

Phytotoxic Effects on Selected Species by Chemical Substances of *Artemisia princeps* var. *orientalis*

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쑥(*Artemisia princeps* var. *orientalis*)에 들어있는 화학물질이 다른 식물에
미치는 독성 효과

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ABSTRACT

To verify allelopathic effects of *Artemisia princeps* var. *orientalis*, a number of laboratory experiments have been performed. Aqueous extracts of leaves, stems and roots of the above species inhibited the seed germination and seedling growth of experimental species, receptor plants. In general, the higher was the concentration of the extracts, the lower was the germination and the growth ratio. Volatile substances released from leaves of the donor plants also inhibited the seed germination and the radicle elongation of receptor plants. Therefore, to find out the inhibitory substances emitted from the wormwood, gas chromatography was employed. As a result of the analysis, 17 chemical substances were isolated from the leaves and most of them were identified as phenolic compounds.

INTRODUCTION

A plant is influenced by a multiplicity of environmental factors, chemical inhibition being only one of many (Hoffman and Hazlett, 1977). Allelopathy, the inhibition of one plant by another through the production and release of toxic organic compounds, is believed to influence vegetation patterning, production, and nutrient cycling in many plant communities (Muller, 1966; Whittaker, 1971; Rice and Pancholy, 1972; Putnam and Duke, 1978; Lodhi and Killingbeck, 1980; Putnam, 1983; Kil and Yim, 1983; Rice, 1984). Both water-soluble and volatile inhibitors have been implicated (Whittaker, 1970; Muller and Chou, 1972; Horsley, 1977; Hoffman and Hazlett, 1977). McPherson and Muller (1969) and Christensen and Muller (1975) have presented strong evidence that toxic materials leached out of leaves by rain are at least part of the reason for poor herb growth under shrubs.

The role of metabolic products in various forms of growth inhibition has been reviewed extensively since 1950 (Börner, 1960). In general, the isolated chemical inhibitors belong to a group of materials known as secondary plant compounds (Moreland *et al.*, 1966; Whittaker and Feeny, 1971; Chandramohan *et al.*, 1973; Robinson, 1983; Swain, 1977). Those implicated as effective allelopathic agents include simple phenolic acids, coumarins, flavonoides, alkaloids, cyanogenic glycosides, and glucosinolates (Moreland *et al.*, 1966; Whittaker, 1970).

The genus *Artemisia* (Compositae tribe Anthmideae) belongs to the useful aromatic and medicinal plants comprising about 300 species which are found in the northern hemisphere (Weyerstahl *et al.*, 1987). In Korea, 30 taxa were reported (Lee, 1979). A species of the genus well known for allelopathy is *A. absinthium* which Bode (1939) and Funke (1943) found to be toxic to some plants but not to others. Also seed germination and seedling growth are inhibited by volatile (Klarich and Weaver, 1973) and nonvolatile substances (Schlatterer and Tisdale, 1969; McCahon *et al.*, 1973) produced by *A. tridentata*.

This study was focussed to determine the effect of water-soluble and volatile inhibitors from *A. princeps* var. *orientalis* on the seed germination and seedling growth of other species, either species. Such tests must be interpreted with caution since the conditions are very different from those in the field (Grümmer, 1961; Harper, 1977; Stowe, 1979), however, they may be useful in a preliminary way to identify potentially toxic plant species. The results of this study are intended to imply the occurrence of allelopathy of *A. princeps* var. *orientalis*.

MATERIALS AND METHODS

To determine the allelopathic effects of *Artemisia princeps* var. *orientalis*, the donor species, the plants were collected in the fields around the Wonkwang University campus. Seeds used for germination and seedling growth experiments, receptor species, were collected in the same places and/or bought from seed company.

