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Identification and Effects of Phenolic Compounds from Some Plants

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수 종 식물의 페놀화합물 분석과 효과

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ABSTRACT

The extracts of selected plants and analyzed phenolic compounds were used to study the effects of allelochemicals on seed germination and seedling growth. HPLC analysis of the aqueous extracts of seven species identified 15 phenolic compounds including caffeic acid. Among them, protocatechuic acid was detected at 65.87ppm and 6.84ppm, in *Erigeron canadensis* and *Pinus rigida*, respectively. And the extract of *P. rigida* showed the strongest inhibitory effect on seed germination.

The extract of *P. rigida* leaves significantly inhibited germination and radicle growth of *Raphanus sativus* var. *hortensis* for. *acanthiformis* in direct proportion to concentration. However, germination of *Cassia mimosoides* var. *nomame* was stimulated by the treated extracts at the same concentrations, but root growth was inhibited at high concentrations.

Except chlorogenic acid, eleven of the twelve phenolic compounds inhibited the germination of *R. sativus* var. *hortensis* for. *acanthiformis*. In the case of *C. mimosoides* var. *nomame*, some phenolic compounds such as chlorogenic acid, vanillic acid, protocatechuic acid, salicylic acid, caffeic acid, ferulic acid, gallic acid and ρ-coumaric acid stimulated germination, while the others reduced it.

Key words: Allelochemical, Phenolic compound, Seed germination, Seedling growth

INTRODUCTION

De Candolle (1832) proposed that allelochemicals produce natural growth inhibitors and suppress seed germination or seedling growth of some species. His results has significantly contributed to the study of physioecology particularly since Cook (1921) established the necessity of crop rotation. In Korea, allelopathic studies took notice in 1963 when Lee and Monsi reported the phenomenon in pine trees at the International Institute.

The allelochemicals as a secondary product are emitted to the environment as an aqueous extract or a volatile substance (Whittaker and Feeny 1971), which control physiological metabolism. Specifically they inhibit seed germination and seedling growth, photosynthesis, cell division and function of the membrane (Bhowmik and Doll 1984, Kapustka and Rice 1976, Muller 1974, Olmsted and Rice 1974). Also Knapp and Furthmann (1954) stated that allelochemicals are significantly important factors which can inhibit or stimulate seed germination and growth in one or more species. Jameson (1968) and Newman (1978) noted that chemical substances stimulated various factors in plants, so that they may be beneficial to seedling growth and to pathways of physiological mechanisms. Also there are many kinds of allelopathic substances such as phenolic compounds, volatile substances, tannins and terpenoids (Einhellig and Rasmussen 1973, Lodhi 1976).

The authors have examined the effects of phenolic compounds on seed germination and protein band pattern (Kim *et al.* 1990). The purpose of the present study was to isolate and identify chemical substances from seven selected species and to investigate the inhibitory or stimulatory effects of these phenolic compounds that may contribute to allelopathic activity.

MATERIALS AND METHODS

Experimental materials

Donor plants for this experiment on the inhibition of seed germination and seedling growth were seven species: *Artemisia princeps* var. *orientalis*, *Chrysanthemum morifolium*, *Erigeron canadensis*, *Larix leptolepsis*, *Pinus rigida*, *Thuja orientalis* and *Cassia mimosoides* var. *nomame*. After several preliminary experiments were performed with the various plants, each plant was tested for quantity of phenolic compounds. *P. rigida* was found to act strongly against some receptor plants even in small amounts, thus it was selected as the donor plant for this study. Receptor plants for the test were twelve species as follows: *Echinochloa crus-galli*, *Amaranthus mangostanus*, *Lycopersicon esculentum*, *Cucumis melo* var. *makuwa*, *Brassica campestris* subsp. *napus* var. *pekinensis*, *Lactuca sativa*, *Oenothera odorata*, *Setaria viridis*, *Rumex acetocella*, *Cassia mimosoides* var. *nomame*, *Raphanus sativus* var. *hortensis* for. *acanthiformis* and *Glycine max*.

