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Allelopathic Potentials of *Larix leptolepis* on Germination of Several Forest Tree Species[1]

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몇가지 森林樹種 種子發芽에 對한 落葉松葉 抽出物의 Allelopathic 效果[1]

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ABSTRACT

Inhibitory effect of *L. leptolepis* fallen leaf extracts on the germination of *Pinus densiflora*, x *Pinus rigitaeda*, *Pinus rigida*, *Pinus thunbergii* and *Larix leptolepis* was investigated. Germination of those seeds in culture room and in pot at field showed the highly significant inhibitory effects by the aqueous extracts from *L. leptolepis* fallen leaves. Among them *L. leptolepis* was the most severely inhibited by the aqueous extracts used.

To identify allelopathic substances, thin-layer chromatography was employed. Gallic, ferulic, t-cinnamic and vanillic acids were identified from fallen leaves of *L. leptolepis*.

From the results, it is assumed that autotoxicity of *L. leptolepis* may act as inhibitory factors on germination of the species in natural stands.

Key words : *allelopathic potentials* ; Larix leptolepis ; *leaf extracts* ; *germination* ; *thin-layer chromatography* ; *natural regeneration*.

要 約

우리나라 重要造林 樹種의 하나이며 天然更新이 어려운 것으로 알려져 있는 낙엽송의 落葉으로 부터 時間別로 水溶性 抽出液을 造製하여 主要森林 樹種 種子의 發芽에 미치는 영향에 對해 調査하였다. 發芽實驗은 培養室과 野外에서 實施하였다. 또한 發芽實驗 結果 抑制 現象을 일으키는 것으로 抽定되는 物質을 박층크로마토그라피를 利用하여 分析하였다.

그 結果 室內 實驗에 있어서 抽出 時間別에 따른 效果는 없었으며 落葉松 種子 자체가 가장 심한 發芽 抑制 現象

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을 나타 냈다. 野外 Pot 實驗에서는 리기다 소나무를 除外한 소나무, 리기테다소나무, 해송 및 낙엽송의 種子 發芽가 현저하게 억제되었다. 特히 낙엽송 種子 發芽는 室內 및 野外 實驗에서 다같이 크게 억제되는 傾向을 보였다.

落葉松葉 抽出液의 物質分析 結果 9가지 物質이 檢出되었으며 그중 gallic 酸, ferulic 酸, vanillic 酸과 tcinnamic 酸등 4가지 페놀 酸이 同定되었다.

INTRODUCTION

Since Molish (1937) adopted the term allelopathy as refering to biochemical interactions between all types of plants including microorganisms, several experimental researches have been performed to find out the influence of this activity in a community. Rice (1984) defined allelopathy as any direct or indirect harmful effect by one plant on another through production of chemical compounds that escape into the environment. There are thousands of such compounds, but only limited number of them have been identified as toxins involved in allelopathy. Phenolic compounds, terpenes, alkaloids, flavonoids, cyanogenic glycoside are generally known allelopathic substances (Rice, 1979 ; Swain, 1977).

Not so many researches have been conducted on allelopathic effects of various woody species. Allelopathy is a cause of slow forest regeneration, there remains a paucity of supportive evidence based on field experimentation. Peterson (1965) reported that *Kalmia angustifolia* occurs frequently on upland sites in eastern Canada, and that there is abnormally poor tree growth on such sites. Mensah (1972) reported that *Acer pseudoplatanus* is allelopathic to *Betula alleghaniensis*. Lodhi (1975) observed that *Celtis laevigata* leaves, leaf leachates, and soil collected from under *Celtis laevigata* significantly reduced seed germination and seedling growth of test species. Ferulic, caffeic, gentisic and p-coumaric acids and scopolin and scopoletin were identified as phenolic phytotoxins produced by *Celtis laevigata*.

In Korea, there are some papers dealing with allelopathic effects of forest species (Kil and Yim, 1983 ; Ko and Kil, 1985 ; Seo, 1985). However, reports have not been found in which possible allelopathic effects of forest tree species were taken into consideration on reforestation or natural regeneration of planted areas in our country.

Larix leptolepis has been planted all over the country but does not grow well and regenerate in the same site it was once planted. This study was undertaken to examine the possibility that leaf extracts of *L. leptolepis* may exert their influence to germination of some common forest species including *L. leptolepis* in our country, and to analyze inhibitory substances if exist.

