Effects of Major Phenolic Acids Identified from Barley Residues on the Germination of Paddy Weeds

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보리 殘餘物속에 含有된 主要 Phenolic Acids가 논 雜草 發芽에 미치는 影響

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ABSTRACT

Effects of major phenolic acids identified from barley residues (straw, root) on the germination of rice and 3 paddy weeds such as *Echinochloa crusgalli, Cyperus serotinus,* and *Potamogeton distintus* were evaluated to obtain the basic informations on the development of naturally occurring herbicides. The predominant phenolic compounds extracted from barley residues in both straw and root were identified as p-coumaric, p-hydroxybenzoic, ferulic, vanillic, and salicylic acids by means of paper chromatography. Total phenol content of barley straw and root at the harvesting stage was 0.169% and 0.127% per dry weight, respectively. During the decomposing process, total phenol content slightly increased and then decreased. The germination of test plants was inhibited by treatments of 4 major authentic phenolic acids identified, most significantly on rice, and less on *E. crusgalli,* and *C. serotinus. P. distintus,* however, was markedly stimulated by them as the concentration increased, and then sprouted buds of pondweeds were changed to dark brownish color, resulting in the death as the treatment prolonged. The greater inhibitory effect appeared on shoot growth rather than germination. The aqueous extracts of barley residues showed the similar inhibitory effect on the germination and shoot growth of rice and three paddy weeds as the treatments of 4 authentic phenolic acids.

Key words: *authentic phenolic acids, Echinochloa crusgalli, Cyperus serotinus, Potamogeton distintus.*

INTRODUCTION

The phenomenon of allelopathy was defined by Rice[24] as any direct or indirect harmful effect by one plant (including microorganism) on another through the production of chemical compounds that escape into the environment. These chemical compounds related to allelopathy can be leached from leaves by rainwater, secreted by roots, and be formed from decaying plant matters[32]. In general, these allelopathic chemicals are supposed to be the secondarly metabolites such as phenolic acids, coumarins, terpenoids, flavonoids, alkaloids, cyanogenic glycocyde, and glucosanolates, etc.[24, 28,36].

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Allelopathy has played an important role in crop production on certain types of crop rotations, in orchard replanting in old orchard land, in crop monoculture, and forest site replanting[23]. In particular, recently agricultural practices such as minimum tillage and mulch production have resulted in reduced yields attributable to the release of allelochemicals from crop residues[3,4,30].

Most crop residues contain water-soluble substances that inhibit the germination and growth of antoher crops or weeds. Research on the possible allelopathic effects of major crops such as barley, wheat, sorghum, oat, sugarcane, sunflower on weeds is generally recent[6,15,19,21,22,30,33]. The concept that some crop plants may be allelopathic to certain weeds is receiving increased attention in the search for alternative weed-control strategy. Recently, several workers attempted to evaluate crop plants for the allelopathic potential for weed control or as the sources of naturally occurring herbicides[21,26].

In particular, Mann and Barners[20] found that barley (*Hordeum vulgare*) inhibited *Holcus molis* in the abscence of competition, even though the latter is better able to utilize nitrogen when supply is limited. They also found that even a small number of barley plants reduced clover growth by more than 50%. Overland[22] showed that allelopathy was involved in the mechanism of weed control by barley used as a smother crop, with the greatest inhibition occurring with chickweed (*Stellaria media*), less with *Capsella bruspastroris* and *Nicotiana tabaccum*, while no significant effect on wheat was observed. He also demonstrated that the active inhibitory compounds were the presence of alkaloids, gramin, with a much greater concentrations of substance in the living than in the dead root leacheates. Todd et al.[31] reported that resistance of barley to the greenbug (*Schizaphis graminm*) is related to the amounts of phenolic and flavonoid compounds and related substances in the barley plants.

Meanswhile, phenolic compounds in plant residues have been mainly studied as allelopathic substances having the potential of protective agents against other plants, insects, and fungi[24,28]. These substances are dominant in plant cell wall and vacuoles of seeds, fruits, and other plant tissues. In some instances, these naturally occurring phenolic compounds affect fundamental plant process such as photosynthesis, synthesis of plant growth regulators as well as inhibition of germination and growth of plants[10,37].

However, in Korea, no much works on allelopathic effects have been made in terms of weed control purpose or a source of naturally occurring herbicides, whereas a few works have been made in determining allelopathic substances in plants such as *Pinus densiflora*[12], *Chrysanthemum morifolum*[17], and *Miscanthus sinensis*[16] etc.

It has been known among Korean farmers that the barley starw spreading on the paddy fields may suppress an important perennial weed, *P. distintus.* Several studies have been reported in the foreign countries that inhibitory substances presented in the crop residues including the grasses may be the phenolic compounds[2,7,14,27].

Thus, the objectives of this experiment were 1) to identify the major phenolic compounds from barley residues,2) to determine the total phenol content in barley residues during the decomposing process, 3) to evaluate the effects of authentic phenolic acids, and 4) to evaluate the effects of aqueous extracts from barley residues on the germination and shoot growth of rice and 3 paddy weeds.

