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Identification and Growth Inhibition of Phytotoxic Substances from Tomato Plant

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토마토植物의 毒性物質 確認과 生長抑制作用

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ABSTRACT

Phenolic compounds such as gallic acid, ferulic acid, *p*-hydroxybenzoic acid, vanillic acid, salicylic acid, tannic acid, and hydroquinone were identified from the aqueous extracts and volatile substances of tomato plant by paper chromatography, high performance liquid chromatography and gas chromatography.

The seed germination and seedling growth of the experimental species, lettuce and egg plant, were severely inhibited in 5×10^{-3} M of phenolic reagents identical to those identified from tomato plant. Germination and growth rate of test species in 5×10^{-4} M and 5×10^{-5} M were higher than that of 5×10^{-3} M. Therefore, 5×10^{-3} M of phenolic compounds would be assumed to be threshold concentration for allelopathic effects.

INTRODUCTION

If a plant produces a chemical which suppresses the growth of metabolism of other plants, the phenomenon is called allelopathy. Normally the effect is harmful, but beneficial effect is possible (Newman, 1978). It has been reported that extracts from *Rumex crispers* leaves were toxic to the growth of corn and sorghum seedling (Einhellig and Rasmussen, 1973), and the growth of corn seedling was inhibited in aqueous leachates of *Chenopodium album* roots (Kossanel *et al.*, 1977). Many researchers have found that the inhibitory substances involved in allelochemic interations were phenolic compounds and volatile substances from leaves, roots, fallen leaves and underneath soil of growing plants. These inhibitory substances were identified by paper chromatography, thin layer chromatography, gas chromatography and mass spectrophotometry (Grümmer, 1961; Lodhi, 1976; Chou and Chen, 1976; Jackson and Willemsen, 1976).

From our previous study it has been found that seed germination and seedling growth of experimental species treated with aqueous extracts and leachates of tomato plant were remakably inhibited in laboratory and vinyl house work (Kim and Kil, 1987).

However it is important not only to know which chemicals have phytotoxic effects, but also at which concentration this harmful action inhibits growth of neighboring species.

In the present paper we identified allelochemic substances in tomato plant by paper chromatography, thin layer chromatography and gas chromatography, and performed germination and growth test with the identical chemicals obtained from tomato plants.

METERIALS AND METHODS

Isolation of phytotoxic substances from tomato plant. Modified method of Lodhi and Rice (1971) was used for the isolation of phytotoxic substances from tomato plants. Ten grams of tomato leaves were soaked in 150 ml of 2 N NaOH and then hydrolyzed under the pressure of 1.5 kg/cm^2 in autoclave for 45 min. After filtering, the filterates were adjusted to pH 2.0 with 1 N HCl. The filtered 30 ml of ether extractions were added with half volumes of 5% $NaHCO_3$ solution. From the separated two layers, the upper ether fraction was discarded and lower $NaHCO_3$ fraction was adjusted to pH 2.0 with I N HCl. This was extracted with 10 ml of ether three times successively and concentrated in rotary evaporator (Corning Co.). The concentrated extracts were developed by paper chromatography (PC). Two dimensional developments were done with 46 × 57 cm Whatman No. 1 paper by descending method at 28℃ +2℃, first, 63 n-butanol: 10 acetic acid: 27 water (BAW) in volume ratio, and second, 6% acetic acid. After development the filter papers were dried in air, illuminated under the 2,537 Å UV lamp and marked for calculating Rf value to compare with known compounds.

High performance liquid chromatography (HPLC) was also used for identifying phytotoxic substances from tomato plants (Kim and Kil, 1984).

The modified Yamamoto apparatus (Yamamoto, 1963) was used for collection of volatile substances from tomato plant (Fig. 1). Tomato plant were washed and put into bottle B. Dry ice was placed in bottle C, and volatile substances from tomato plants were collected in small bottle D. Collected substances were injected into mobile phase in HPLC (Waters Co. 440 type) as follows; methanol, 1% acetic acid, Bondpak C18 in column, UV absorbance detector, flow speed in 254 nm/0.05 Aufs were 1.5 ml/min, chart speed was 1.0 cm/min, respectively.

