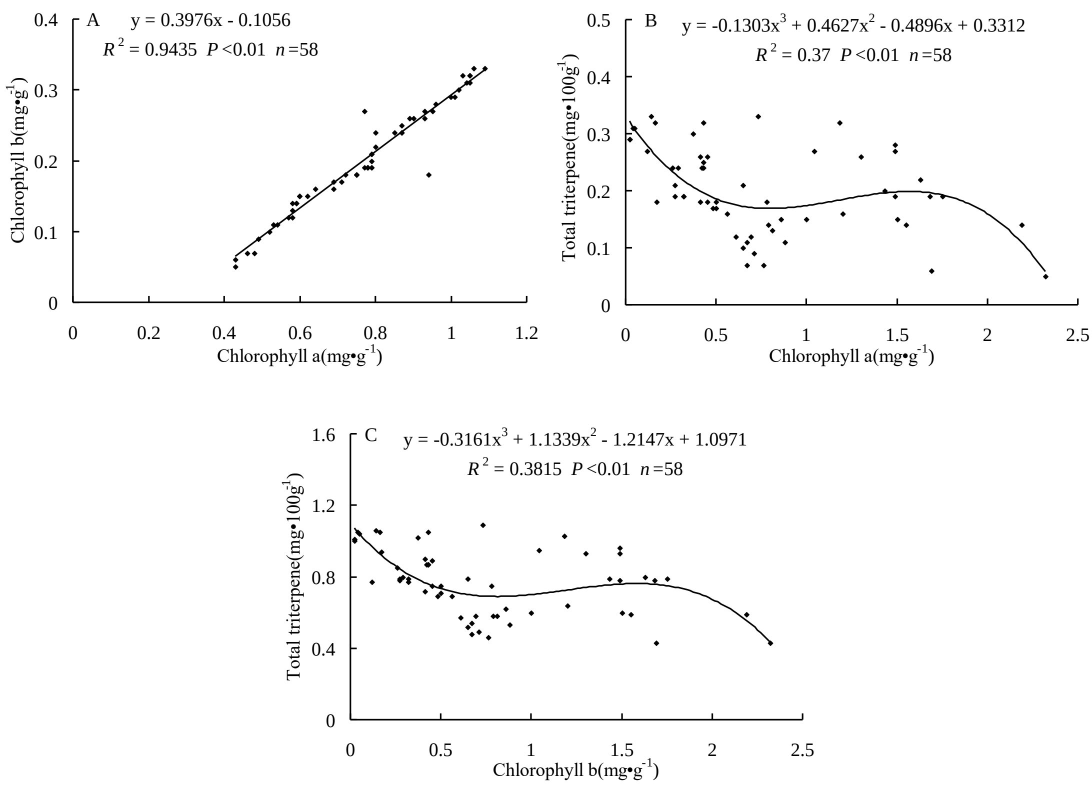

***Figure 2.** Relevance of Chl. a, Chl. b and total triterpene in 3 kinds of seedlings (A, B, C)*

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Discussion

In recent years, it has been found that allelochemicals can inhibit the absorption and transport of amino acids, and can interfere with the protein synthesis (Li et al., 2010), inhibit or damage the synthesis of photosynthetic pigments and accelerate the decomposition of photosynthetic pigments (Poonpaiboonpipat et al., 2013). It also affects the shape and structure of plant cells, and change the transport of nutrients and nutrient accumulation efficiency (Singh et al., 2009; Sunar et al., 2013; Grana et al., 2013), eventually causing a strong change in plant cells and growth of plants. The soil leaching liquor in *P. koraiensis* plantation had suppressive effects on the 7 measured indices, such as germination percentage, germination potential, hypocotyl and radicle length, seedling height, root length and dry mass (Chen et al., 2016). The leaf litter, semi-decomposed litter and surface soil infiltration solution of *P. tabulaeformis* significantly inhibited the growth, chlorophyll content (especially Chl. a content) and net photosynthetic rate of *P. tabulaeformis* seedlings (Jia et al., 2003). Japanese red pine (*P. koraiensis*) produces resin acids, such as abscisic acid-β-d-glucopyranosyl ester (Kato-Noguchi et al., 2011), 9α,13β-epidioxyabeit-8(14)en-18-oic acid and abscisic acid-β-Dglucopyranosyl ester (Kimura et al., 2015), 15-hydroxy-7-oxodehydroabietate and 7-oxodehydroabietic acid (Kato-Noguchi et al., 2017), which are transported to the surface soil through fallen leaves, inhibiting the growth of roots and shoots of other plants, resulting in sparse understory vegetation. The cedar (*Cedrus deodara*) leaves volatiles have a strong allelopathic effect, and its main components are terpenoids (Li et al., 2015). Their leaf water leaching liquid affects root length, shoot length and chlorophyll content, and reduce seed germination and plant dry weight (Talukdar and Talukdar, 2012; Uddin and Robinson, 2017). Allelochemicals reduce plant absorption and increase the availability of soil nutrients (Aslam et al., 2017; Mohammadkhani and Servati, 2018). Our study showed that pine needle water leaching solutions, such as *P. tabuliformis*, *P. thunbergii* and *P. koraiensis*, significantly inhibit their Chl. a and Chl. b, and will be a norm, which provides a new explanation for the decline in the coniferous forests of northern China. We propose to carry on research in this direction, such as dividing the concentration of the pine needle litter water extract, separating and identifying more new allelochemicals, thus revealing the plant physiological and biochemical reactions and countermeasures of the three major *Pinus* species in northern China.

Conclusions

P. thunbergii needle water leaching solution treatment significantly reduced chl. a and chl. b contents in *P. thunbergii* seedlings. It greatly reduced the total triterpene content in *P. tabuliformis* seedlings while enhanced the total triterpene content in *P. tabuliformis.* In addition, it significantly reduced the total triterpene content in *P. koraiensis* seedlings while enhanced the total triterpene content in *P. koraiensis* seedlings.

P. tabuliformis needle water leaching solution treatment significantly reduced Chl. a and Chl. b contents in *P. thunbergii* seedlings and Chl. a and Chl. b contents in *P. tabuliformis* seedlings as well. However, it strengthened the total triterpene content in *P. tabuliformis* seedlings, with the decrease of Chl. a and Chl. b contents in *P. koraiensis* seedlings.

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Although *P. koraiensis* needle water leaching solution treatment significantly reduced Chl. a and Chl. b contents in *P. thunbergii* seedlings, it dramatically increased total triterpene content in *P. thunbergii* seedlings. And it also caused the total triterpene content in *P. tabuliformis* seedlings to decrease and the total triterpene content in *P. tabuliformis* seedlings to increase. Moreover, it decreased the total triterpene content in *P. koraiensis* seedlings while it increased the total triterpene content in *P. koraiensis* seedlings.

Comprehensive assessment showed that *P. thunbergii* needle litter water leaching solution had a significantly low inhibition on *P. thunbergii* while a significantly strong inhibition on *P. tabuliformis* and *P. koraiensis*. It also showed a significantly low inhibition on *P. thunbergii*, a moderate inhibition on *P. tabuliformis* and a significantly strong inhibition on *P. koraiensis*. *P. koraiensis* needle litter water leaching solution had a significantly strong inhibition on *P. thunbergii*, *P. tabuliformis* and *P. koraiensis*.

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