Germination and Seedling Growth in Extracts

Leaves, stems and roots of *A. princeps* var. *orientalis* were separated and chopped into small pieces. Two hundred grams of each samples were soaked in 1,000 ml of water at 20°C for 24 hrs. Each aqueous extracts were filtered through sieve and the filtrates were diluted to 10, 30, 50 and 70% of the extracts. And the original filtrate was used as 100% extract (pH 5.2). Fifty seeds of the different species were placed on filter paper which was on Petri dishes (ϕ 12 cm), wetted with the extracts and germinated at 20°C. Each experiment was performed four replications for each receptor species. The controls were treated in the same way except that D.W. was used instead of the aqueous extracts. The experiment extened over a period of 10 days to allow maximum seed germination. The results were taken by counting seed germination and measuring the length of seedling growth in millimeters.

In seedling growth test seeds were sown in plastic pots with vermiculite. After germination was completed, the plants were thinned to the ten largest seedling, and were grown for 4-5 weeks. The extracts or water were poured into each pot every 2-3 days. The results were taken by measuring the total length of seedling growth and dry weight. Ratio of germination, elongation, and dry weight were calculated as follows:

$$\text{Relative Germination Ratio (RGR)} = \frac{\text{Germination ratio of test}}{\text{Germination ratio of control}}$$

$$\text{Relative Elongation Ratio (RER)} = \frac{\text{Mean length of test}}{\text{Mean length of control}}$$

$$\text{Relative Dry Weight Ratio (RWR)} = \frac{\text{Dry weight of test}}{\text{Dry weight of control}}$$

Germination and Radicle Growth in Volatile Substance

To test the effects of volatile substances of the *A. princeps* var. *orientalis* chamber and contents were modeled after the setup described in Baker (1966) (Fig. 1). In the glass chamber (1,800 ml) the fifty seeds of the test species were placed on the filter paper which was on the absorbent cotton was moistened sufficiently. Sliced leaves of *A. princeps* var. *orientalis* in different quantities (5, 10, 15, 20, 25, 30 g) were placed in glass beakers (100 ml) standing in the glass chamber. Control was the same setup with an empty beaker with no leaves in it. The chamber was covered with vinyl wrap and placed at room temperature (ca. 25 °C). After 2-4 days of sowing germination was checked and radicle elongation was measured. The results were calculated as RGR and RER.

Identification by Gas Chromatography

The analysis of extract was performed using a gas chromatograph (Hewlett Packard 5890, U.S.A.) equipped with J & W fused silica capillary column, methylsilicone banded column (B-1) 60 m, 0.25 mm i.d. Oven temperature was 100-300°C with 4°C/min. The head pressure of column was 30 psi and split ratio was 1:50. Integrator is Hewlett Packard 3392A and sample injection volumn was 0.5-1.0 µl. Otherwise, the extraction and procedures of phytotoxic substances and analysis by gas chromatography were as previous study (Kil and Yim, 1983).

RESULTS

Germination and Seedling Growth in Extract

Aqueous extracts of *Artemisia princeps* var. *orientalis* leaves, stems, and roots have shown inhibitory effect on germination and elongation of test plants (Fig. 2: Table 1,2,3). Germination of *Achyranthes japonica*, *Chrysanthemum boreale*, *Plantago asiatica* seeds was reduced severely by

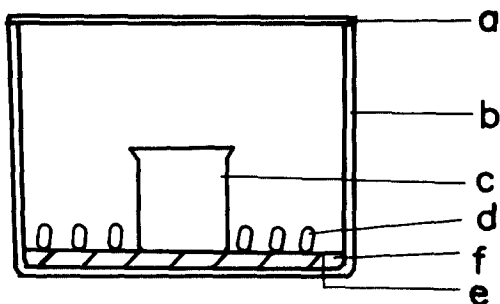


Fig. 1. Diagrammatic section through test chamber: a, vinyl wrap; b, glass chamber; c, container for wormwood leaves; d, test seeds; e, filter paper; f, absorbent cotton, moistened with water.

