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Allelopathic Potential of Red Pepper (*Capsicum annuum* L.)

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Abstract

The yield of red pepper has been decreasing due to continuous cropping in Korea. Although this decline had been mainly attributed to the occurrence of diseases, it was suggested that allelopathy may account for the decline. The growth inhibition of red pepper was assayed by a germination test using water or organic solvent extracts of leaf, stem, root and cultivates soil. In addition, the allelopathy of red pepper from other sources of competition was analyzed by using continuous root exudate trapping system. Methanol extracts of stem and root of red pepper strongly inhibited the radicle growth of red pepper. Methanol extracts of leaf and root and water extract of root inhibited the hypocotyl growth. The methanol and water extracts of red pepper root contained several phenolic acids such as p-hydroxybenzoic acid and vanillic acid. In the absence of an XAD-4 resin column, the growth of red pepper was inhibited by the root exudate as compared with the presence of the column. In addition, this exudate inhibited the germination of red pepper, and it showed a characteristic absorption peak as in the case of the methanol extract of cultivated soil at 254 nm. I was assumed that the red pepper decline under continuous cropping was due to allelopathy.

Discipline: Soils, fertilizers and plant nutrition / Horticulture
Additional key words: continuous cropping, decline problem, phenolic acid

Introduction

Red pepper is not only the material used for *Kimchi* (Korean pickles) but also a seasoning for many dishes. It is the most important vegetable used for spices in Korea and it is cultivated in 30% of the Korean vegetable fields. After the Seoul Olympic Games, the consumption of vegetables became diversified, and the cultivated area of red pepper decreased slightly. However, since the demand for green pepper increased rapidly along with the increase of meat consumption, the cultivated area of green pepper has increased in greenhouses in winter.

The yield and the quality of red pepper have decreased due to continuous cropping in Korea. This decline was mainly attributed to the occurrence of diseases such as late blight,

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anthracnose and mosaic. However, it was assumed that allelopathy was one of the reasons for this decline. Recently, although reports on the allelopathy of vegetables such as asparagus[5,6], pea[4], etc. have been published by a large number of researchers, there are only a few reports on the allelopathy of red pepper[1,2]. Therefore, experiments were carried out to investigate the occurrence of allelopathy in red pepper. The growth inhibition of red pepper was assayed by using the germination test with water or organic solvent extracts of leaf, stem, root and cultivated soil. In addition, the allelopathy of red pepper from other sources of competition was analyzed by using a continuous root exudate trapping system and the growth inhibiting substances were studied.

Materials and methods

1) Analysis of allelopathy by using extracts of red pepper

(1) Influence of methanol extract of leaf, stem and root of red pepper

Red pepper ('Dahong' grown in Korea) was used in this experiment. In order to inactivate the enzymes which degrade some of the constituents of red pepper and to minimize the changes in the content of organic chemicals, leaves, stems, and roots were dried at 60°C for 48 hr. A 2 g aliquot of the dried samples was extracted with 20 m*l* of 90% methanol for 1 hr. The extracts were filtered with Toyo No.2 filter papers. Twenty red pepper seeds were placed in 4.5 cm diameter petri dishes containing 1,000 ppm test solution on Toyo No. 2 filter papers. The seeds were incubated at 28°C under dark conditions. The number of germinated seeds was counted and the hypocotyl and radicle length was measured on the 8th day. On the other hand, methanol extracts were analyzed by HPLC with an ODS column.

(2) Influence of water extract of root of red pepper

Five g, 1 g, 100 mg and 10 mg of dried root of red pepper were extracted with 100 m*l* of distilled water for 1 hr. The extracts were filtered with Toyo No.6 filter paper. These extracts were analyzed by the same method as that previously described.

(3) Influence of organic solvent extract of root of red pepper

(a) Influence of methanol-chloroform-water extract of root of red pepper

A 2 g aliquot of dried red pepper root was extracted with 20 m*l* of mixed organic solvent (methanol : chloroform : water = 2 : 1 : 0.8) for 1 hr. The supernatant solution was fractionated into a water phase, chloroform phase, and phenolic phase, as shown in Fig. 1. These fractions were analyzed by the germination test and HPLC.