Identification of phenolic compounds by HPLC

Aqueous extracts were made from the leaves of 7 species (donor plants). One liter of distilled water was added to 200g of leaves at 80℃, then distillated for 48 hrs, and each aqueous extract was filtered through a 150mm filter paper. The supernatant filtrated in 1,000g centrifugation (Centrikon T.-1045, Kontron Co) for 30 minutes was used as the aqueous extracts of this experiment. We used commercial compounds obtained from Sigma Chemical Co., USA. as a standard. Purification of the sample was carried out with the Kil method (1992). HPLC (Waters, U.S.A.) was used to identify the allelochemicals from the seven species. The HPLC conditions were as follows: detector UV absorbance, 250, 254, 284nm, columm, μ Bonda-Pak C_{18} Radial Pak (0.8×10m), mobile phase, acetonitrate and sodium acetate buffer (A pump: acetonictrile, B pump: 0.02M sodium acetate buffer, pH 4.3 with acetic acid), flow rate, 1.3ml /min and injection volume, 20μl.

Bioassay with *Pinus rigida* extracts

The germination test was carried out in glass Petri dishes (d, 12cm) on two sheets of filter paper wetted with various concentrations of the aqueous extracts. Distilled water was used for the control. Each dish containing 50 seeds were placed in a 28℃ incubator (Hotpat) and the germination test was repeated 3 times. Seedlings of *R. sativus* var. *hortensis* for. *acanthiformis* and *C. mimosoides* var. *nomame* as the control were grown with Hoagland solution (Hoagland and Arnon 1950). The others were treated with 3%, 12%, 25%, 50%, 75% and 100% extract solutions (Einhellig and Rasmussen 1973) as the test. The plants were harvested 12 days after seedling growth, and dried in an oven at 50℃ for 10 hrs. For bioassay, the modified method of Lodhi (1976) was used. The extracts were diluted to concentrations of 10^{-3}M and 10^{-4}M stock for the 12 phenolic acid which were known to be toxic to plants. The germination rate was calculated after incubation at 28℃ for 10 days. Seedling length was measured in millimeters.

RESULTS

Isolation and identification of phenolic compounds

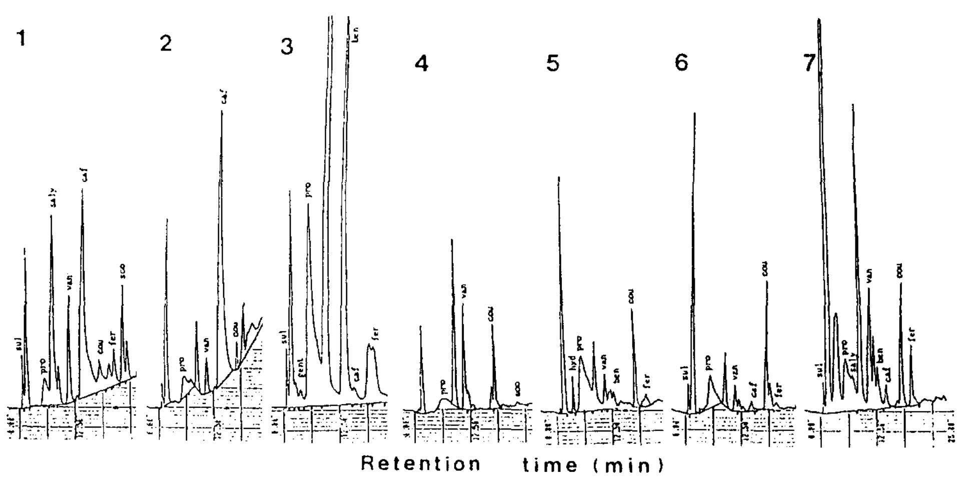

Fig. 1. High performance liquid chromatography identification of chemical compounds from 7 species. Keys: 1: *Artemisia princeps* var. *orientalis*, 2: *Chrysanthemum morifolium*, 3: *Erigeron canadensis*, 4: *Larix leptolepsis*, 5: *Pinus rigida*, 6: *Thuja orientalis*, 7: *Cassia mimosoides* var. *nomame*, sul.: sulfosalicylic acid, pro.: protocatechuic acid, sal.: salicylic acid, van.:vanillic acid, caf.: caffeic acid, cou.: *p*-coumaric acid, fer.: ferulic acid, sco.: scopoletin, gen.: gentistic acid, ben.: benzoic acid, cat.: catechol, hyd.: *p*-hydroxybenzoic acd, syr.: syringic acid, chl.: chlorogenic acid.