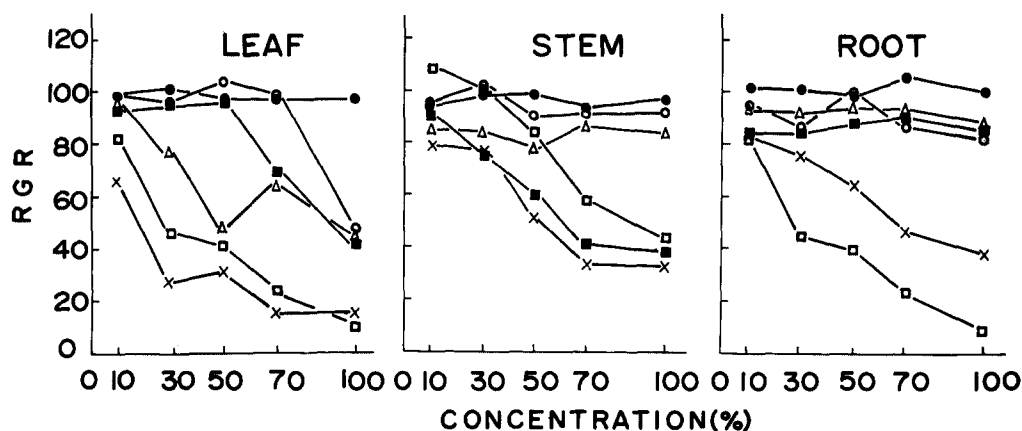


Fig. 2. Relative germination ratio of selected species tested in Petri dish with aqueous extracts from *Artemisia princeps* var. *orientalis*. ●—●, *Echinochloa crus-galli*; ○—○, *Oenothera odorata*; X—X *Achyranthes japonica*; □—□, *Chrysanthemum boreale*; ■—■, *Hordeum vulgare* var. *hexastichon*; △—△, *Lactuca sativa*.

the leaf extract in accordance with the concentration. Especially seeds of *P. asiatica* showed no germination at all in 70 and 100% extracts.

Echinochloa crus-galli was scarcely influenced by the extracts of leaves, stems and roots of *A. princeps* var. *orientalis* (Fig. 2 and Table 1,2,3). In the extract of stems, germination of *C. boreale* seeds were promoted slightly in 10% and 30% extracts. While it was inhibited gradually from 50% to 100% in extract concentration. *Oenothera odorata* showed above 90% RGR in all test extracts while *A. japonica*, *C. boreale* and *Hordeum vulgare* var. *hexastichon* were inhibited severely in high concentrations, 50, 70, and 100% extract. In root extract, *A. japonica* and *C. boreale* were heavily inhibited, and the other four plants didn't respond to toxic effect from the donor plant.

Elongation of seedling grown in Petri dishes with leaf extract was mostly inhibited (Table 1). It showed a very similar tendency to the stem extract (Table 1,2). Seedling growth of *E. crus-galli* was little influenced in leaf, stem, and root extracts.

Table 1. Mean seedling length (mm) of experimental species tested in Petri dish with different concentrations of wormwood leaves extracts

No Species	Control	Concentration (%)				
		10	30	50	70	100
1 <i>Echinochloa crus-galli</i>	55.4 ^a	67.4 ^a	60.6 ^a	53.9 ^a	48.6 ^{ab}	28.0 ^b
2 <i>Oenothera odorata</i>	23.8 ^a	23.0 ^a	20.9 ^a	18.0 ^{ab}	11.7 ^{bc}	10.3 ^c
3 <i>Achyranthes japonica</i>	55.2 ^a	34.7 ^b	34.9 ^b	35.7 ^b	30.0 ^{bc}	18.0 ^c
4 <i>Chrysanthemum boreale</i>	23.0 ^a	17.3 ^b	13.7 ^{bc}	10.6 ^c	9.8 ^c	9.7 ^c
5 <i>Hordeum vulgare</i> var. <i>hexastichon</i>	57.9 ^a	54.7 ^{ab}	39.9 ^{abc}	30.4 ^{bc}	29.9 ^{bc}	18.6 ^c
6 <i>Lactuca sativa</i>	82.0 ^a	88.8 ^a	67.1 ^b	37.3 ^c	58.2 ^b	39.4 ^c

*Means within rows followed by same letters are not significantly different at the 5% level by Duncan's multiple-range test. LSD to each species (No) correspond to 23.72, 6.32, 13.25, 3.72, 23.20, 10.50, respectively.