Extraction procedure of tomato plant that gas chromatography (GC) was the same as Kil and Yim (1983), except that the ether extracts were transferred into 250 ml conical flask and concentrated about 2 ml in volume. These concentrated extracts were poured into centrifuge tubes and vapor dried under N_2 gas. Residues attached to the wall of centrifuge tube were melted with 100 ml of HPLC acetonitrile. In this solution 150 ml of BSTFA (N,D-bistrimethylsilyl-trifloro acetamide) were added and injected into the gas chromatography apparatus after five minutes. Hewlett Packard 5840 A was used, column was 6 ft×1/8 inch i.d, OV-1

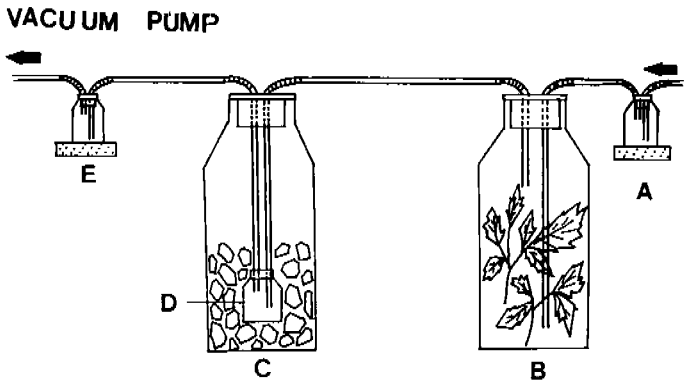

Fig. 1. Apparatus for collecting volatile substances from tomato plant. A, trap; B, container; C, dry ice; D, collecting bottle; E, trap.

90/100 mesh, flow speed of N_2 gas was 15 ml/min., sensitivity of flame ionization detection was 2 × 10, temperature of injection detector was 205-250℃, temperature of column was 6℃ /min., 100℃ to 200℃ in rising temperature speed.

Germination and growth test. With the identical chemical reagents (Sigma Co.) which were identified from tomato plants, germination and seedling growth test was attempted in the laboratory with 5×10^{-3} M, 5×10^{-4} M and 5×10^{-5} M of each phenolic compound, tannic acid, hydroquinone, *p*-hydroxybenzoic acid, vanillic acid, and ferulic acid which were prepared for germination and seedling growth experiments. The procedure of the test included control was the same as Kim and Kil (1987). Fifty seeds of lettuce were germinated in pot added with vermiculite and their seedling were grown with different concentrations of five chemicals. The results of germination were calculated percentage of control. Also, after growing the seedlings for a given period, they were harvested and oven dried at 80℃ until keeping constant weight. All these experiments were done four times repeatedly.

RESULTS

Identification of phytotoxic substances. Five phenolic acids, gallic acid, ferulic acid, *p*-hydroxybenzoic acid, vanillic acid and salicylic acid were identified from tomato plants by PC (Table 1). And the other five chemical substances from leaves, stems and roots of tomato plants were detected (Table 2).

Seven kinds of chemical substances were isolated and identified by PC and HPLC. Among them ferulic acid, *p*-hydroxybenzoic acid and vanillic acid were found by both PC and HPLC method. In addition tannic acid and hydroquinone were identified from valatile substances of tomato plants by GC (Fig. 2).