Table 1. Quantitative snalysis of chemical compound from different species by high performance liquid chromatography

Substance	Species							
	RT	Ap	Cm	Ec	Ll	Pr	To	Cmn
1. Sulfosalicylic acid	3.17	Tr	–	14.63	–	–	Tr	Tr
2. Gallic acid	3.8	–	Tr	Tr	–	–	–	–
3. Gentistic acid	4.6	–	–	–	–	–	–	Tr
4. Hydroquinone	5.76	–	–	–	–	0.72	–	–
5. Protocatechuic aicd	7.0	Tr	1.35	65.87	20.63	6.84	2.56	2.91
6. Salicylic acid	8.5	11.15	–	Tr	–	–	–	Tr
7. Catechol	8.8	–	–	–	Tr	–	–	–
8. Vanillic acid	11.4	1.76	0.60	–	12.36	0.61	0.4	6.3
9. Chlorogenic acid	11.7	–	–	–	–	–	–	–
10. Syringic acid	12.8	–	–	–	–	Tr	–	–
11. Benzoic acid	13.0	–	–	16.94	–	2.06	–	2.37
12. Caffeic acid	13.8	7.11	8.23	Tr	Tr	Tr	Tr	Tr
13. *p*-Coumaric acid	16.9	0.68	0.19	–	16.78	1.10	1.16	1.17
14. Ferulic acid	18.8	0.45	–	1.88	–	0.17	Tr	0.65
15. Scopoletin	21.1	4.63	–		1.5	–		–

Keys : Tr, Trace; RT, Retention time; Ap, *Artemisia princeps* var. *orientalis*; Cm, *Chrysanthemum morifolium*; Ec, *Erigeron canadensis*; Li, *Larix leptolepsis*; Pr, *Pinus rigida*; To, *Thuja orientalis*; Cmn, *Cassia mimosoides* var. *nomame*.

The components of the seven species were analyzed using HPLC (Fig. 1, Table 1). The concentration of salicylic acid was the maximum amount of 11.15ppm in *A. princeps* var. *orientalis* among phenolic compounds. Caffeic acid was 8.23ppm in *C. morifolium*. Protocatechuic acid was 20.63ppm and 2.56ppm, in *L. leptolepsis* and *T. orientalis*, respectively. Vanillic acid was 6.3ppm in *C. mimosoides* var. *nomame*. Protocatechuic acid was 6.84ppm in *P. rigida* compared to 65.87ppm in *E. canadensis*. The chemical substances of *P. rigida* showed a significantly low amount of substances compared to the other aqueous extracts.

Germination and growth test in aqueous extracts

The germination rates of *R. sativus* var. *hortensis* for. *acanthiformis* treated with *P. rigida* were not remarkably different (Fig. 2). Except for *C. mimosoides* var. *nomame*, seed germination of the 11 receptor species was inhibited when treated with 50% and 100% concentrations of the *P. rigida* extract (Fig. 2, 3). The seed germination and seedling growth of *R. sativus* var. *hortensis* for. *acanthiformis* was retarded the most severely at all the conentrations of the extract of *P. rigida* (Fig. 4A). *C. mimosoides* var. *nomame* was stimulated at a threshold concentration below 25% (Fig. 4B, C). The early and late stage germination rates for control was 20% and 80%, respectively. The germination rates of these plants treated with 50% extract was 60% and 70%, respectively. Based on the above results, the threshold concentrations of *R. sativus* var. *hortensis* for. *acanthiformis* and

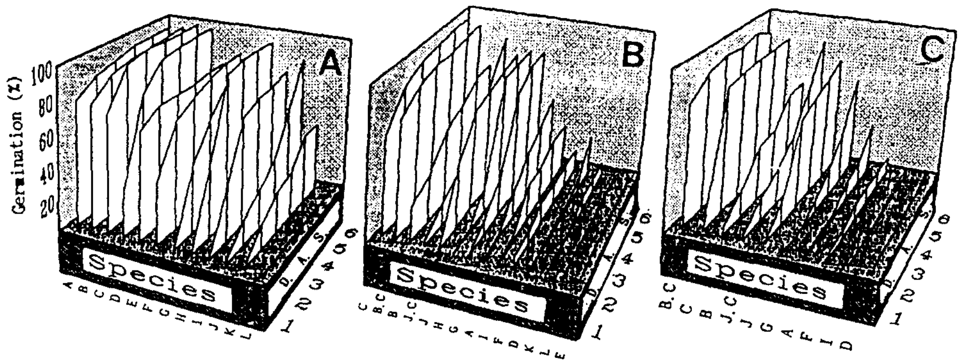

Fig. 2. Effect of sterile water (A), and 50% (B) and 100% (C) of *P. rigida* extract on the germination rate of 12 species. Keys: A, *Amaranthus mangostanus*; B, *Raphanus sativus* var. *hortensis* for. *acanthiformis*; C, *Glycine max*; D, *Lycopersicon esculentum*; E, *Lactuca sativa*; F, *Brassica campestris* subsp. *napus* var. *pekinensis*; G, *Echinochloa crus-galli*; H, *Oenothera odorata*; I, *Cucumis melo* var. *makuwa*; J, *Cassia mimosoides* var. *nomame*; K, *Setaria viridis*; L, *Rumex acetocella*; B.C; Control of *Raphanus sativus* var. *hortensis* for. *acanthiformis*; J.C; Control of *Cassia mimosoides* var. *nomame*; D.A.S; Days after sowing.