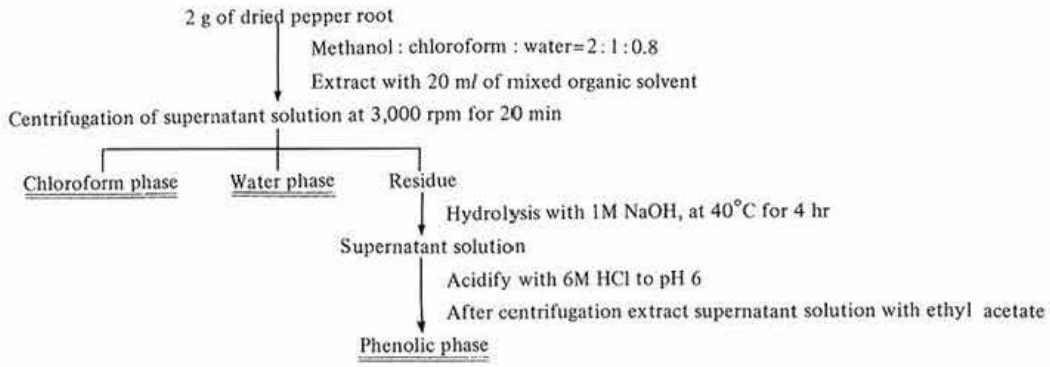

Fig. 1. Fractionation method of red pepper root using mixed organic solvent

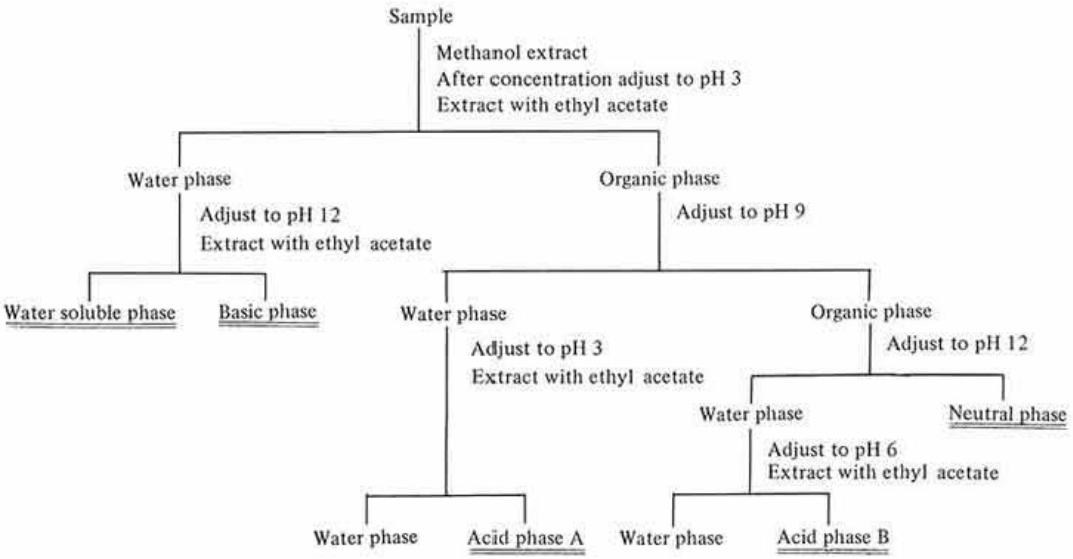

Fig. 2. Fractionation method using methanol-ethyl acetate

(b) Influence of methanol-ethyl acetate extract of root of red pepper

Twenty g of dried red pepper root was extracted with 200 m*l* of methanol for 1 hr. The extract was fractionated into an acid phase, neutral phase, basic phase, and water soluble phase, as shown in Fig. 2. These fractions were analyzed by the germination test and HPLC.

2) Analysis of allelopathy of red pepper by using root exudate

(1) Influence of root exudate of red pepper in soil

Six seedlings of red pepper, 30 cm tall at the 15 leaf age were planted in a 1/2,000 a Wagner pot filled with 8 kg of Andosol. After 40 days, 100 g of fresh soil was extracted with 150 m*l* of water or methanol. Water and methanol extracts were dried using a vacuum freezing drier and rotary evaporator, respectively. These extracts were analyzed by the germination test and HPLC.