MATERIALS AND METHODS

Barley residues (straw and root) used were collected from the experiment farm belonging to Kyungpook National University in the 10th of June, 1983 and washed with tap water several times to remove impurites. For the methanolic and aqueous extraction, the barley residues were dried for 3 hrs in a dry oven maintained at 60°C and crushed to pass through a 2mm sieve. To prepare the decomposed products, 200ml distilled water was added to 10g of dry sample prepared as the above-mentioned procedures, wraped with aluminium foil and incubated at 30°C for 120 days. The sample were thoroughly mixed every 5 days to prevent precipitation.

Seeds of rice (Nakdong) and *E. crusgalli,* tubers of *C. serotinus* and bulbs of *P. distintus* used were collected from the experimental farm of the Kyungpook Provincial Office of Rural Development in the month of November, 1982 and stored at 4°C refrigerator until used.

Identification of major phenols. To identify the major phenolic compounds from barley residues, the extraction of phenolic compounds was performed by a modified method of Kuwastuka and Shindo[14] (Fig. 1). 10g of dry sample or 10g of the decomposed sample was homogenized with 700ml of 70%

Dry sample
decomposed sample
  homogenized with 70% MeOH, stored for 48 hrs under N_2 gas
  filtered in *Vacuo*

Filtrate — Residues
  concentrated at 40°C *in Vacuo*
Concentrate
  extracted with chloroform under N_2 gas
Non-chloroform soluble fraction — Chloroform soluble fraction
  adjusted to pH 2
  extracted with ether under N_2 gas (3 times)
Ether soluble fraction — Non-ether soluble fraction
  saturated with NaCl
  anhydrous sodium sulfate
Ether extract

Fig. 1. Flow diagram showing procedures for extraction and separation of phenolic compounds in the barley residues.

methanol under N_2 gas and stored for 48 hrs. The mixture was filtered *in Vacuo.* The filtrate was concentrated to about 200ml at 40°C *in Vacuo* and treated with chroloform for getting rid of a pigment etc.. Chroloform soluble fraction (lower layer) was discarded, and non-chloroform soluble fraction (upper layer) was adjusted to pH 2 with 1N HCl. The acidified solution was extracted with 200ml of ether three times. The ether soluble fractions was saturated with sodium chloride, treated with anhydrous sodium sulfate and evaporated to dryness.

To identify the major phenolic compounds from the ether fraction, ether extracts was dissolved with ethylacetate. The solution was spotted on Whatman No. 1 papers. The chromatograms were developed in two dimensions with n-butanol: acetic acid : water (BAW, 4: 1:2 v/v) as the first solvent and with 2% acetic acid as the second solvent, adapted from a modified method of Rice and Pancholy[25]. The air-dried chromatograms were sprayed with a immediately prepared chromatographic reagent made of equal volume of 0.5% $FeCl_3$ and 0.5% $K_3Fe(CN)_6$. Identification of major phenolic compounds was confirmed by chromatograms with authentic phenolic compounds.

Total phenol content. To measure the changes of the total phenol content of barley residues during the decomposing process, total phenol content was determined by a modified method of Swain and Hillis[29]. A 0.5ml of the ether extracts obtained by the same method as mentioned in the prior experiment put into a test tube, and 3ml of Folin's reagent diluted 5 times with distilled water and 1ml of 2% Na_2Co_3-4 % NaOH mixture were added in this order. After the reaction for 30min. In the water bath maintained at 30°C, the optical density was measured at 750mm by spectrophotometer. The calibration curve was made with standard solutions of vanillic acid which is one of major phenolic acids identified from barley residues.

Effects of major phenolic acids. To evaluate the effects of major phenolic acids identified from barley residues on the germination and shoot growth of rice and 3 paddy weeds, 4 authentic phenolic acids such as p-coumaric, p-hydroxybenzoic, ferulic, and vanillic acids were used. Authentic phenolic acids purchased from Tokyo Chemical Company, Japan, were dissolved with pure ethanol, depending on their solubility. Test plants of untreated control were also treated with the same amount of ethanol at concentrations equal to the chemical treatments. Various concentrations such as 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}M and mixture of 4 phenolic acids were prepared.

Ten ml of each concentration of phenolic acids was applied on petri dishes having Whatman No. 1 filter paper which contained 20 seeds of rice and *E. crusgalli,* 10 tubers of *C. serotinus* and 10 bulbs of *P. distintus* per petri dish. All the petri dishes were allowed to germinate at 25°C under dark condition. Percentage germination and shoot length were determined at 10 days after treatments for rice, *E. crusgalli,* and *C. serotinus,* and for *P. distintus* 15 and 30 days after treatments which demands a more time to sprout. Germination counts were made when radicle or coleptile attained a length of 2mm or more.