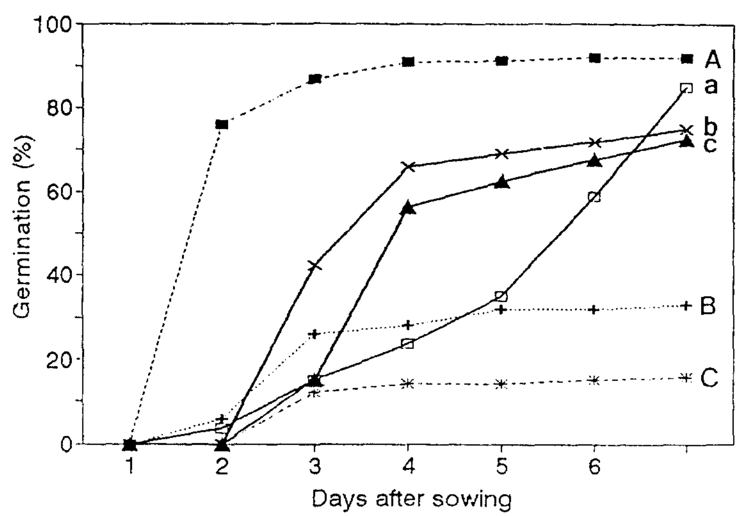

Fig. 3. Changes of germination rate of *R. sativus* var. *hortensis* for. *acanthiformis* and *C. mimosoides* var. *nomame* with different concentrations of *P. rigida* extract. A, B, C; Treatments of sterile water (A), 50% extract (B) and 100% extract (C) on the seed germination of *R. sativus* var. *hortensis* for. *acanthiformis*. a, b, c; Treatments of sterile water (a), 50% extract (b), 100% extract (c) on the seed germination of *C. mimosoides* var. *nomame*.

C. mimosoides var. *nomame* were determined to be 60% and 25%, respectively. Seedling growth and dry weight were also investigated during the 12 days after treatment with

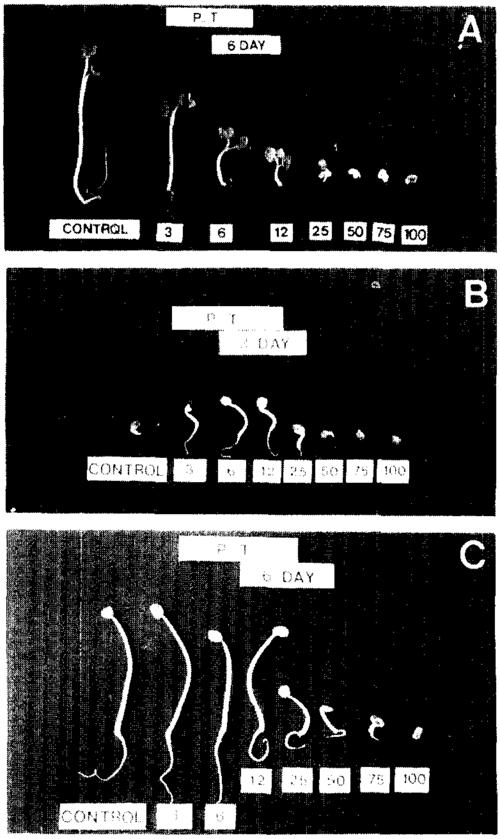

Fig. 4. Comparison of seedling growth at 6 days (A) after sowing of *R. sativus* var. *hortensis* for. *acanthiformis* and at 3 days (B), 6 days (C) after sowing of *C. mimosoides* var. *nomame* with different concentrations of *P. rigida* extract. P.T : Treatment of *P. rigida* extract.

various concentrations of the *P. rigida* extract. The seedling growth of *R. sativus* var. *hortensis* for. *acanthiformis* was reduced over 25% extracts (Fig. 5A, Fig. 6). As shown in Fig. 5B, the germination rate of *C. mimosoides* var. *nomame* was elevated between the first to third days after treatment in the test plants (3, 6, 12 and 25%) compared to control (Fig. 5B). These results show a stimulatory effect on seed germination. However, there was an inhibitory effect in the 5th and 6th days of late stage germination (Fig. 5).