Table 2. Mean seedling length (mm) of experimental species tested in Petri dish with different concentrations of wormwood stems extracts

No Species	Control	Concentration (%)				
		10	30	50	70	100
1 <i>Echinochloa crus-galli</i>	58.9 ^{ab}	73.6 ^a	61.3 ^{ab}	48.8 ^{ab}	50.5 ^b	49.6 ^b
2 <i>Oenothera odorata</i>	25.9 ^a	27.5 ^a	27.0 ^a	24.3 ^a	22.6 ^a	20.0 ^a
3 <i>Achyranthes japonica</i>	47.8 ^a	44.7 ^{ab}	34.9 ^{bc}	35.9 ^{bc}	31.3 ^c	28.5 ^c
4 <i>Chrysanthemum boreale</i>	23.5 ^a	19.1 ^b	17.7 ^{bc}	15.0 ^{bcd}	13.9 ^{cd}	11.3 ^d
5 <i>Hordeum vulgare</i> var. <i>hexastichon</i>	67.7 ^a	57.9 ^{ab}	43.8 ^{bc}	38.1 ^{bcd}	26.1 ^{cd}	18.1 ^d
6 <i>Lactuca sativa</i>	76.4 ^a	85.0 ^a	79.8 ^a	76.5 ^a	83.5 ^a	67.3 ^b

*Means within rows followed by same letters are not significantly different at the 5% level by Duncan's multiple-range test. LSD to each species (No) correspond to 16.17, 9.28, 10.13, 3.92, 21.49, 8.23, respectively.

Table 3. Mean seedling length (mm) of experimental species tested in Petri dish with different concentrations of wormwood roots extracts

No Species	Control	Concentration (%)				
		10	30	50	70	100
1 <i>Echinochloa crus-galli</i>	60.9 ^{ab}	67.0 ^{ab}	69.2 ^a	63.8 ^{ab}	58.9 ^{ab}	54.4 ^b
2 <i>Oenothera odorata</i>	23.8 ^a	27.8 ^a	27.0 ^a	28.1 ^a	28.1 ^a	24.9 ^a
3 <i>Achyranthes japonica</i>	49.0 ^a	49.1 ^a	44.2 ^a	42.8 ^a	32.0 ^b	35.7 ^b
4 <i>Chrysanthemum boreale</i>	27.8 ^a	18.2 ^a	14.4 ^b	13.0 ^b	14.6 ^b	13.5 ^b
5 <i>Hordeum vulgare</i> var. <i>hexastichon</i>	64.0 ^a	65.1 ^a	55.0 ^a	53.0 ^a	48.1 ^a	43.2 ^a
6 <i>Lactuca sativa</i>	80.3 ^b	88.7 ^{ab}	92.8 ^a	93.3 ^a	95.6 ^a	89.0 ^{ab}

*Means within rows followed by same letters are not significantly different at the 5% level by Duncan's multiple-range test. LSD to each species (No) correspond to 13.21, 4.94, 7.04, 2.63, 22.51, 10.36, respectively.

The effect of aqueous extract on growth of seedling in pot is shown in Fig. 3. RER of lettuce grown in 10, 30, 50, 70, and 100% aqueous leaf extract was shown 77.1, 85.1, 79.1, 71.9, and 0.0% of control, respectively. The inhibition was generally stronger for leaf extracts than for stem extracts and/or root extracts. Growth in dry weight of lettuce seedling was greatly inhibited by leaf, stem and root extracts of wormwood plants. Comparing these results which elongation of