MATERIALS AND METHODS

Fallen leaves of *L. leptolepis* were collected in late March 1986 in pure stands of 40 year old in average age located at Seoknam Temple in Ulju-Gun, Gyeongsangnam-Do. Aqueous solutions were prepared by soaking 200g of fallen leaves of larch in respective 1,000 ml of water bath for each 24, 48, 72, 96, and 120 hours at room temperature. Seeds used for germination test were supplied from Gyeongsangnam-Do Branch Office of National Federation of Forest Associations Union and from Forest Research Institute in Seoul, Korea.

We have two ways for germination test, one was in culture room, the other was in pot at field. In culture room, twenty seeds of each three species were put on the filter paper wet with 5 ml of each solutions in 12 cm diameter petri dish, respectively three replications. Germination rates were investigated for three weeks. Control was treated in the same manner using distilled water. All tests were performed in culture room at 26±1℃. In pot test at field, each vinyl pot (7 cm diameter, 20 cm height) was filled by the height 8 cm with soils collected from under larch stand, and then the surface of soils was covered with ca. 1.5 cm thickness of fallen leaves of larch. Before pot soils were covered with fallen leaves, thirty seeds of each five test species including *Pinus rigida* and *P. thunbergii* add to

three species of culture room test were placed on 3 different locations, i.e. on soil surface, on, and in middle of fallen leaves respectively. Two more species as common forest species in our country were added to pot experiments to know their response. It might be helpful to select suitable species to planting site after harvesting larch trees. Twenty four hours extracts was applied to each pot every day, while control was supplied with only water. Each experiment was repeated three times and germination was recorded after three weeks.

To determine phytotoxins in germination tests, fallen leaves collected were air-dried for 2 to 3 days in shade and stored 0℃ below. The extraction procedures for phenolic compounds are shown in Fig. 1 as same method by Krygier *et al*. (1982). Thin-layer chromatography was empolyed for the isolation and identification of phenolic compounds. The solvent system adopted for separation of phenolic compounds were benzene-methanol-acetic acid (20 : 4 : 1 and 15 : 8 : 2). After first and second developments, Rf values were measured and compared with those of authentic phenolic compounds.