Effect of phenolic compounds

The germination rate of *R. sativus* var. *hortensis* for. *acanthiformis* was suppressed mostly at 10^{-3}M and 10^{-4}M concentrations of protocatechuic acid. Control showed a germination rate above 85% compared to 45% with the treatment of protocatechuic acid (Fig. 7A). On

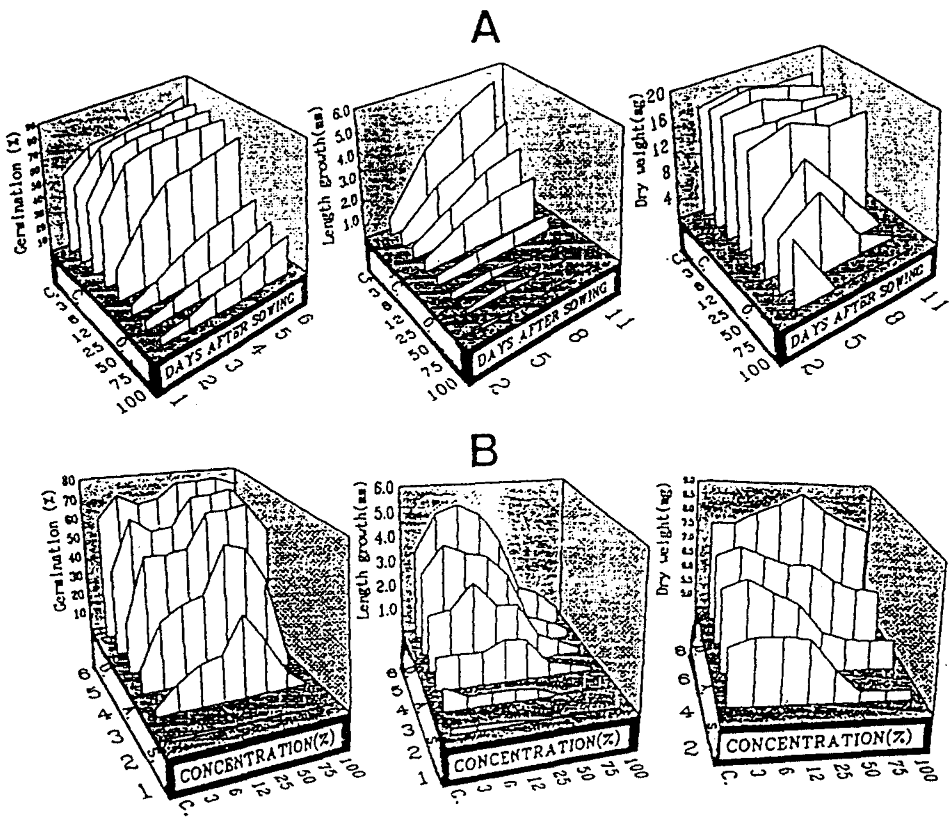

Fig. 5. Comparison of germination rate, length growth, and dry weight during the seed germination of *R. sativus* var. *hortensis* for. *acanthiformis* (A) and *C. mimosoides* var. *nomame* (B) with different concentrations of *P. rigida* extract. C.O: Concentration, C: Control, D.A.S: Days after sowing.

the other hand, chlorogenic acid was only a little better than control. Therefore, it is suggested that all of the 11 compounds except chlorogenic acid inhibit the germination rate of *R. sativus* var. *hortensis* for. *acanthiformis* (Fig. 7A, 8). In the case of *C. mimosoides* var. *nomame*, *ρ*-hydroxybenzoic, benzoic, syringic acids and catechol were appeared to inhibit the germination rate while 7 compounds including coumaric acid stimulated it (Fig. 7B, 9). Generally speaking, it was found that the germination of *C. mimosoides* var. *nomame* was stimulated by the treatment of several phenolic compounds.