Effects of aqueous extracts. In order to obtain a basic evidence as allelopathic potential of barley residues on the paddy weeds, the germination test with aqueous extracts on rice and three paddy weeds was carried out. To prepare the aqueous extracts of barley residues, 10g of dry sample was added to the flask containing 200ml of distilled water, stored for 48 hrs at 30°C, and filtered *in Vacuo.* The filtrate was diluted to various concentrations such as a undilution, a 1/10 dilution, and a 1/100 dilution with distilled water. The rest procedures used were the same method as mentioned in the above experiment.

RESULTS AND DISCUSSION

Identification of major phenols. The major phenolic compounds separated from barley residues by paper partition chromatography presented in Fig. 2. Ten spots were detected with the $FeCl_3$-K_3Fe (CN)6 reagent. The color of the spots varied from light to deep blue, depending on the quantity of phenolic compounds. Spot 1,2,3,4, and 5 were detected in both the straw and root. However, spot 6,7, and 8 were detected only in the straw, whereas spot 9 and 10 only in the root.

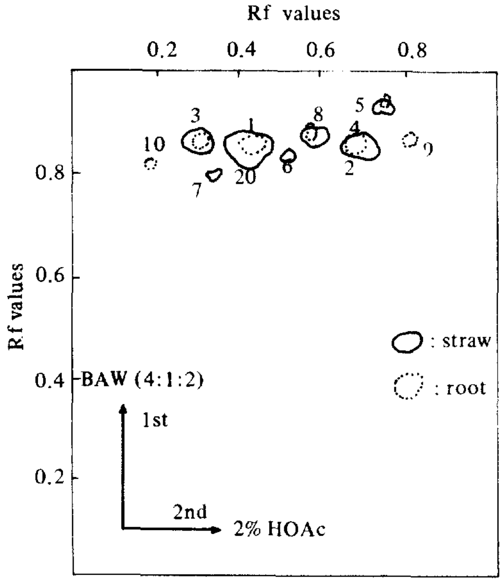

Fig. 2. Two-dimensional paper chromatogram of phenolic acids in the barley residues.

Table 1 shows the Rf values and the color development under ultraviolet light with and without ammonium vapor. Considering Rf value and color development, p-coumaric (spot 1), p-hydroxybenzoic (spot 2), ferulic (spot 3), and vanillic (spot 4) acids seemed to be the the major phenolic compounds detected in both straw and root portions as compared with those of authentic compounds. Spot 5 seemed to be salicylic acid referred to Kil'results12). The rest spots presented on this paper chromatogram could not be definitely identified because of difficulty in obtaining the standard compounds.

The major phenolic compounds identified from barley residues seemed to be similar to earlier reports2,7,27). The color reaction with the spray of $FeCl_3$-K_3Fe $(CN)_6$ reagent indicates that straw portion may contain higher total phenol content than that of root. Secondly, it was assumed that p-coumaric, p-hydroxybenzoic, ferulic, and vanillic acids

Table 1. Identification of major phenolic acids in the barley residues by PPC.

Spot no. Identification	$FeCl_3$-$K_3Fe(CN)_6$	Rf values		Color under	
		BAW(4:1:2)	2%HOAc	UV	NH_3 + UV
(Straw)					
1. p-coumaric acid	blue	.86	.46	violet	yellow
2. p-hydroxybenzoic acid	blue	.87	.65	violet	-
3. ferulic acid	blue	.88	.38	blue	yellow
4. vanillic acid	blue	.87	.58	violet	-
5. salicylic acid	blue	.92	.72	-	-
6. unidentified	blue	.85	.50	-	yellow
7. "	blue	.80	.32	-	-
8. "	blue	.91	.51	violet	-
(Root)					
1. p-coumaric acid	blue	.87	.44	violet	yellow
2. p-hydroxybenzoic acid	blue	.86	.68	violet	-
3. ferulic acid	blue	.87	.37	blue	yellow
4. vanillic acid	blue	.86	.55	violet	-
5. salicylic acid	blue	.92	.70	-	-
9. unidentified	blue	.87	.75	-	-
10. "	blue	.84	.15	violet	-
(Authentic compounds)					
p-coumaric acid	blue	.87	.45	violet	yellow
p-hydroxybenzoic acid	blue	.88	.66	violet	-
ferulic acid	blue	.86	.40	blue	yellow
vanillic acid	blue	.90	.59	violet	-

mgiht be the predominant phenolic acids based on degree of color development.

Total phenol content. Table 2 shows that total phenol content evaluated at the seedling, harvest stages and decomposing process of barley residues. At the seedling of 4-5 leaf stages, the total phenol content of aerial parts of barley was 12.77mg per 10g of dry sample, while that of root was 10.04mg. At the harvesting stage, it increased up to 16.79mg and 12.74mg, in the straw and root portions, respectively. It increased further up to 20.74 and 15.53mg, in the straw and root at 15 days after decomposition of the sample progressed. Thereafter, total phenol content steadly decreased as the decomposition proceeded. Finally, at 120 days after decomposition, it decreased to 4.79mg in straw and 4.01mg in root.