(2) Effect of root exudate of red pepper by using a continuous root exudate trapping system

Effect of root exudate of red pepper on the

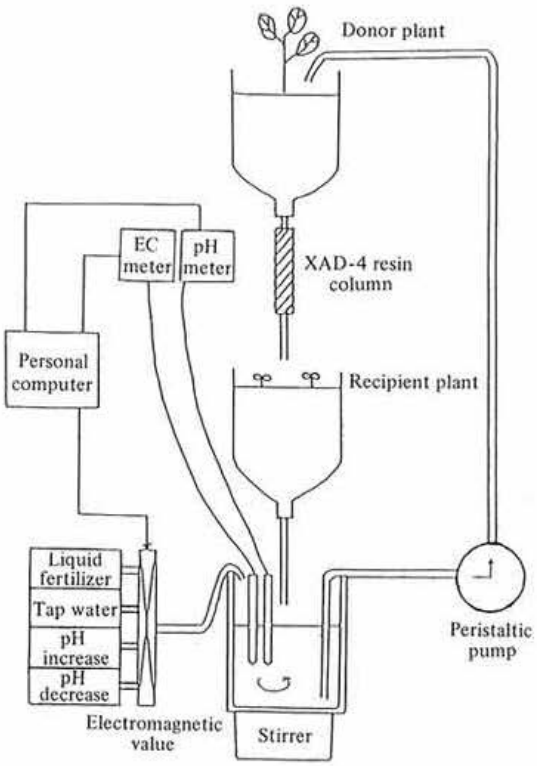

Fig. 3. Continuous root exudate trapping system for analysis of allelopathy

germination and the growth of red pepper itself was studied by using a continuous root exudate trapping system (CRETS)[3] (Fig. 3), with or without a column of Amberlite XAD-4 resin. Three seedlings of red pepper, 15 cm tall at the 8–9 leaf age were planted in washed sand. Amberlite XAD-4 resin column was or was not connected between the donor and recipient pots. Constituents of the solution were: N; 9.3 me, P; 2.55 me, K; 3.8 me, Ca; 4.1 me, Mg; 1.85 me/*l*, MnO; 0.75 ppm, B_2O_3; 0.75 ppm, Fe; 1.35 ppm. EC of the culture solution was 1.22 mS/cm, flow rate was 450 m*l*/hr. One week after planting, 30 seeds of red pepper were sown as recipient plants, and 8 seedlings were picked out after the germination analysis. Thirty seven days after sowing, the growth of red pepper was examined. Amberlite XAD-4 resin was washed with methanol and the solution obtained was analyzed by the germination test and HPLC.

Results and discussion

1) Analysis of allelopathy by using extracts of red pepper

(1) Influence of methanol extracts of leaf, stem and root of red pepper

Germination rate was not affected by the methanol extracts of leaf, stem and root of red pepper (Table 1). On the other hand, radicle growth was inhibited by the stem and root extracts and hypocotyl growth was also inhibited by each extract. Inhibition rate of the root extract was the strongest.

