Allelopathic Effects of Fir Tree (*Abies holophylla*)

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ABSTRACT : It was found that seed germination and seedling growth of selected species were inhibited by phytotoxic substance released from fir trees. The aqueous extracts of leaves caused significant inhibition in the seed germination of the receptor plants, Whereas seed germination of some species was not inhibited in extracts of leaves, but seedling elongation of the receptor plants was also decreased by the aqueous extract. Dry weight growth was slightly increased in lower concentration of the extract, while that was proportionally inhibited by higher concentration of the extracts. Chemical substances of fir trees were shown the biological toxicity. The GC method was employed for analysis of phytotoxic chemicals and sixteen chemical substances were identified such as benzoic, phenylacetic, etc. Also 33 volatile substances were identified from the fir tree. These chemical compounds are assumed the substances related to allelopathic potential of *Abies holophylla* plant species.

Key words : *Abies holophylla*, Allelopathic effect, GC, Water extract

INTRODUCTION

If a plant produces a chemical which suppresses the growth of other plants, the phenomenon is called allelopathy. Normally, the effect is harmful, but beneficial effect is possible (Newman 1978).

Allelopathy is expressed through the release of allelochemicals by the donor plant in the vicinity of a receptor plant species. Aside from their many roles in allelopathy - influencing soil microbial ecology, nutrient dynamics, and other abiotic and biotic factors - allelochemicals play key roles in structuring of other trophic levels, especially affecting predators, pests and mediating competitive circumstances (Dakshimi *et al*. 1999).

Fir trees are widely distributed high mountain, green field, gardens in Korea and are known to exhibit allelopathic effects or produce a number of other terpenoid allelochemicals.

Allelopathic chemicals are released from plant tissues in a variety of ways, including exudation of volatile chemicals from living plant parts (Muller *et al*. 1964, Whittaker 1971, Rice and Pancholy 1972, Putnam and Duke 1978, Lodhi and Killingbeck 1980, Putnam 1983, Kil and Yim 1983, Heisey and Delwiche 1984, Rice 1984), exudation of water soluble toxins from ground parts, leaching of water soluble chemicals from above ground parts in response to the action of rain, fog or dew (Muller 1966, Al-Naib and Rice 1971, Kumari and Kohli 1987).

It has been shown that pine needle causes a toxic effect on the growth of some plants such as *Oenothera odorata* and *Celosia argentea* than *Aster tartaricus* and *Platycodon grandiflorum* (Kil, 1982). But there has been no report on the fir tree concerning phytotoxic effect.

The purpose of this study is to get some evidences for allelopathic characteristics of fir trees : (1) growth inhibitor has been studied indirectly by allelopathic experiments (2) identification of the chemical substances of fir trees by GC / MS.

MATERIALS AND METHOD

Water Extracts of the Fir Tree

Fresh leaves of fir tree were collected from mid part of the tree, in August, 2002, at garden of Wonkwang Health College and kept in refrigerator at 0℃ to 2℃ until experiment use.

It was selected by hand for good leaves. 100 g of fir tree green leaves were put into 2000 ml of distilled water, so that water extracts could be obtained from different extracting time, that is, 24, 48, 72, 96, 120 hrs, respectively. After two sheets of filter paper (11 cm in diameter) were spread on petri plate (12 cm in diameter) 100 g of sample seeds such as *Siegesbeckia glabrescens*, *Lactuca sativa*, *Oenothera odorata*, *Rumex acetosella*, *Arundinella hirta* and *Achyanthes japonica* were sown on it, 100 ml of water extracts were applied to each petri plate and then the controls were treated in the same way except that distilled water (DW) was used instead of the water extract.

All plates were sealed and kept in a 25℃ growth chamber. Used seeds for this experiment were provided from collection in the field

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by authors. And the seeds were selected by hand carefully.

The water extract and DW were applied to each plate every two days and seed germination rate was recorded every day. This experiment was repeated four times for each tested species. Other information, for example, experimented condition on seeds, germination, and leaf of fir tree were followed as Kil (1982).