Table 2. The total phenol contents at the seedling, harvesting stages and during decomposing process of barley residues.

barley parts / Time determined	Straw	Root
	(mg/10g)	
Seedling stage[1)]	12.77	10.04
Harvesting stage[2)]	16.79	12.74
15 DAD[3)]	20.74	15.33
30 DAD	18.76	14.02
45 DAD	9.74	8.38
120 DAD	4.79	4.01

1) 75 days before harvest, 4-5 leaf stages
2) Harvest day; June 10, 1983
3) Days after decomposition begins

The above results almost coincided with other's several reports on various content of total phenol. It can be assumed that an increase of total phenol at the early time of decomposition may be resulted from the decomposition of lignin which is a high polymer made up of several different types of phenyl propane unites. Ishiura[9] reported that the total phenol and flavanol content in *Rhus, Euonymus* and *Acer* leaves increased rapidly at the early growth stages but thereafter the content was kept rather constant. Kuwatsuka and Shindo[14] reported that the content of the whole phenolic acids was decreased to one-third of its original during the decomposing process of rice straw for 45 days at 50°C. Guenzi et al.[8] found that corn and sorghum residues had considerably more toxic material at harvest and required 22 to 28 weeks of decomposition before the water-soluble part of the residues was relatively nonphytotoxic.

Meanwhile, the pH value which was 4.7 at 15 days after the start of decomposition was alkalified as the decomposition proceeds, showing the similar trend in both straw and root (Fig. 3).

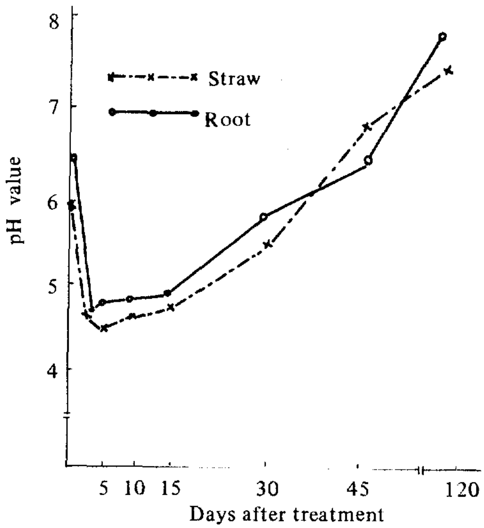

Fig. 3. Changes of pH value during the decomposing process of barley residues.

Kimber[13] found the pH of some extracts of residues shifted from the range of 6.2-6.9 to 4.8-5.9 in 5 days decomposition, resulting in increased toxicity of the extracts. Thus, he concluded that the toxic substances involved played a significant inhibitory role under acidic condition, but once formed those are inhibitory over a fairly broad range of pH's. Crafts and Reiber[5] reported that the activation of the salts of phenolic herbicides resulted from the shift in pH toward the acid side.

Table 3. Effects of four phenolic acids on the germination and shoot growth of *Oryza sativa.*[1)]

Phenolic acids Conc.(M)	p-coumaric acid		p-hy.benzoic acid		ferulic acid		vanillic acid		mixture of 4 phenols	
	% germ.	shoot (mm)	% germ.	shoot (mm)	% germ.	shoot (mm)	% germ.	shoot (mm)	% germ.	shoot (mm)
10^{-2}	10.3[d]	0.1[b]	20.5[c]	1.1[c]	12.8[b]	0.3[b]	12.8[b]	0.5[b]	15.4[c]	0.9[c]
10^{-3}	33.3[c]	3.5[b]	61.5[b]	6.5[b]	66.7[a]	8.3[a]	79.5[a]	5.9[b]	33.3[bc]	3.0[b]
10^{-4}	87.2[b]	9.5[a]	59.0[b]	5.9[b]	82.1[a]	11.7[a]	79.5[a]	7.8[a]	61.5[abc]	7.4[b]
10^{-5}	89.7[ab]	13.6[a]	76.9[ab]	7.9[a]	71.8[a]	11.2[a]	92.3[a]	8.8[a]	64.1[ab]	5.9[b]
Untreated control	96.7[a]	13.2[a]	96.7[a]	13.2[a]	96.7[a]	13.2[a]	96.7[a]	13.2[a]	96.7[a]	13.2[a]

1) Each value is an average of 20 seeds with three repl., and determined at 10 days after treatment. Means within a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Effects of major phenolic acids. Both germination and shoot growth of rice were significantly inhibited in a similar trend as the concentrations of phenolic acids increased (Table 3). 10.3% of rice seeds was germinated when treated with p-coumaric acid at 10^{-2}M. At the same level of acids, no significant difference among phenolic acids used was observed. However, both germination percentage and shoot growth increased significantly as the concentration decreased from 10^{-2}M to 10^{-5}M. The mixture of 4 phenolic acids also resulted in greater inhibitory effect in overall on the germination and shoot growth of rice than each of them.

From the practical point of view, all the test plants including rice were inhibited by major phenolic acids used, while in the field conditions, the transplanted rice can escape the toxic effects caused by barley residues due to the advanced age of it. On the contrary, paddy weeds may be affected by the treatment of barley residues because it is about time to germinate.