DISCUSSION

Alsadawi *et al.* (1983) identified phenolic compounds (caffeic, *ρ*-coumaric, ferulic and gallic acids) which reduced the growth of other species, and it was noted that chemical

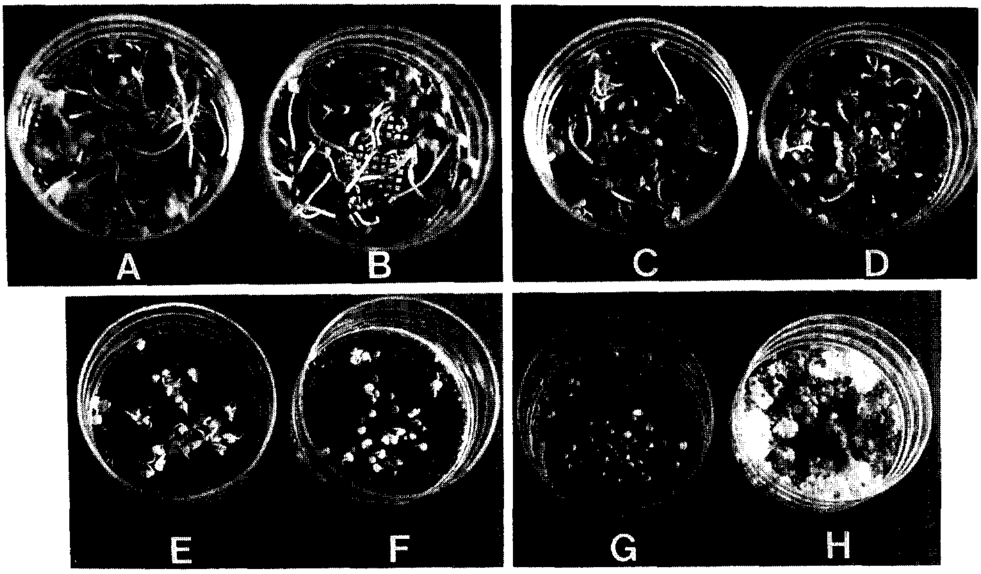

Fig. 6. Growth of *R. sativus* var. *hortensis* for. *acanthiformis* with different concentrations [control(A), 3%(B), 6%(C), 12%(D), 25%(E), 50% (F), 75%(G), 100%(H)] of *P. rigida* extract.

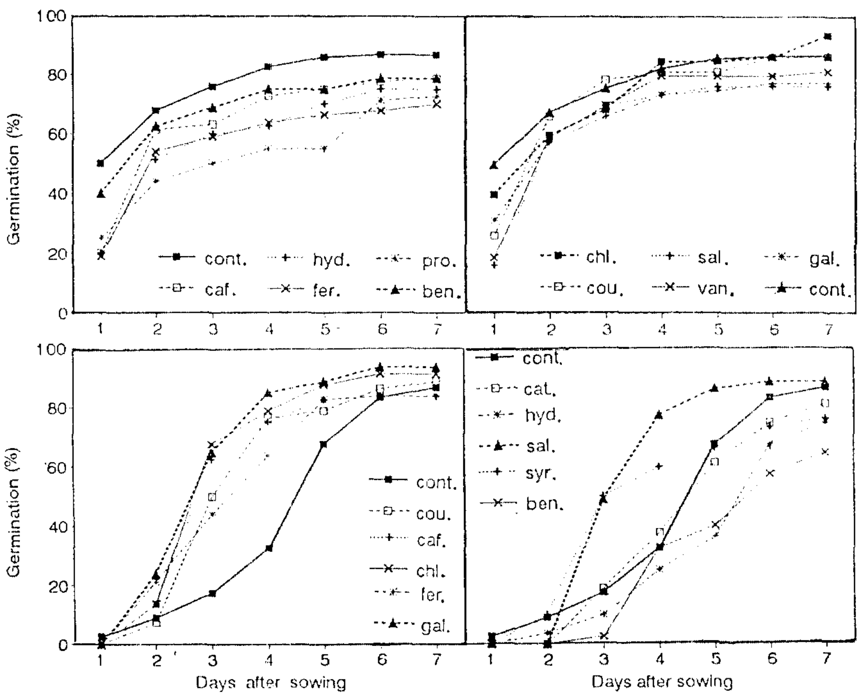

Fig. 7. Germination rate of *R. sativus* var. *hortensis* for. *acanthiformis* (A) and *C. mimosoides* var. *nomame* (B) at 10^{-3}M concentration of chemical compounds. Keys: sal.: salicylic acid, caf.: caffeic acid, fer.: ferulic acid, pro.: proto catechuic acid, gal.: gallic acid, ben.: benzoic acid, hyd.: ρ-hydroxybenzoic aicd, van.: vanillic acid, cou.: ρ-coumaric aicd, chl.: chlorogenic acid.