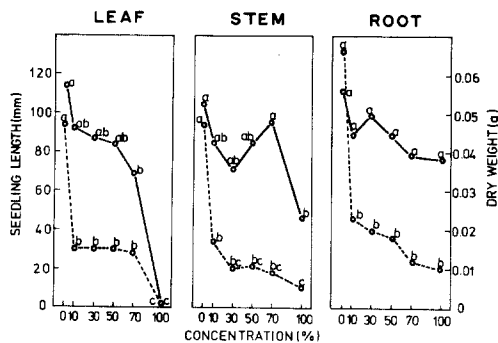


Fig. 3. Mean seedling length (mm) and dry weight (g) of lettuce tested in pot with aqueous extracts from wormwood. ○—○, Elongation; ○---○, Dry weight. The letters are compared within each series by one-way analysis of variance followed by Duncan's multiple-range test. Same letter indicated means that are not significantly different ($p < 0.05$).

the same species tested, dry weight was drastically decreased. Dry weight was more significant statistically than seedling elongation (Fig. 3).

Germination and Radicle Elongation in Volatile Substances

Germination responses in low concentrations such as 5, 10, 15, and 20 g/1,800 ml were generally not correlated with in high concentrations such as 25 and 30 g/1,800 ml of wormwood leaves (Fig. 4). Particularly the seeds of *Achyranthes japonica* and *Plantago asiatica* did not germinate at all in high concentrations such as 20, 25, 30 g/1,800 ml. In general the higher was concentration of volatile compounds, the lower was germination ratios in all test specis. At 25 and 30 g/1,800