And the results of experiments were determined by counting the number of germinated seeds, measuring the whole length of seedlings in millimeter and weighing of seedling dry weight, respectively. From these data the relative germination ratio (RGR), relative elongation ratio (RER) and relative dry weight ratio (RWR) were calculated according to Kil and Yun`s method (1992) by following equation :

RGR = germination percentage of tested plant / germination percentage of control × 100

RER = mean length of tested seedlings / mean length of control seedlings × 100

RWR = dry weight of tested seedlings / dry weight of control seedlings × 100

All the results of the experiments were means of four replicates. The analysis of water extract of the fir tree was performed on gas chromatography qualitatively a Hewlett-Packard 5890 using SE-54 column (50 cm×0.33 μm×0.2 mm, i.d). Temperature was programmed from 45℃(5 min) to 300℃(3 min) at 4℃/min. Carrier gas was helium, flow rate, 0.5 ml/min, with FID. Injector temperature was 250℃. Split ratio was 1:10 and head pressure was 34 psi. Injection volume of all samples was 0.20 μl. Identification of each peak was made by the comparison of retention times and mass spectra of the peaks with those of commercial compounds obtained from Sigma, Aldrich and Fluka chemical companies.

RESULTS AND DISCUSSION

Germination and Seedling Growth

The germination of six species, including *Siegesbeckia glabrescens* were determined using water extracts of fresh leaves of fir trees (Fig. 1). All of these except *Arundinnella hirta* showed an inhibition effect of RGR. In general, the more was the concentration of the extracts in extraction time, the worse was germination effect of the selected species. In other words, the chemical substances from the fir tree had the biological toxic activity.

Kil and Yim (1983) also reported the suppression of herbaceous species by *Pinus densiflora* to allelopathy.

The seedling growth of *Lactuca sativa* used in this experiment was significantly lowered when they were cultured in different concentrations of the exacts of fir tree leaves. But the others were not much or stimulated their seedling growth comparing with water extracts of the fir tree (Table 1).

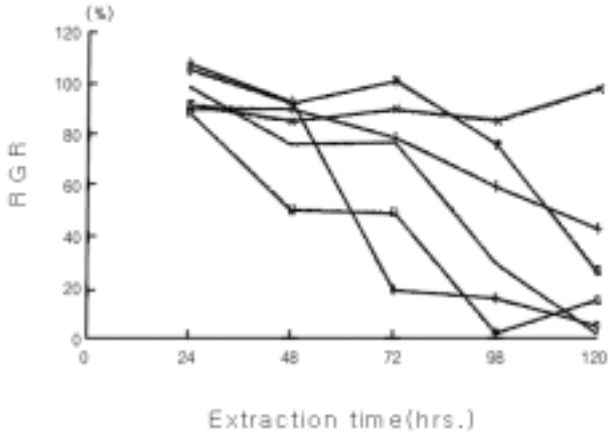

Fig. 1. Relative germination ratio (RGR) of receptor plant tested in extracts of *Abies holophylla* soaked for various length of time. Key to species : —●— , *Siegesbeckia glabrescens* ; —+—, *Lactuca sativa* ; —✳— , *Oenothera odorata* ; —□— , *Rumex acetosella* ; —×— , *Arundinnella hirta* and —◇— , *Achyranthes japonica*.

Seedling growth in dry weight cultured with water extracts of the fir tree was showed different ways in three patterns, that is, stimulated growth as *Arundinella hirta*, slightly responded growth according to extraction hours of the fir tree leaves, the other species were remarkably inhibition effect of seedling growth treated in the extracts comparing with the control (Fig. 2).

Kil and Yun (1992) stated that the tested species could be classified into three types according to the sensitivity of receptor plants to the wormwood toxicity.