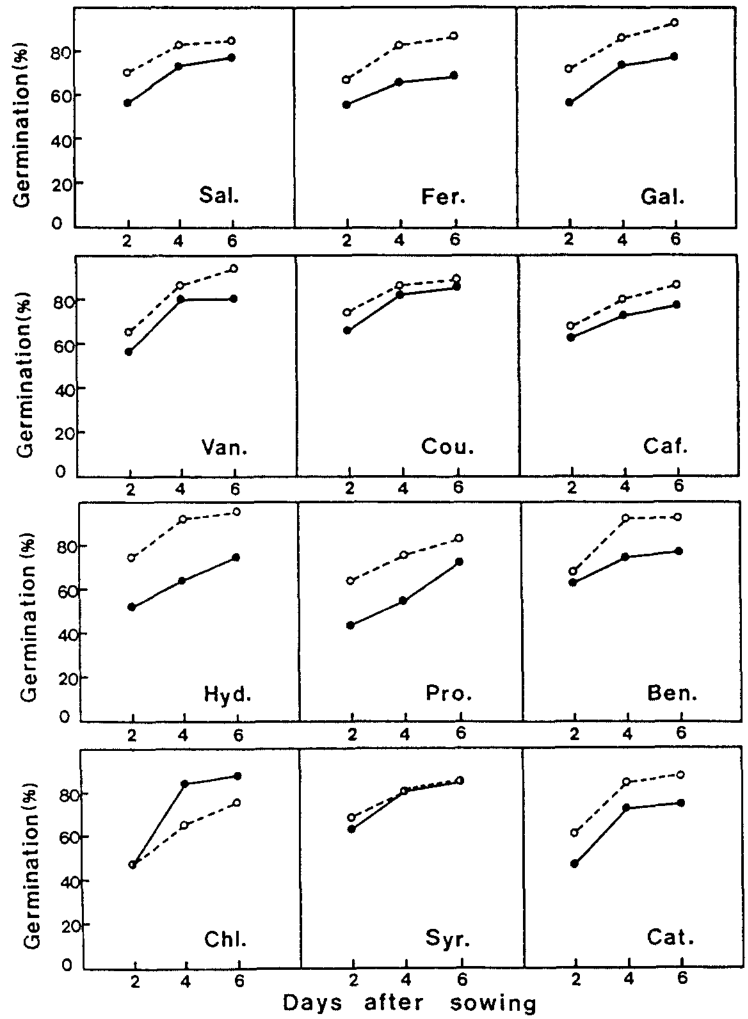

Fig. 8. Comparison of germination rate of *R. sativus* var. *hortensis* for. *acanthiformis* with different concentrations of chemical compounds. Keys: Sal.: salicylic acid, Caf.: caffeic acid, Fer.: ferulic acid, Pro.: protocatechuic acid, Gal.: gallic acid, Ben.: benzoic acid, Hyd.: *p*-hydroxybenzoic aicd, Van.: vanillic acid, Cat.: catechol, Cou.: *p*-coumaric acid, Chl.: chlorogenic acid, Syr.: syringic acid, 10^{-3}M : •——•, 10^{-4}M : ○······○.

substances were released into the environment as secondary products of the plants. The present study has tried to analyze the phenolic compounds from the 7 selected species. Fifteen phenolic compounds were isolated by HPLC. Benzoic acid was at 65.87ppm in *E. canadensis* with *P. rigida* having a lesser quantity. Yet it was found to be the strongest germination inhibitor among the donor plants. Such results suggest a synergistic interaction between phenolic compounds which warrants further investigation.