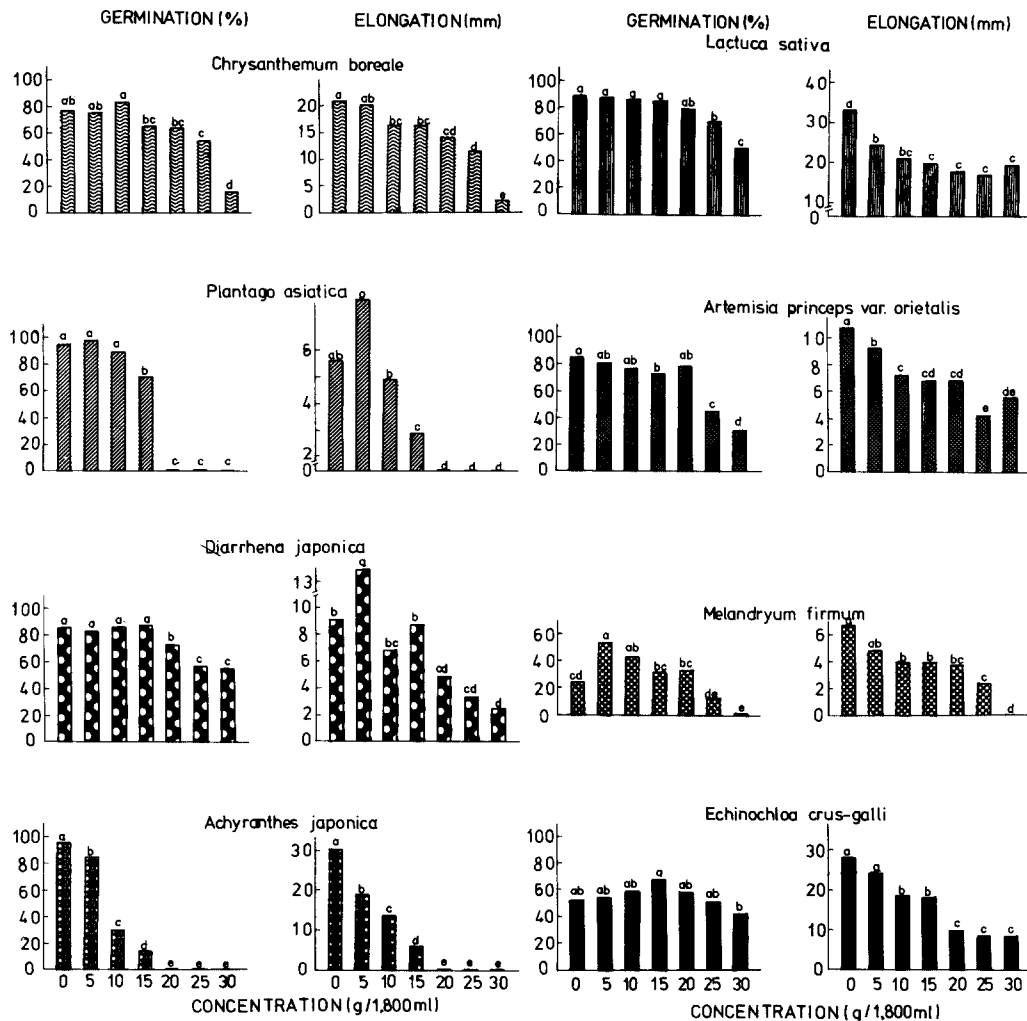


Fig. 4. Germination percentage (5, left) and mean radicle length (mm, right) of different species tested in volatile substances of wormwood leaves treating for various concentrations. The letters are compared within each series by one-way analysis of variance followed by Duncan's multiple-range test. Same letter indicated means that are not significantly different ($p < 0.05$).

ml, inhibitory effect was evident in most test species. RGR of *Echinochloa crus-galli* and *Melan-dryum firum* was generally promoted in 5, 10, 15, 20 g/1,800 ml, while that of *M. firum* represented 55.4% and 5.4% of control in 25 g and 30 g/1,800 ml, respectively.

Radicle elongation of all experimental plants were generally inhibited in volatile compounds of wormwood leaves except *Diarrhena japonica* and *Plantago asiatica* in 5 g and with statistical significance.

Identification of Allelochemicals by Gas Chromatography

Seventeen chemical compounds were identified in the leaf extract of *Artemisia princeps* var. *orientalis* by gas chromatography (Fig. 5).