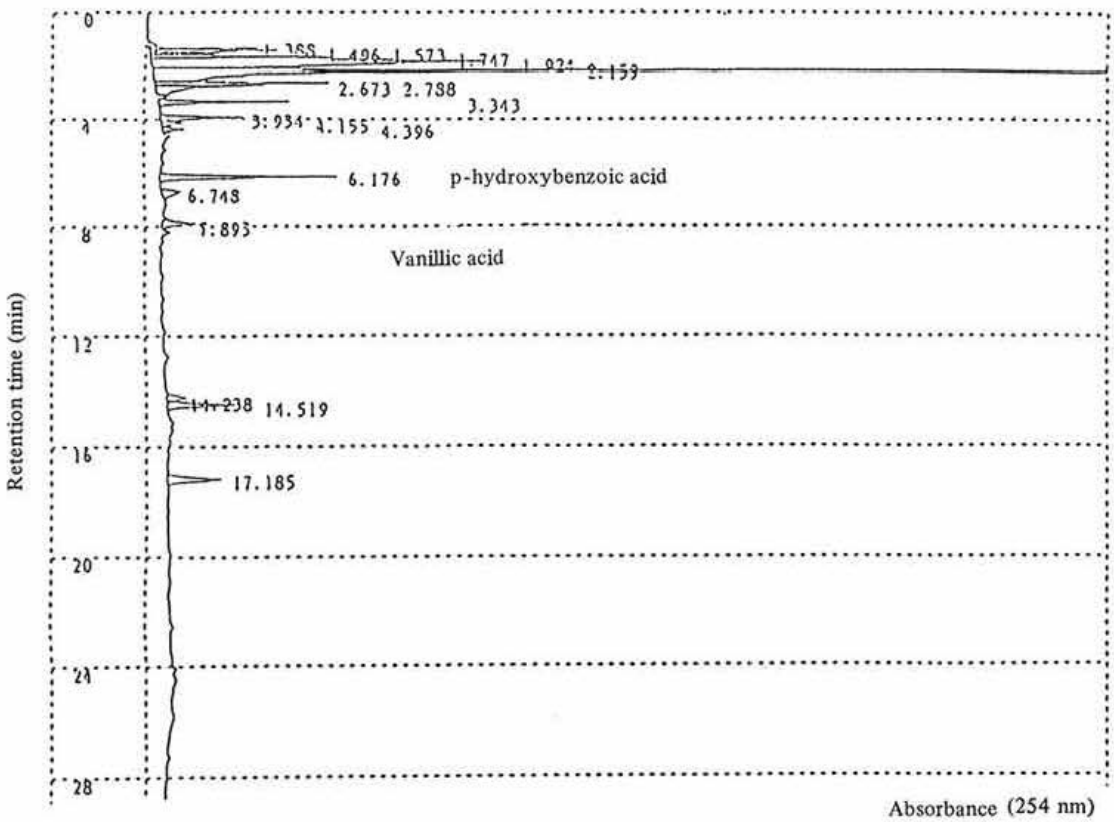

Fig. 4. HPLC analysis of methanol extracts of red pepper root

The methanol extracts of red pepper root contained several phenolic acids such as p-hydroxybenzoic acid and vanillic acid, at the rates of 32, 11 mg/kg, respectively (Fig. 4). However, the components of the leaf and stem methanol extracts of red pepper could not be identified.

In general, although leaf and stem residues are removed from the field, root residues remain in the field. Consequently, root exudates or root residues are considered to play a major inhibitory role in the allelopathy of red pepper.

(2) Influence of water extract of root of red pepper

Germination rate was not affected by the concentration of the extract (Table 2). Radicle growth showed a 60% inhibition by the water extract of red pepper at the concentration of 5 g/d*l*. However, the growth of red pepper was rather accelerated by the water extract of red pepper root by 20–30% at the concentration of 0.01–0.1 g/d*l*. Phenolic acids such as p-hydroxybenzoic acid and vanillic acid which were detected (Fig. 5) were considered to be allelochemicals.

(3) Influence of organic solvent extract of root of red pepper

(a) Influence of methanol-chloroform-water extract of root of red pepper

Germination rate was not affected by these fractions. Water phase (sugars and amphoteric substances) and chloroform phase (lipid substances) inhibited the radicle growth of red pepper at 1,000 ppm (Table 3), unlike the phenolic phase. This phenomenon was ascribed to the fact that the concentration of the phenolic phase was low (100 ppm). The phenolic phase also contained phenolic acids such as p-hydroxybenzoic acid, vanillic acid, etc.

Table 1. Effect of methanol extracts of leaf, stem and root of red pepper on the germination of red pepper

Extract	Germination rate (%)	Radicle length (cm)	Hypocotyl length (cm)
Leaf	93.0 a*	1.96 a	0.98 c
Stem	93.3 a	1.42 b	1.49 b
Root	95.3 a	1.08 b	1.11 c
D.W.	93.3 a	2.26 a	1.85 a

Extract ratio was 1:10.

*Means in the same column followed by the same letter are not significantly different (P = 0.05) by Duncan's multiple range test.

