Seasonal Variation in Allelopathic Activity of Japanese Red Pine Needles

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Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) was reported to have strong allelopathic activity. However, the allelopathic activity of the pine has not been monitored throughout a year. In this research, the seasonal change in the allelopathic activity of the extracts of Japanese red pine needles was determined. The extracts of pine needles inhibited the growth of cress (*Lepidium sativum* L.) and the inhibitory activity of the extracts showed the seasonal variation. The inhibitory activity of June extract was significantly greater than that of December extract. The extracts also inhibited the barnyard grass (*Echinochloa crus-galli* (L.) Beauv.), but the seasonal change was not apparent. The present research suggests Japanese red pine needles have allelopathic activity throughout a year and the patterns of the change in the inhibitory activity were different with cress and barnyard grass, which may be due to the different sensitivity of cress and barnyard grass to a mixture of allelopathic substances in the pine needles.

Keywords : allelopathy, growth inhibition, Japanese red pine forest, needles, variation

INTRODUCTION

Pine species grow widely in both northern and southern hemispheres and sometimes form unique forest floors. Compare to the other forests, the forest floors of pine are sparse. The growth of undergrowth plant species on the floors are restricted (Rice, 1984). One of the reasons for the sparse forest floors was considered to be due to strong allelopathy of pine species. Several putative allelopathic active substances have been reported (Chu-Chou, 1978; Harris et al., 2003; Jia et al., 2003; Nektarios et al., 2005; Caboun, 2006; Fernandez et al., 2006; 2013).

Japanese red pine (*Pinus densiflora* Sieb. et Zucc., Pinaceae family) is distributed in Japan, Korea and China, and most common pine species in Japan. The red pine is a typical pioneer plant and can grow in relatively nutrient-poor soils. In addition, the pine also form sparse forest floors (Rice, 1984). It has also reported that the extracts of pine needles inhibited the growth several plant species and the red pine has strong allelopathic activity (Lee and Monsi, 1963; Kil and Yim, 1983). Therefore, the allelopathy of Japanese red pine may also be one of the reason to form such forest floors. However, allelopathic activity of the needle has not been monitored throughout a year. The information is probably necessary to understand allelopathy of the red pine. In this study, allelopathic activity of Japanese red pine needles was determined throughout a year.

MATERIALS AND METHODS

Plant materials

Needles of red pine (*Pinus densiflora* Sieb. et Zucc.) were randomly collected from 20 trees in area of 10×10 m², in mountainous terrain in Takamatsu, Japan every three months from June 2012 to March in 2013. Annual average temperature was 16.3°C in a range of 5.5°C (January mean temperature) to 28.1°C (August). Annual mean rainfall was 1082.3 mm in a range of 37.3 mm (December) to 150.6 mm (June). The climate data of 1981 to 2010 was obtained from Takamatsu local meteorological observatory (34° 19' N, 134° 03' E, 8.7 m alt.), which is about 3.5 km south west of the sampling place. A dicotyledonous plant, cress (*Lepidium sativum* L.) was chosen as test plant for bioassay because of its known germination behaviour and sensitivity to allelochemicals. A monocotyledonous plant, barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) was chosen as test plant for bioassay due to its weedy characteristics.

Extraction

Needles of red pine (45 g dry weight) were extracted with 300 mL of 80% (v/v) of aqueous methanol for 2 days. After filtration using filter paper (No. 2; Toyo Ltd., Tokyo), the residue was extract again with 300 mL of cold methanol for 2 days and filtered. The two filtrates were combined and evaporated with a rotary evaporator at 40°C.

Bioassay for seedling growth

The extracts obtained from 3, 10 or 100 mg dry weight pine needles were dissolved in methanol, and added to a sheet of filter paper in 2.8 cm Petri dishes. After evaporation of methanol, the filter paper in the Petri dish was moistened with 0.6 mL of 0.05% (v/v) Tween 20. Ten seeds of

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cress or 10 germinated seeds of barnyard grass were then arranged on the filter paper in Petri dishes. Barnyard grass was germinated in dark at 25°C for 48 h. The hypocotyl and root lengths of cress, and coleoptile and root lengths of barnyard grass were measured at 48 h after incubation in dark at 25°C. Control seeds or seedlings were arranged on the filter paper moistened with the aqueous solution of Tween 20 without the extracts. The bioassay was repeated two times with 10 plants for each determination. Inhibitory activity (%) was calculated by the formula: $[1 - (\text{plant length treated the extracts})/(\text{control plant length})] \times 100$. Statistical analysis was performed using SPSS version 11.5. Significant differences were examined by Tukey's HSD tests at $P < 0.05$. Significant differences between control

and treatments were analyzed by a Tukey's-Kramer Multiple comparison test at $P < 0.05$.