However, the germination of *E. crusgalli* was slightly inhibited, whereas the shoot growth was significantly inhibited when treated with 4 phenolic acids (Table 4). More than 50% of *E. crusgalli* seeds was germinated when treated with four authentic phenolic acids. Under the highest concentration like 10^{-2}M, *E. crusgalli* seemed to be more tolerant to phenolic acids than rice.

Table 4. Effects of four phenolic acids on the germination and shoot growth of *Echinochloa crusgalli*[1)]

Phenolic acids	p-coumaric acid		p-hy. benzoic acid		ferulic acid		vanillic acid		mixture of 4 phenols	
	% germ.	shoot (mm)	% germ.	shoot (mm)	% germ	shoot (mm)	% germ	shoot (mm)	% germ	shoot (mm)
Conc. (M)										
10^{-2}	75.0^{a}	6.6^{d}	86.7^{a}	9.9^{d}	80.0^{a}	6.0^{c}	50.0^{b}	6.4^{c}	50.0^{b}	5.2^{c}
10^{-3}	70.0^{b}	19.6^{c}	88.3^{a}	31.9^{b}	91.7^{a}	28.1^{b}	86.7^{a}	28.9^{b}	80.0^{a}	30.2^{b}
10^{-4}	86.7^{a}	23.8^{bc}	73.3^{b}	24.1^{c}	78.3^{a}	31.9^{ab}	73.3^{ab}	30.9^{b}	78.3^{a}	32.8^{b}
10^{-5}	76.7^{a}	28.1^{b}	81.7^{a}	37.7^{ab}	83.3^{a}	35.8^{ab}	80.0^{a}	33.0^{ab}	91.7^{a}	31.6^{b}
Untreated control	91.7^{a}	42.3^{a}	91.7^{a}	42.3^{a}	91.7^{a}	42.3^{a}	91.7^{a}	42.3^{a}	91.7^{a}	42.3^{a}

1) Each value is an average of 20 seeds with three repl., and determined at 10 days after treatment.

Means within a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Phenolic acids inhibited very slightly germination of *C. serotinus* compared with rice and *E. crusgalli*. No significant differences among levels of phenolic acid used on germination were observed except for 10^{-2}M (Table 5). However, varied inhibition on shoot growth was observed depending upon kinds of phenolic acids used.

On the contrary, the authentic phenolic acids used promoted the germination of *P. distintus*, particurly, at the hgihest concentration such as 10^{-2}M, showing more than 90% of bulb germination, as compared with the untreated control, 50% (Table 6.). However, the pondweeds sprouted at the higher concentrations were changed to dark brown color, and then resulted in the death, showing the highest inhibitory effect among weeds tested (Fig. 4). The black portion of Fig. 4 represents the % killed portion of *P. distintus* after sprouting observed at the 30 days after treatment.

Meanwhile, Wang et al.[33)] reported that maximum levels for p-coumaric and ferulic acids of 30.3 and 6.5 umol/100g of soil were found in sugarcane fields. Whitehead35) reported soil concentrations of 4x 10^{-5} and 3×10^{-5}M for p-coumaric and ferulic acids,

Table 5. Effects of four phenolic acids on the germination and shoot growth of *Cyperus serotinus*.[1)]

Phenolic acids Conc.(M)	p-coumaric acid		p-hy.benzoic acid		ferulic acid		vanillic acid		mixture of 4 phenols	
	% germ.	shoot (mm)	% germ.	shoot (mm)	% germ	shoot (mm)	% germ	shoot (mm)	% germ	shoot (mm)
10^{-2}	83.3ᵃ	12.7ᵇ	76.7ᵇ	15.2ᶜ	46.7ᶜ	3.3ᶜ	73.3ᵇ	8.4ᶜ	96.7ᵃ	24.2ᵇ
10^{-3}	93.3ᵃ	29.9ᵃ	96.7ᵃ	24.2ᵇ	76.7ᵇ	21.8ᵇ	96.7ᵃ	22.8ᵇ	90.0ᵃ	21.4ᵇ
10^{-4}	96.7ᵃ	33.3ᵃ	96.7ᵃ	24.5ᵇ	93.3ᵃᵇ	31.7ᵃ	93.3ᵃ	20.2ᵇ	96.7ᵃ	31.0ᵃ
10^{-5}	96.7ᵃ	36.7ᵃ	93.3ᵃᵇ	29.9ᵃᵇ	90.0ᵃᵇ	34.2ᵃ	96.7ᵃ	26.3ᵇ	93.3ᵃ	24.5ᵇ
Untreated control	100.0ᵃ	34.2ᵃ	100.0ᵃ	34.2ᵃ	100.0ᵃ	34.2ᵃ	100.0ᵃ	34.2ᵃ	100.0ᵃ	34.2ᵃ

1) Each value is an average of 10 tubers with three repl., and determined at 10 days after treatment. Means within a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 6. Effects of four phenolic acids on the germination of Potamogeton distintus[1)]