Growth. The effects of different concentrations of chemical reagents identical to those

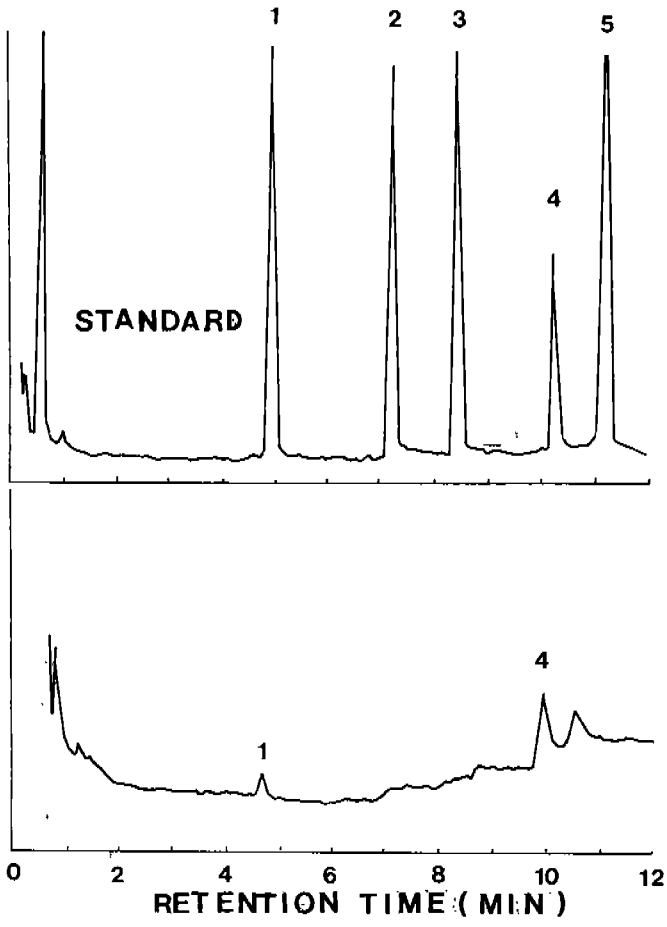

Fig. 2. Standard phenolic compounds (above) and volatile substances of tomato plants (below) analyzed by gas chromatography.
Key: 1, hydroquinone; 2, *p*-hydroxybenzoi acid; 3, vanillic acid; 4, Tannic acid; 5, Ferulic acid.

Table 1. Comparison of Rf values of standard phenolic compounds and extract from tomato leaves by PC

Name of Phenolic Acid	Standard		Tomato	
	BAW[1]	AA[2]	BAW[1]	AA[2]
Gallic acid	0.62	0.54	0.62	0.56
Ferulic acid	0.85	0.52	0.86	0.52
P-hydroxybenzoic acid	0.87	0.66	0.87	0.68
Vanillic acid	0.88	0.59	0.88	0.58
Salicylic acid	0.92	0.69	0.91	0.68

1) Solvent of n-butanol-acetic acid-water.
2) Solvent of 6% acetic acid.

identified from tomato plants on seed germination and seedling growth were shown in Table 3.

Table 3 shows that lettuce seeds did not germinate in 5×10^{-3} M of tannic acid, *p*-hydroxybenzoic acid and vanillic acid. But 26.8% and 50.5% germination occurred in 5×10^{-3} M of hydroquinone and ferulic acid, respectively. Germination of test plots compared

Table 2. Identification of phenolic compounds from extract of tomato leaves, stems and roots by HPLC

Relative Retention Time (min)	Materials	Phenolic Compounds
	Leaves	
2.05		Tannic acid
3.42		Hydroquinone
4.78		*P*-hydroxybenzoic acid
5.16		Vanillic acid
8.79		Ferulic acid
	Stems	
2.44		Tannic acid
	Roots	
2.38		Tannic acid
3.38		Hydroquinone
4.74		*P*-hydroxybenzoic acid

Table 3. Effects of phenolic compounds on germination and seedling growth of *Lactuca sativa* grown in Petri dish