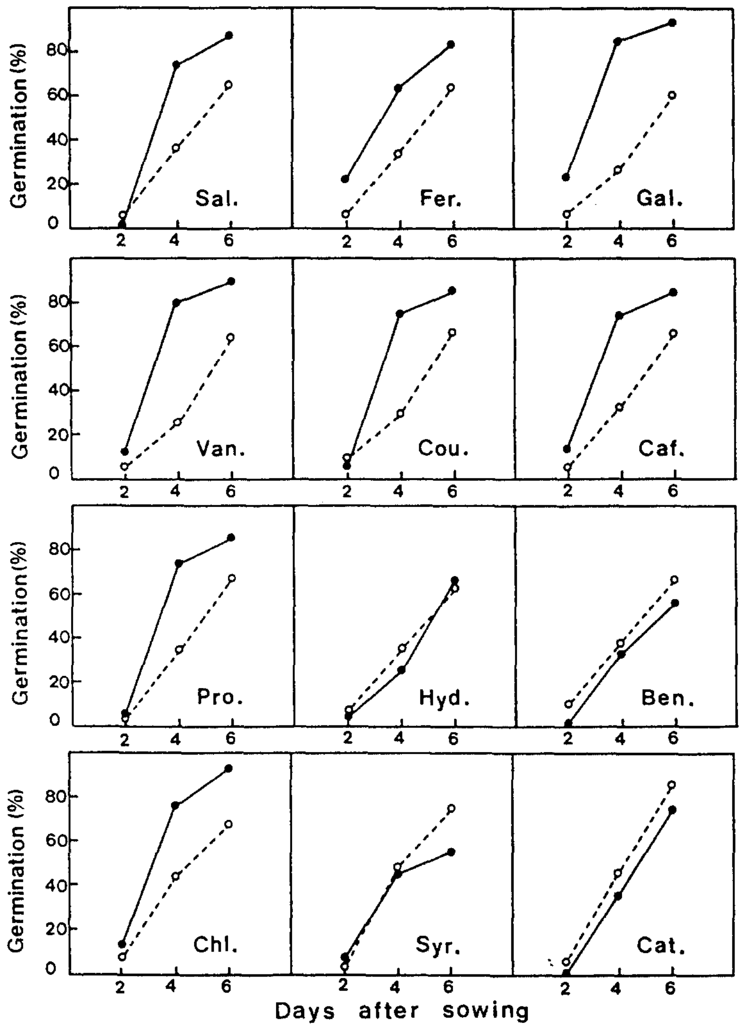

Fig. 9. Comparison of germination rate of *C. mimosoides* var. *nomame* with different concentrations of chemical compounds. Keys to chemicals are the same as in Fig. 8.

Our results revealed that increasing concentrations of the *P. rigida* extract led to increasing inhibition of seed germination and seedling growth in *A. mangostanus, L. esculentum, L. sativa* and *R. sativus* var. *hortensis* for. *acanthiformis*. This is in agreement with Whittaker and Feeny (1971) and Ishimine *et al.* (1985), who reported that allelopathic activity depended on the nature of the test species and on the concentration of the allelochemicals. The germination rate of *R. sativus* var. *hortensis* for. *acanthiformis* showed greater inhibition, 70% more than those of other species. The germination and growth of *C. mimosoides* var. *nomame* was significantly accelerated at 3%, 6% and 12% concentrations in the early stages, whereas it exhibited a retarded germination rate at concentrations of

25% and more. Accordingly, in the case of *C. mimosoides* var. *nomame* among the 12 receptor plants, it was shown that early germination was elevated only with the *P. rigida* extract (Fig. 3). Except for the growth stimulating phenomenon are in accordance with Lodhi (1976) concerning yield enlargement. These results suggest a species related characteristic of *C. mimosoides* var. *nomame*, and that the accelerated response renders it unsuitable for the production of crops. On the other hand, our experiment showed that chlorogenic acid stimulated only the germination rate, and that all of the 11 phenolic compounds inhibited the germination of *R. sativus* var. *hortensis* for. *acanthiformis*. While 3 phenolic compounds (ρ-hydroxybenzoic, syringic acids and catechol) and benzoic acid retarded the seed germination rate, 8 phenolic compounds accelerated that of *C. mimosoides* var. *nomame*. This is in agreement with Olmsted and Rice (1974), who reported that ρ-coumaric acid showed the highest inhibition rate in a germination experiment of *Bromus japonica* and *Ambrosia artemisiifolia* var. *elatior*.

적 요

종자의 발아와 유근생장에서 allelochemicals의 효과를 규명하기 위하여 식물추출액을 선발하고 phenolic compound를 분석하였다. HPLC를 이용한 7종 식물의 성분분석결과는 caffeic acid를 포함하여 15개의 phenolic compound로 동정되었다. 이들 중 protocatechuic acid는 망초에서 65.87ppm이었고 리기다소나무는 6.84ppm이었다. 리기다소나무의 추출액은 여러식물의 종자발아율을 가장 크게 억제하였다. 무우에 있어서 chlorogenic acid는 촉진효과를 나타내고 그외 11종은 모두 억제효과를 보였으며 차풀의 경우는 chlorogenic, scopoletin, vanillic, protocathechuic, salycilic, caffeic, ferulic acid는 종자의 발아를 촉진하였다.

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