Phenolic acids Conc.(M)	p-coumaric acid		p-hy.benzoic acid		ferulic acid		vanillic acid		mixture of 4 phenols	
	15DAT	30DAT	15DAT	30DAT	15DAT	30DAT	15DAT	30DAT	15DAT	30DAT
10^{-2}	93.3ᵃ	100ᵃ	83.3ᵃ	96.7ᵃ	90.0ᵃ	96.7ᵃ	96.7ᵃ	96.7ᵃ	73.3ᵃ	93.3ᵃ
10^{-3}	70.0ᵇ	76.7ᵇ	63.3ᵇ	70.0ᵇ	73.3ᵃ	80.0ᵃᵇ	60.6ᵇ	80.0ᵃᵇ	60.0ᵃᵇ	66.7ᵇ
10^{-4}	56.7ᵇ	80.0ᵃᵇ	53.3ᵇ	70.0ᵇ	56.7ᵇ	70.0ᵇ	63.3ᵇ	73.3ᵃᵇ	56.7ᵃᵇ	66.7ᵇ
10^{-5}	50.0ᵇ	66.7ᵇ	53.3ᵇ	76.7ᵃᵇ	73.3ᵃ	83.3ᵃᵇ	70.0ᵃᵇ	76.7ᵃᵇ	73.3ᵃ	83.3ᵃᵇ
Untreated control	50.0ᵇ	73.3ᵇ	50.0ᵇ	73.3ᵇ	50.0ᵇ	73.3ᵇ	50.0ᵇ	73.3ᵇ	50.0ᵇ	73.3ᵇ

1) Each value is an average of 10 bulbs with three repl., and determined at 15, 30 days after treatment. Means within a column followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.

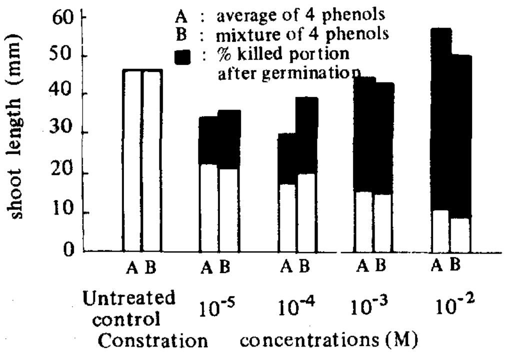

Fig. 4. Effects of four major phenolic acids on the shoot growth of *P. distintus* at 30 days after treatment.

respectively. From these results, the total phenol content in the soil which cultivated the barley may be 10^{-5} to 10^{-4}M which is equivalent to about 50% inhibitory effect of shoot growth of *P. distintus.*

Fig. 5 represents the average germination of plants tested with 4 authentic phenolic acids. The germination of rice markedly decreased as the concentrations increased, whereas *E. crusgalli* and *C. serotinus* slightly decreased. However, the germination of *P. distintus* was promoted as the concentrations increased. Further, it can be seen that shoot growth was more affected by phenolic acids than germination of plants tested (Fig. 6). The highest inhibitory effect on shoot growth was observed in *P. distintus* followed by rice, *E. crusgalli,* and *C. serotinus.*

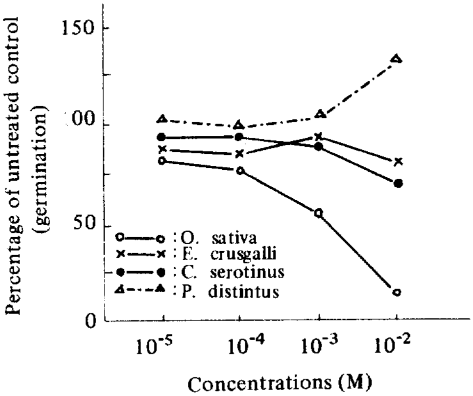

Fig. 5. The average germination rate of rice and three paddy weeds treated with four major phenolic acids.

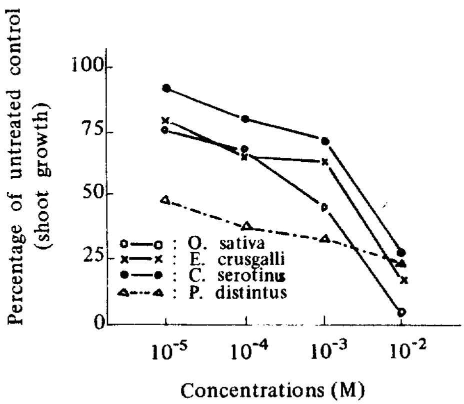

Fig. 6. The average shoot growth of rice and three paddy weeds treated with four major phenolic acids.