The first group, susceptible species, showed low germination and seedling in wormwood extracts. The second group, nonsusceptible species, showed comparatively high germination and seeding growth

Table 1. Seedling length (mm) of receptor plants grown in petri dishes at different concentrations of *Abies holophylla* leaves extract

	Control	Extraction time (hrs.)				
		24	48	72	96	120
Siegesbeckia glabrescens	4.2[a]	4.5[a]	3.6[c]	3.2[d]	2.5[e]	2.1[f]
Lactuca sativa	8.2[a]	7.7[a]	6.3[ab]	6.5[ab]	5.3[b]	4.9[b]
Oenothera odorata	4.2[b]	4.4[a]	4.1[b]	2.3[c]	2.4[c]	1.3[d]
Rumex acetosella	4.1[a]	4.1[a]	4.4[a]	4.3[a]	3.6[a]	3.2[a]
Arundinella hirta	7.3[a]	8.7[a]	8.7[a]	8.8[a]	7.5[a]	7.3[a]
Achyranthes japonica	8.8[a]	8.7[a]	8.4[a]	8.5[a]	8.1[a]	8.2[a]

[a] Mean followed by the same letter are not significantly different at the 5% level by Duncan's multiple-range test.

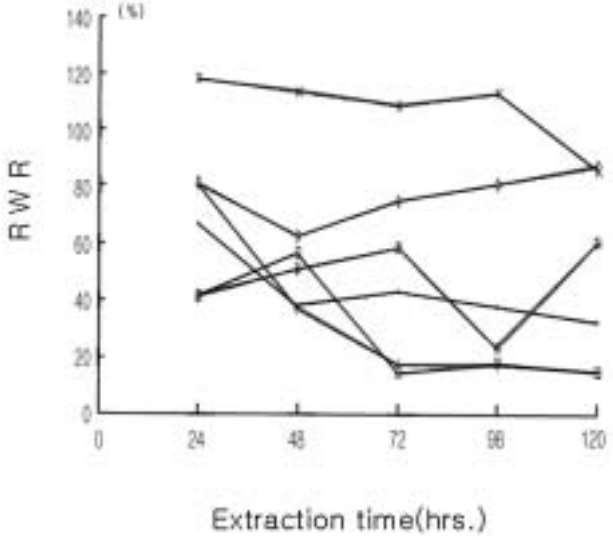

Fig. 2. Relative dry weight ratio (RWR) of receptor plants grown in pot at different concentration of *Abies holophylla* leaves extract. Key to species : —●—, *Siegesbeckia glabrescens* ; —+—, *Lactuca sativa* ; —✳—, *Oenothera odorata* ; —□—, *Rumex acetosella* ; —×—, *Arundinella hirta* and —◇—, *Achyranthes japonica*.

in the extracts. The third group, intermediate species, showed moderate germination and seedling growth in the extracts.

The GC/MS methods were employed for the analysis and identification of phytotoxic substances from the tree leaves. Sixteen chemical compounds were identified from water extracts of *Abies holophylla* leaves (Fig. 3). This shows relative amount, relative retention time and frequency of the compounds, e. g. benzoic acid, phenolic compounds such as p-hydroxy benzoic acid, gentisic acid, syringic acid and p-coumaric acid were markedly present in water extracts of *Abies hollophylla* leaf. These compounds would be responsible for the phytotoxic effects of the fir tree in this study. It is well known that phenolic compounds are a main inhibitor of seed germination and seedling growth (Olmsted and Rice 1970, Whittaker 1971). From red pine, benzoic acid and eleven kinds of phenolic compounds have been identified by GC (Kil and Yim 1983).

In shorts, the phytotoxins of fir tree have inhibitory effects on the seed germination and seedling growth of different herbaceous plants in higher concentration of the extracts, but it was not much inhibited at lower one. Therefore, the water extracts and phenolic compounds of fir tree are considered to be important allelochemicals.

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Fig. 3. Gas chromatogram obtained from a sample of the combined water extracts of *Abies holophylla* leaf.
1, benzoic acid; 2, phenylacetic acid; 3, catechol; 4, salicylic acid; 5, t-cinnamic acid; 6, m-hydroxybenzoic acid; 7, p-hydroxybenzoic acid; 8, phlorogluicinol; 9, p-phenylbenzaldehyde; 10, vanillic acid; 11, gentisic acid; 12, protocatechuic acid; 13, syringic acid; 14, p-coumaric acid; 15, ferulic acid and 16, caffeic acid. Other peaks in the chromatogram did not appear regularly from one analysis to another.

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