Table 2. Effect of water extracts of roof of red pepper on the germination of red pepper

Extract conc. (g/d*l*)	Germination rate (%)	Radicle length (cm)	Hypocotyl length (cm)
5.0	93.3 a*	0.59 c	1.19 c
1.0	93.3 a	1.48 b	1.71 a
0.1	89.0 a	2.15 a	1.63 a
0.01	89.0 a	2.04 a	1.58 a
D.W.	93.3 a	1.58 b	1.35 b

Extract ratio was 1:10.

*See the footnote of Table 1.

Table 3. Effect of organic solvent extracts of root of red pepper on the germination of red pepper

Fraction	Conc. (ppm)	Germination rate (%)	Radicle length (cm)	Hypocotyl length (cm)
Water	1,000	80.0 a*	1.41 b	1.36 a
Chloroform	1,000	68.9 a	1.27 b	0.92 b
Phenolics	100	88.9 a	2.17 a	1.54 a
D.W.		84.4 a	2.26 a	1.55 a

* See the footnote of Table 1.

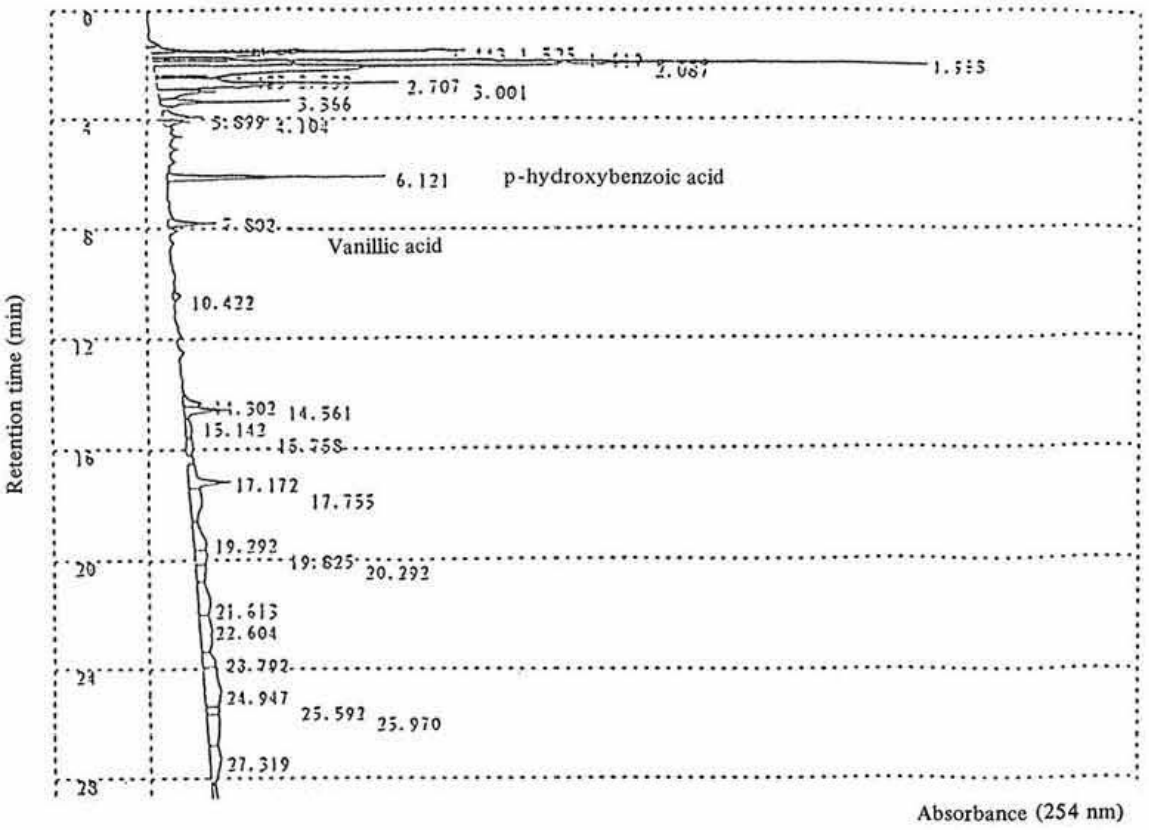

Fig. 5. HPLC analysis of water extracts of red pepper root

Table 4. Effect of methanol-ethyl acetate extracts of root of red pepper on the germination of red pepper