Compounds	Concentration (M)	Germination (% of control)	Elongation S+R*(cm)
Tannic acid	5×10^{-3}	0.0	0.0
	5×10^{-4}	92.2	41.8
	5×10^{-5}	95.0	116.5
Hydroquinone	5×10^{-3}	26.8	13.3
	5×10^{-4}	98.2	97.6
	5×10^{-5}	100.7	115.6
P-hydroxybenzoic acid	5×10^{-3}	0.0	0.0
	5×10^{-4}	97.4	64.0
	5×10^{-5}	98.7	113.8
Vanillic acid	5×10^{-3}	0.0	0.0
	5×10^{-4}	94.1	62.4
	5×10^{-5}	98.3	105.1
Ferulic acid	5×10^{-3}	50.5	7.7
	5×10^{-4}	98.3	87.9
	5×10^{-5}	98.0	109.7

Each point represents an average of four determinations. S, shoot; R, root of seedling.

with the control was almost the same in 5×10^{-4} M and 5×10^{-5} M of all phenolic compounds. Elongation of lettuce seedling grown in 5×10^{-4} M was suppressed but the inhibition level differed among the chemicals. At 5×10^{-5} M, there was no inhibitory effect on seedling elongation.

The results of germination and seedling elongation of the egg plant in various concentrations of phenolic compounds were presented in Table 4. Seeds of the egg plant did not germinate in 5×10^{-3} M tannic acid, hydroquinone, *p*-hydroxybenzoic acid, vanillic acid and ferulic acid,

Table 4. Effects of phenolic compounds on germination and seedling growth of *Solanum melongena* grown in Petri dish

Compounds	Concentration (M)	Germination (% of control)	Elongation S+R*(cm)
Tannic acid	5×10^{-3}	0.0	0.0
	5×10^{-4}	41.7	19.2
	5×10^{-5}	93.9	115.9
Hydroqinone	5×10^{-3}	0.0	0.0
	5×10^{-4}	0.0	0.0
	5×10^{-5}	97.3	35.6
P-hydroxybenzoic acid	5×10^{-3}	0.0	0.0
	5×10^{-4}	34.7	45.3
	5×10^{-5}	97.3	38.7
Vanillic acid	5×10^{-3}	0.0	0.0
	5×10^{-4}	83.4	26.1
	5×10^{-5}	86.9	54.7
Ferulic acid	5×10^{-3}	0.0	0.0
	5×10^{-4}	22.6	18.0
	5×10^{-5}	29.5	41.7

Each point represents an average of four determinations. S, shoot; R, root of seedling.

Table 5. Effects of phenolic compounds on germination and seedling growth of *Lactuca sativa* grown in pot

Compounds	Concentration (M)	Germination (% of control)	Elongation (% of control)	Dry Weight (% of ontrol)
Tannic acid	5×10^{-3}	0.0	0.0	0.0
	5×10^{-4}	72.2	65.5	27.6
	5×10^{-5}	96.6	75.1	43.0
Hydroquinone	5×10^{-3}	0.0	0.0	0.0
	5×10^{-4}	79.3	65.1	16.9
	5×10^{-5}	82.9	76.0	41.5
P-hydroxybenzoic acid	5×10^{-3}	0.0	0.0	0.0
	5×10^{-4}	96.6	81.3	29.2
	5×10^{-5}	94.6	80.5	38.4
Vanillic acid	5×10^{-3}	0.0	0.0	0.0
	5×10^{-4}	94.6	67.5	30.7
	5×10^{-5}	94.6	70.4	32.3
Ferulic acid	5×10^{-3}	0.0	0.0	0.0
	5×10^{-4}	94.6	68.3	33.8
	5×10^{-5}	91.5	74.8	44.6

Each point represents an average of four determinations. S, shoot: R, root of seedling.

while germination in 5×10^{-4} M of hydroqinone, ferulic acid, *p*-hydroxybenzoic acid, tannic acid and vanillic acid were 0.0, 22.6, 34.7, 41.7 and 83.4%, respectively. However, germina-

tions in 5×10^{-5} M of phenolic acids were good except for ferulic acid. Seedling elongation of egg plant was inhibited in all used chemical concentrations except for the 5×10^{-5} M of tannic acid.