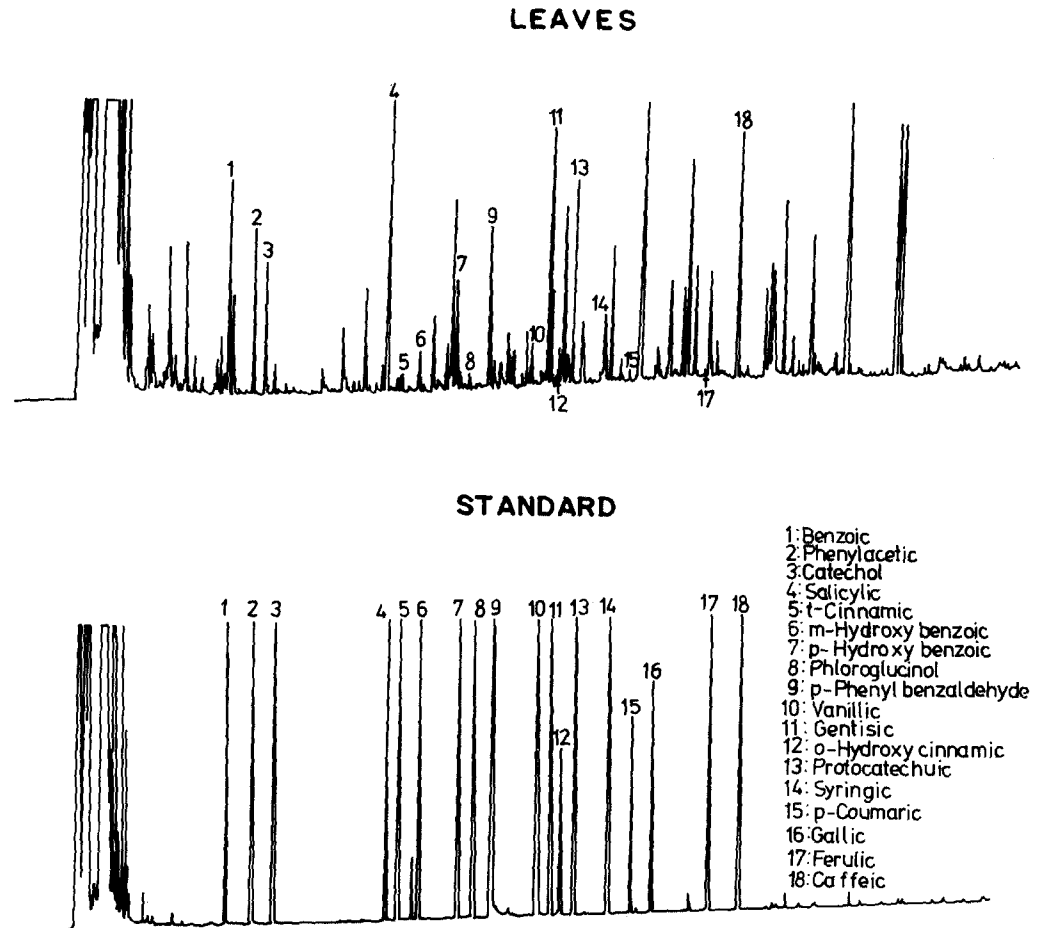


Fig. 5. Chromatograms of chemical substances from *Artemisia princeps* var. *orientalis* leaves by gas chromatography.

DISCUSSION

Results of these experiments suggested an allelopathic potential of aqueous extract and volatile substances from the wormwood, which significantly reduced seed germination and seedling elongation, although different species showed different responses.

Chemicals with allelopathic potential are present in virtually all plant tissues, including leaves, stems, roots, rhizomes, flowers, fruits, and seeds (Putnam, 1985). Ashraf and Sen (1978) concluded that the degree of inhibitory effect was in the order leaves>stems> roots which is similar to the result of present experiments.

In this study, generally the higher was the concentration of aqueous extracts, the greater was the inhibitory effect on the seed germination and seedling growth, which has similar tendency to the result of Kim (1988) in which the higher concentration of fallen leaves powder of 5 oak tree species is the lower RGR, RWR of test plants.

There have been many reports on volatile substances of *Artemisia* which affected on germination or seedling growth of other species, e.g. volatile substances inhibit seed germination, radicle elongation, and total seedling elongation, etc. (Funke, 1943; Halligan, 1975, 1976; Heisey and Delwiche, 1983; Hoffman and Hazlett, 1977; Muller *et al.*, 1964; Weaver and Klarich, 1977).

Muller and Muller (1964) identified terpenes, e.g. α -pinene, camphene, β -pinene, dipentene, cineole and camphor from extracts of fresh cut leaves of *Salvia leucophylla*, *S. apiana* and *S. melifera*. It has been demonstrated that these chemicals inhibit the growth of *Cucumis*. Tames *et al.* (1973) found that the tubers of yellow nutsedge contained compounds which inhibited the growth of oat coleoptile and the germination of seeds of seven crop species. It has been identified p-hydroxybenzoic, vanillic, syringic, ferulic and p-coumaric acid in the extract. Ethylene is the volatile allelochemical produced by various fruits, such as apples and pears (Molisch, 1937), and by decomposing litter of *Pinus radiata* (Lill and McWha, 1976). In this study, benzoic acid and 16 phenolic acids were identified by gas chromatography from *Artemisia princeps* var. *orientalis*.

摘 要

쑥에 들어있는 화학물질에 의한 allelopathy 효과를 조사하기 위해 잎, 줄기, 뿌리의 수용추출액으로 종자발아와 유식물생장 실험을 실시한 결과, 일반적으로 추출액의 농도가 높을수록 발아율과 생장율이 감소되었다. 또한 쑥의 잎으로부터 나오는 휘발성물질에서도 종자발아와 유근신장이 억제되었는데 실험식물의 종에 따라서 그 정도에는 차이가 있었다.

쑥으로부터 나오는 독성물질을 찾기 위하여 gas chromatography로 분석한 결과 benzoic acid와 16가지 phenolic compound가 확인되었다.

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