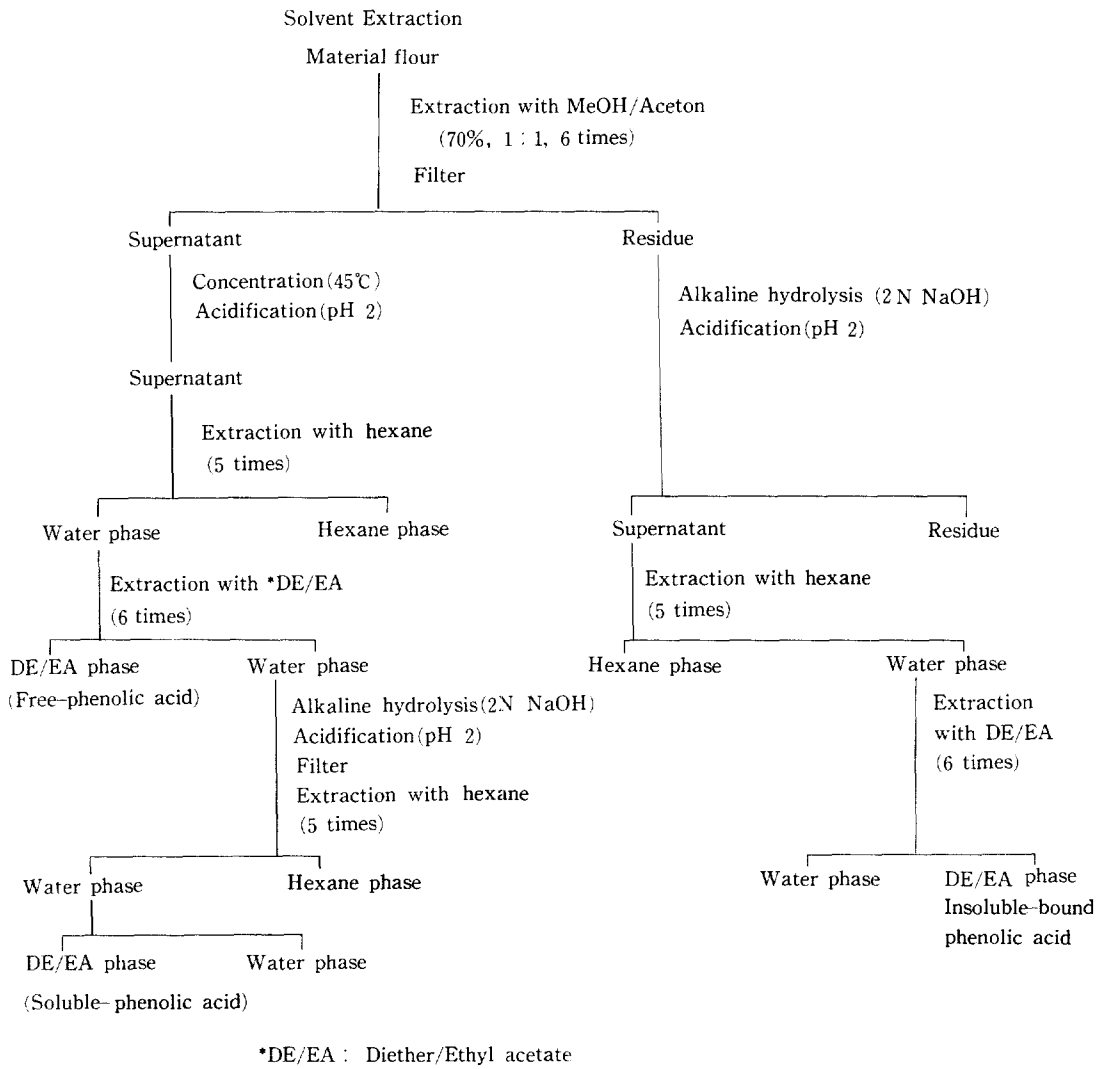

Fig. 1. Extraction procedure for phenolic compounds from fallen leaves of *Larix leptolepis*.

Table 1. Germination percentages of three forest seeds in the presence of aqueous extracts from fallen leaves of *Larix leptolepis*

Species / Extracted time (hours)	*Pinus densiflora* (%)	x *Pinus rigitaeda* (%)	*Larix leptolepis* (%)
24	23.5a*	41.5a, c	1.5a
48	31.2a	26.5a, b	0.0a
72	35.0a	28.0a, b	0.0a
96	26.5a	15.0b	0.0a
120	30.0a	18.0b	1.5a
Control	45.0b	40.0c	10.0b

*Values followed by different letters are significantly different at P=0.05.

RESULTS AND DISCUSSION

The effect of *L. leptolepis* fallen leaves extracts on germination of three test species is shown in Table 1.

While x *Pinus rigitaeda* showed no significantly different germination rate between treated and control solution, *Pinus densiflora* and *Larix leptolepis* were inhibited for the germination. Especially that of *L. leptolepis* was deeply inhibited by extracts of its fallen leaves.

These results are well in accord with those of Ko and Kil (1985). They proved that fresh leaf, root, and stem leachates of *Larix leptolepis* exert allelopathic potentials on germination and growth of test species; *Larix leptolepis*, *Rhododendron yedoense* and *Amorpha fruticosa* etc.. Accordingly, it is probable that *L. leptolepis* produces autotoxins.

No significant differences were obsreved in inhibitory effects on seed germination of all test species in the presence of the fallen leaves solutions for 24 to 120 hours, except x *Pinus rigitaeda* which revealed no significant difference on the germination between extracted and control solution. The longer extracting period was, the heavier the brown color of each solution was. It can be supposed that most of water-soluble allelopathic compounds in fallen leaves of *L. leptolepis* leach out within 24 hours. There are many researches reported about allelopathic effects of leaf leachates of woody species on germination or radicle growth of selected species (Jobidon, 1986; Gabor and Veatch, 1981).

Table 2 showed the result of seed germination of *Pinus densiflora*, x *Pinus rigitaeda*, *Pinus rigida*, *Pinus thunbergii*, and *Larix leptolepis* in vinyl pots filled with transported field soil and fallen leaves of larch.

There was no significance in four species of the rate of germination between on soil surface and in leaves except *Pinus densiflora*. On leaves the rate of germination was highly inhibited about all test tree species. The germination of larch seeds on the leaves was found to be greatly inhibited among all

Table 2. Germination percentages of five test species by different sowing locations on transported field soils and fallen leaves

Species / Treatment	*Pinus densiflora* (%)	x *Pinus rigitaeda* (%)	*Pinus rigida* (%)	*Pinus thunbergii* (%)	*Larix leptolepis* (%)
On soil surface	26.7a*	48.9a	91.1a	28.9a	15.5a
In leaves	10.0b, c	42.2a	95.6a	28.9a	15.6a
On leaves	4.4c	17.8b	73.3b	17.8a	0.0b
Control	50.0d	73.3c	96.7a	60.0b	40.0c

*Values followed by different letters are significantly different at P=0.05.