Germination, seedling elongation and dry weight of lettuce in pot test were compared amomg the different chemical solution (Table 5). The concentration of 5×10^{-3} M of all chemicals used for the experiment showed severe phytotoxic effects on lettuce plant. There was no germination in all 5×10^{-3} M plots in this study. Dry weight of the lettuce was more suppressed by chemicals than germination and subsequent seedling elongation.

DISCUSSION

From red pine benzoic acid and eleven kinds of phenolic compounds have been identified by GC (Kil and Yim, 1983). Comparing these chemicals from red pine with seven kinds of chemicals from tomato plant, *p*-hydroxybenzoic acid, vanillic acid and ferulic acid were common substances from both sides. All these substances are phenolic compounds. It is well known that phenolic compounds are a main inhibitor of germination and seedling growth (Olmsted and Rice, 1970; Whittaker and Feeny, 1971; Tinnin and Muller, 1972; Horsley, 1977; Carballeira, 1980). The results of this study have clearly shown that 5×10^{-3} M of phenolic compounds would be proposed a threshold concentration. However, the low concentration of phenolic compounds improved the growth of experimental donor plants.

Germination of lettuce and egg plant in Petri dish showed a similar tendeny each other but somewhat different results in detail. It was because sprouting days after sowing was different between two species, namely, germination of lettuce have taken for two days after sowing and harvested after seven days, however, egg plant, for twenty days to harvest.

Germination percentages of sorghum treated in 5×10^{-3} M of *p*-coumaric acid and ferulic aid have depended upon a time lapse, i.e. at 24 h after seed sowing was 34%, but at 48 h, 59% (Rasmussen and Einhellig, 1977). In the bioassay with juglone, seedlings of experimental species were killed in 10^{-3} M (Rietveld, 1983; Rietveld *et al.*, 1983). These results have coincided to the same tendency with this study. Other researchers have reported that radicle elongation of cucumber tested in 0.12 M and 0.25 M of ferulic acid has inhibited 7% and 14%, respectively (Blum *et al.*, 1984). And 10^{-3} M of ferulic acid was very toxic to cell cultures (Danks *et al.*, 1975), dry weights of soybean seedling were severely inhibited in 10^{-3} M and 5×10^{-4} M of ferulic acid (Einhellig and Rasmussen, 1979), growth inhibitor isolated from hackberry leaves was certified ferulic acid (Lodhi, 1975).

적 요

토마토 식물의 수용추출액과 휘발성물질이 다른 식물의 발아와 생장을 억제한다는 사실을 이미 밝힌 바 있었다 (Kim and Kil, 1986). 본 연구에서는 토마토식물에 함유되어 있는 발아 및 생장억제 물질을 검출

하고 이를 사용한 실험을 통하여 allelochemical임을 확인했다.

토마토 잎, 줄기, 뿌리의 수용액과 휘발성물질에서 PC, HPLC, GC에 의하여 검출된 물질은 gallic acid, ferulic acid, *p*-hydroxybenzoic acid, vanillic acid, salicylic acid, tannic acid, hydroquinone 등 7종류였다.

이들 phenolic compound와 같은 화학약품으로 5×10^{-3} M, 5×10^{-4} M, 5×10^{-5} M 용액을 만들어 상치와 가지의 종자발아와 유식물의 생장을 실험해본 결과 5×10^{-3} M 에서는 발아가 안되거나 심하게 억제 되었으나 5×10^{-4} M에서는 대조구에 비하여 억제되는 경향이었고 5×10^{-5}M에서는 발아율과 생장율 양쪽 다 높은 값을 나타냈다. 따라서 5×10^{-3} M의 phenolic compound는 본 실험에서 역치농도로 생각된다.

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