Based on the results and observation, it can be assumed that the inhibitory effect of major phenolic acids identified from barley residues occured in subsequent growth after germination than germination itself of plants. On the other hand, in contrast to growth regulators, the phenolic compounds may occur in very high concentrations in plants, either as free phenolics or as derivatives such as glycosides. These phenolic compounds and related lignins, tannins, and flavonoids are derived from the shikimic acid pathway or by condensation of acetate unite, and are based on the benzen ring[10]. Kefeli and Turetskaya[11] reported that the natural phenolic growth inhibitors extracted from *Salix rubra* and apple trees suppress the activity of IAA and gibberellin. Lee[18] reported p-coumaric and ferulic acids can decrease bound indole-3 acetic acid (IAA) formation in maize, but caffeic and protocatechuic

Table 7. Effects of aqueous extracts of barley residues on the germination of rice and three paddy weeds.[1)]

Plants	*O. sativa*		*E. crusgalli*		*C. serotinus*		*Potamogeton distintus*			
							straw		root	
Concent.	straw[2)]	root[2)]	straw	root	straw	root	15DAT	30DAT	15DAT	30DAT
	%									
Undiluted	59.5^{b}	97.3^{ab}	61.7^{a}	43.3^{b}	96.7^{a}	100^{a}	66.7^{a}	83.3^{a}	53.3^{a}	73.3^{a}
1/10 dil.	64.9^{b}	86.5^{ab}	71.7^{a}	61.7^{ab}	100^{a}	100^{a}	33.3^{b}	56.7^{a}	16.7^{b}	33.3^{b}
1/100 dil.	95.0^{a}	78.4^{b}	78.3^{a}	73.3^{ab}	100^{a}	100^{a}	6.7^{c}	33.3^{ab}	40.0^{ab}	56.7^{a}
Untreated control	98.0^{a}	98.0^{a}	80.0^{a}	80.0^{a}	100^{a}	100^{a}	33.3^{b}	80.0^{a}	33.3^{b}	80.0^{a}

1) Each value is an average of three replications, and determined at 10 days after treatment, but 15, 30 DAT for *p. distintus.*

Means within a column followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.

2) Portion of barley used for aqueous extraction.

Table 8. Effects of aqueous extracts of barley residues on shoot growth of rice and three paddy weeds.[1)]

Plants / Concen.	*O. sativa*		*E. crusgalli*		*C. serotinus*		*P. distintus*	
	straw[2)]	root[2)]	straw	root	straw	root	straw	root
	mm							
Undiluted.	1.6^{b}	8.2^{a}	11.0^{b}	25.3^{a}	9.8^{b}	16.4^{a}	25.2^{ab}	13.7^{ab}
1/10 dil.	3.0^{b}	6.3^{ab}	25.4^{a}	23.0^{a}	12.0^{ab}	8.2^{b}	13.9^{ab}	6.5^{b}
1/100 dil.	5.5^{ab}	3.8^{b}	33.3^{a}	33.3^{a}	11.0^{ab}	11.0^{ab}	6.2^{b}	20.8^{ab}
Untreated control	8.7^{a}	8.7^{a}	35.4^{a}	35.4^{a}	16.7^{a}	16.7^{a}	32.0^{a}	32.0^{a}

1) Each value is an average of three replications, and determined at 10 days after treatment, but 15,30 DAT for *P. distintus.*
Means within a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

2) Portion of barley used for aqueous extraction.

acids had little or no effect. Generally, phenolic substances can exert multiple actions on IAA metabolism.

Effects of aqueous extracts. The aqueous extracts of barley residues seemed to contain water-soluble phenolic compounds and also other water-soluble organic matters, showing much similar to natural conditions. Table 7 and 8 represent the germination and shoot growth of plants tested with varied dilution of aqueous extracts. The germination and shoot growth were inhibited by treatment of aqueous extracts, showing the different trend depending on extract source of barley residues, extract concentrations, and plants tested. The germination and shoot growth of *E. crusgalli* were inhibited as the concentrations of extract from both straw and root portion increased. *C. serotinus* germination was not affected by any dilution treatment of extracts, whereas its significant inhibition of shoot growth was observed.

P. distintus germination was stimulated at the higher concentrations, particularly at the early time. However, accelerated sprouts of pondweeds by the higher concentrations became died as the treatment proceeded, showing the similar symptoms with treatment of authentic phenolic acids. The black portion of Fig. 7 shows the percentage of killed portion after the sprouting of *P. distintus* treated with aqueous extracts. As compared to effects of authentic phenolic acids, the concentration of the undiluted aqueous extracts exerted the equal effect to 10^{-3} to 10^{-4}M of authentic phenolic acids.

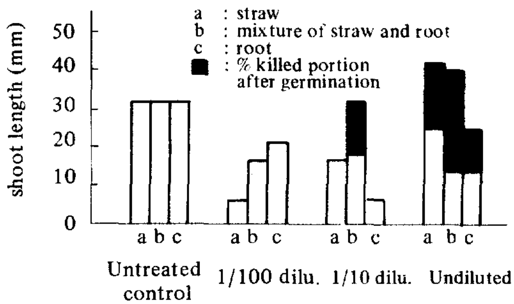

Fig. 7. Effects of aqueous extracts of barley residues on the shoot growth of *P. distintus* at 30 days after treatment.