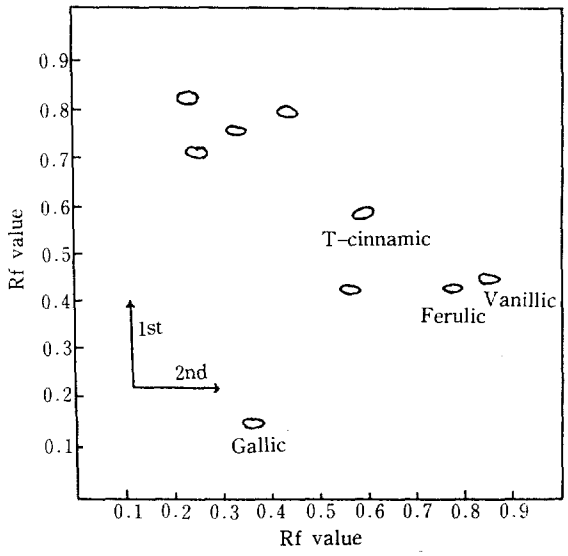

Fig. 2. Thin-layer chromatogram of phenolic compounds in the leaf extracts of *L. leptolepis*

test species.

Apart from the fact that pine species exert their inhibitory effects on other weeds and woody species (Fisher, 1980; Gabor and Veatch, 1981), it is noticeable that *L. leptolepis* might exude autotoxic substances. Bevege (1968) suggested that the autotoxicity of hoop pine (*Araucaria cunninghamii*) may help to explain its slow regeneration in a hoop pine rainforest. Chu-Chou (1978) reported growth retardation of roots from old *P. radiata*. Ko and Kil (1985) showed the possibility of autotoxicity of *L. leptolepis* in their experiments. Thus, it is assumed that the autotoxicity may act as one of factors affecting failure of natural regeneration of *L. leptolepis*.

To analyze phytotoxins of fallen leaves of *L. leptolepis*, thin-layer chromatography was employed. Nine spots were found out on the TLC plate (Fig. 2). Among them four substances were identified by comparision with Rf values of authentic phenolic compounds i.e. gallic, ferulic, vanillic, and t-cinnamic acids (Table 3).

Rice (1984) described that phenolic acids such as p-hydroxybenzoic, vanillic, p-coumaric, ferulic acids have been the most commonly identified allelopathic compounds produced by higher plants. He mentioned also that tannins and cinnamic acid have been identified as growth and germination

Table 3. Rf value of phenolic compounds (standard) and *L. leptolepis* leaf extracts by thin-layer chromatography

No. Chemical Substance	Standard		*L. leptolepis*	
	BMA (20:4:1)	BMA (15:8:2)	BMA (20:4:1)	BMA (15:8:2)
Gallic acid	0.18	0.34	0.16	0.34
Ferulic acid	0.45	0.79	0.43	0.76
Vanillic acid	0.46	0.83	0.46	0.85
T-cinnamic acid	0.56	0.60	0.57	0.63

inhibitors in serveral plants. Lodhi (1976) isolated inhibitors from aqueous extracts of red oak leaves such as caffeic acid, ferulic acid and etc.. Kill and Yim (1983) reported that benzoic and 11 phenolic acids were identified from pine leaves by gas chromatography.

Accordingly, it is certain that the phenolic acids identified in this experiment would act as inhibitors in the germination of the test species.

CONCLUSION

This study was undertaken to evaluate inhibitory effect of *L. leptolepis* fallen leaf extracts on the germination of some coniferous species planted very widely in Korea. Germination percentages of the test coniferous species in growth chamber showed singnificant inhibitory effects but not difference leaching time. *L. leptolepis* was the most severely inhibited among the test species by the aqueous extracts used. The similar tendency was observed in the pot experiments used as transported field soils and fallen leaves of *L. leptolepis*.

To identify the allelopathic substances, thin-layer chromatography was employed. Gallic, ferulic, t-cinnamic, and vanillic acids were isolated from fallen leaves of *L. leptolepis*. It is assumed that autotoxicity of *L. leptolepis* may act as a factor affecting failure of natural regeneration of the species.

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