RESULTS AND DISCUSSION

All extracts of the pine needle inhibited root and hypocotyl growth of cress seedlings except for the extract of December (Fig. 1). The sensitivity of cress roots and hypocotyls against the extracts was not different. However, the inhibitory activity differed with the month although the pattern of the changes was similar in the roots and hypocotyls. The inhibitory activity of March extract was significantly greater than that of December extract. The inhibitory activity of March extract at concentration of 10 mg

Fig. 1 Effects of aqueous methanol extracts of pine needles on (a) hypocotyl and (b) root of cress. The concentrations of tested samples were 3 and 10 mg dry weight equivalent extracts per mL. Hypocotyl and root length of cress was determined after 48 h of incubation in the dark at 25°C. Means $\pm$ SE from 2 independent experiments with 10 plants for each determination are shown. Different letters in the same panel show significant difference ($P < 0.05$) according to Tukey's HSD test. Asterisks show significant difference ($P < 0.05$) with control length and treatment length according to Tukey-Kramer Multiple comparison test.

Fig. 2 Effects of aqueous methanol extracts of pine needles on (a) coleoptile and (b) root of barnyard grass. The concentrations of tested samples were 3 and 10 mg dry weight equivalent extracts per mL. Coleoptile and root length of barnyard grass was determined after 48 h of incubation in the dark at 25°C. Means $\pm$ SE from 2 independent experiments with 10 plants for each determination are shown. Different letters in the same panel show significant difference ($P < 0.05$) according to Tukey's HSD test. Asterisks show significant difference ($P < 0.05$) with control length and treatment length according to Tukey-Kramer Multiple comparison test.

dry equivalent extract per mL was 82.5 and 68.1% for roots and hypocotyls, respectively. Inhibitory activity of the extracts against barnyard grass was lower than cress (Fig. 2). When the concentration of the extract was increased to 100 mg dry equivalent extract per mL, strong inhibition was observed on the growth of barnyard grass. However, there was no significant difference in the inhibitory activities of the extracts on the barnyard grass through a year with all concentrations.

Allelopathic activity of extracts of Japanese red pine needles had been investigated and several allelopathic active substances, such as phenolic acids, 9 α ,13 β -epidioxyabiet-8(14)-en-18-oic acid and abscisic acid- β -D-glucopyranosyl ester, were isolated (Lee and Monsi, 1963; Kil and Yim, 1983; Kato-Noguchi et al., 2009; 2011). 9 α ,13 β -Epidioxyabiet-8(14)-en-18-oic acid inhibited the growth cress and barnyard grass seedlings at concentrations greater than 0.1 mM. Effectiveness of the compound on cress roots and hypocotyls was 1.1- and 2.4-fold greater than that of barnyard grass roots and coleoptiles, respectively (Kato-Noguchi et al., 2009). Abscisic acid- β -D-glucopyranosyl ester inhibited the growth of cress and barnyard grass seedlings at concentrations greater than 0.1 μ M. The effectiveness of this compound on cress roots and hypocotyls was 4.8- and 4.5-fold greater than that of barnyard grass roots and coleoptiles, respectively (Kato-Noguchi et al., 2011). It was also reported that the concentrations of some substances, such as phenolic acids, in plant leaves were high at the beginning of the growing season and decreased with the mature processes of the leaves (Cooper-Driver et al., 1977; Ohmae et al., 1996; Riipi et al., 2002). Thus, concentration and/or composition of those allelopathic substances in the extracts probably differ with the months. This may be one of the possible reasons to explain the change of the allelopathic activity of Japanese red pine needles against cress throughout a year.

On the same time, the effectiveness of those allelopathic substances may be different from the target plant species. Thus, it may be possible that there are some unknown allelopathic active substances, which varied in the concentration with month and inhibit the growth of cress but not barnyard grass. In this case, the allelopathic activity of the needles against cress would be changed significantly throughout a year, but the allelopathic activity against barnyard grass would not be changed significantly.

The present research suggests Japanese red pine needles have allelopathic activity throughout a year and the allelopathic activity showed the changes in a year. However, the patterns of the change in the inhibitory activity monitored with cress and barnyard grass were different,

which may be due to the different sensitivity of cress and barnyard grass to a mixture of allelopathic substances in the pine needles.

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