Meanwhile, the mixture of straw and root extracts exerted more inhibitory effects on test plants than separate treatment of straw and root extracts. Fig. 8 and 9 show effects of aqueous extracts on the germination and shoot growth of rice and 3 paddy weeds treated with the mixed extracts of straw and root. The germination of all the tested plants was markedly inhibited by the undiluted concentrations except for *P. distintus* which was stimulated at the hgiher concentrations. The shoot growth of all the tested plants including *P. distintus* was inhibited as the concentrations increased. But, the least concentration such as 1/100 dilution slightly increased shoot growth except for *P. distintus,* showing marked inhibition.

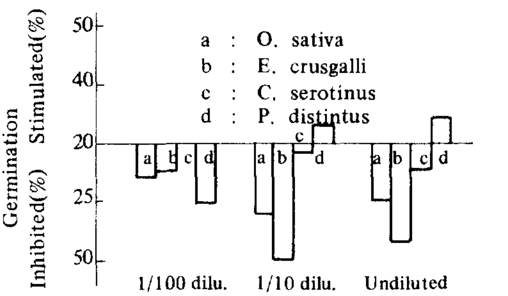

Fig. 8. Effects of aqueous extracts (mixture of straw and root) of barley residues on the germination of rice and three paddy weeds.

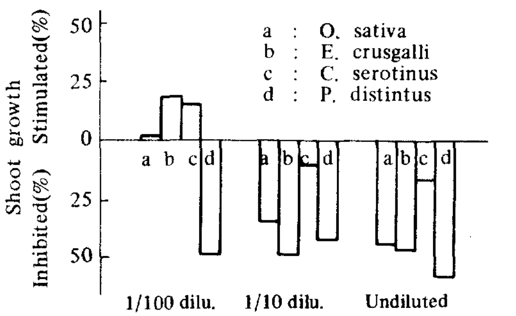

Fig. 9. Effects of aqueous extracts (mixture of straw and root) of barley residues on the shoot growth of rice and three paddy weeds.

The phenolic herbicides have been used as a group of contact herbicide since sodium dinitro-orthocresylate was used against broadleaf annual weeds in cereal crops, flax and peas in 1938[34]). Apparently there are two possible ways of mode of action of phenolic herbicides; 1) at high dosage rates by destroying membranes of treated tissues with resultant leakage of sap and dessication of the plant, and 2) by preventing the formation of ATP from inorganic phosphorus by oxidative phosphorylation[1]).

Based on the results and observations of this experiment, the following suggestings can be made. Firstly, the similar inhibitory effects were observed in both treated with authentic phenolic acids and with aqueous extracts of barley residues. Secondly, the inhibiting symptoms of *P. distintus* treated with authentic phenolic acids almost coincided with those of aqueous extracts of barley residues. Thirdly, it can be assumed that the inhibitors in the barley residues may be the major phenolic acids identified in this experiment or their derivatives. And, further studies in terms of the physiological, biochemical and anatomical approaches will shed more shed light on understanding the inhibitory effects of barley residues on paddy weeds, particuraly *P. distintus.*

摘　　要

보리殘餘物속에 含有되어 있는 phenolic compounds의 分離·同定과 殘餘物의 分解過程中 total phenol 含量 測定 그리고 主要標準 phenol 物質 및 殘餘物의 水溶抽出液이 벼와 主要 논雜草의 發芽 및 生育에 미치는 影響을 究明하여 植物體內에 存在하는 天然除草劑 開發에 관한 基礎資料를 얻고저 本 試驗을 遂行하였던 바 얻어진 結果는 다음과 같다.

1. 보리殘餘物속의 主要 phenolic compounds는 보릿짚과 뿌리 모두 p-coumaric, p-hydroxybenzoic, ferulic, vanillic 및 salicylic酸 等이 分離·同定되었다.

2. 보리 收穫時 殘餘物의 乾物重에 對한 total phenol 含量은 幼苗期보다 增加되어 보릿짚이 0.168 %, 뿌리가 0.127 % 있으며 分解初期 約干 增加되다가 分解가 進前되면서 보릿짚 및 뿌리 모두 顯著히 減少되었으며 어떤 時期에서도 보릿짚이 뿌리보다 total phenol 含量이 높았다.

3. 主要 phenolic acids는 피와 너도방동산의 發芽에는 多少 抑制를 보였다. 反面 가래는 處理初期에 高濃度일수록 發芽가 促進되다가 組織이 褐變·枯死되어 높은 抑制效果를 보여, 新鞘生育으로 본 抑制效果는 가래, 피, 너도방동산이의 順으로 높았다.

4. 殘餘物 水溶抽出液의 抑制力은 抽出部位와 供試雜草에 따라 약간 相異하였으나 보릿짚과 뿌리의 混合處理에서 높았는데, 피와 너도방동산이에는 高

濃度일수록 抑制效果가 높았으나 低濃度에서 新梢生育을 약간 促進시켰다. 가래에는 處理初期에 發芽를 促進시켰으나 組織이 枯死되어 높은 抑制效果를 나타내었다

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