Fraction	Conc. (ppm)	Germination rate (%)	Radicle length (cm)	Hypocotyl length (cm)
Acid-A	500	88.7 a*	0.82 c	0.67 d
Acid-B	500	88.7 a	1.77 a	1.29 b
Base	500	91.3 a	1.56 b	1.37 ab
Neutral	500	93.3 a	1.62 ab	0.90 c
Water soluble	500	93.3 a	1.44 b	1.55 a
D.W.		95.3 a	1.58 b	1.47 ab
Acid-A	100	95.5 a	1.32 e	0.85 c
Acid-B	100	88.9 a	2.14 a	1.78 a
Base	100	95.5 a	1.97 ab	1.28 b
Neutral	100	93.3 a	1.78 bc	1.43 b
Water soluble	100	88.9 a	1.55 d	1.46 b
D.W.		95.3 a	1.58 cd	1.47 b

*See the footnote of Table 1.

(b) Influence of methanol-ethyl acetate extract of root of red pepper

Germination rate of red pepper was inhibited by all the fractions regardless of the concentration (Table 4). The acid fraction A, which contained phenolic acids, strongly inhibited the growth of red pepper, especially by 15% at 100 ppm and by 50% at 500 ppm. Hypocotyl growth was also inhibited by the neutral fraction (500 ppm) by 40%. The acid phase A contained ferulic acid, but only a small amount of p-hydroxybenzoic acid for unknown reasons.

The root extract of red pepper contained several phenolic acids such as p-hydroxybenzoic acid, vanillic acid and ferulic acid. These phenolic acids inhibited the growth of red pepper and were considered to be allelochemicals.

2) Analysis of allelopathy of red pepper by using root exudate

(1) Influence of root exudate of red pepper in soil

Allelochemicals of red pepper must be identified in cultivated soil. Germination rate of red pepper was not affected by the water or methanol extract of cultivated or non-cultivated soil (Table 5). Extraction at 10,000 ppm resulted in the inhibition of hypocotyl growth by 70%. At 1,000 ppm, however, the growth was rather accelerated by 10% by the methanol extract, while by 25% by the water or methanol extract, respectively.

On the other hand, radicle growth was inhibited by 10% by the water or methanol extract, by 15% by the water extract at 1,000 ppm and by 80% at 10,000 ppm. There was a characteristic absorption peak, as in the case of the methanol extract of cultivated soil at 254 nm, with a retention time of 14.3 min (Fig. 6). However the substances responsible for this peak have not been identified yet.

(2) Influence of root exudate of red pepper by using a continuous root exudate trapping system

The EC of the culture solution was set at a high level due to the decrease of the NO_3^- concentration for the last 10 days. The final pH of the solution was 7.3, NO_3^- concentration of the recipient plot decreased gradually after 30 days, but rapidly decreased in the donor plot. However, the concentration of

Table 5. Effect of water and methanol extracts of cultivated soil of red pepper on the germination of red pepper

Solvent	Fraction	Conc. (ppm)	Germination rate (%)	Radicle length (cm)	Hypocotyl length (cm)
Water	Cultivated soil	10,000	91.0 a*	0.29 c	0.33 c
		1,000	89.0 a	1.28 b	1.65 a
		100	89.0 a	1.48 a	1.36 b
	Non-cultivated soil	10,000	89.0 a	0.34 c	0.35 c
		1,000	89.0 a	1.25 b	1.64 a
		100	93.0 a	1.42 a	1.32 b
Methanol	Cultivated soil	10,000	89.0 a	0.35 c	0.42 c
		1,000	91.0 a	1.31 b	1.53 a
		100	89.0 a	1.48 a	1.33 b
	Non-cultivated soil	10,000	91.0 a	0.30 c	0.43 c
		1,000	89.3 a	1.28 b	1.54 a
		100	91.3 a	1.44 a	1.26 b
	D.W.		89.0 a	1.47 a	1.37 b

*See the footnote of Table 1.

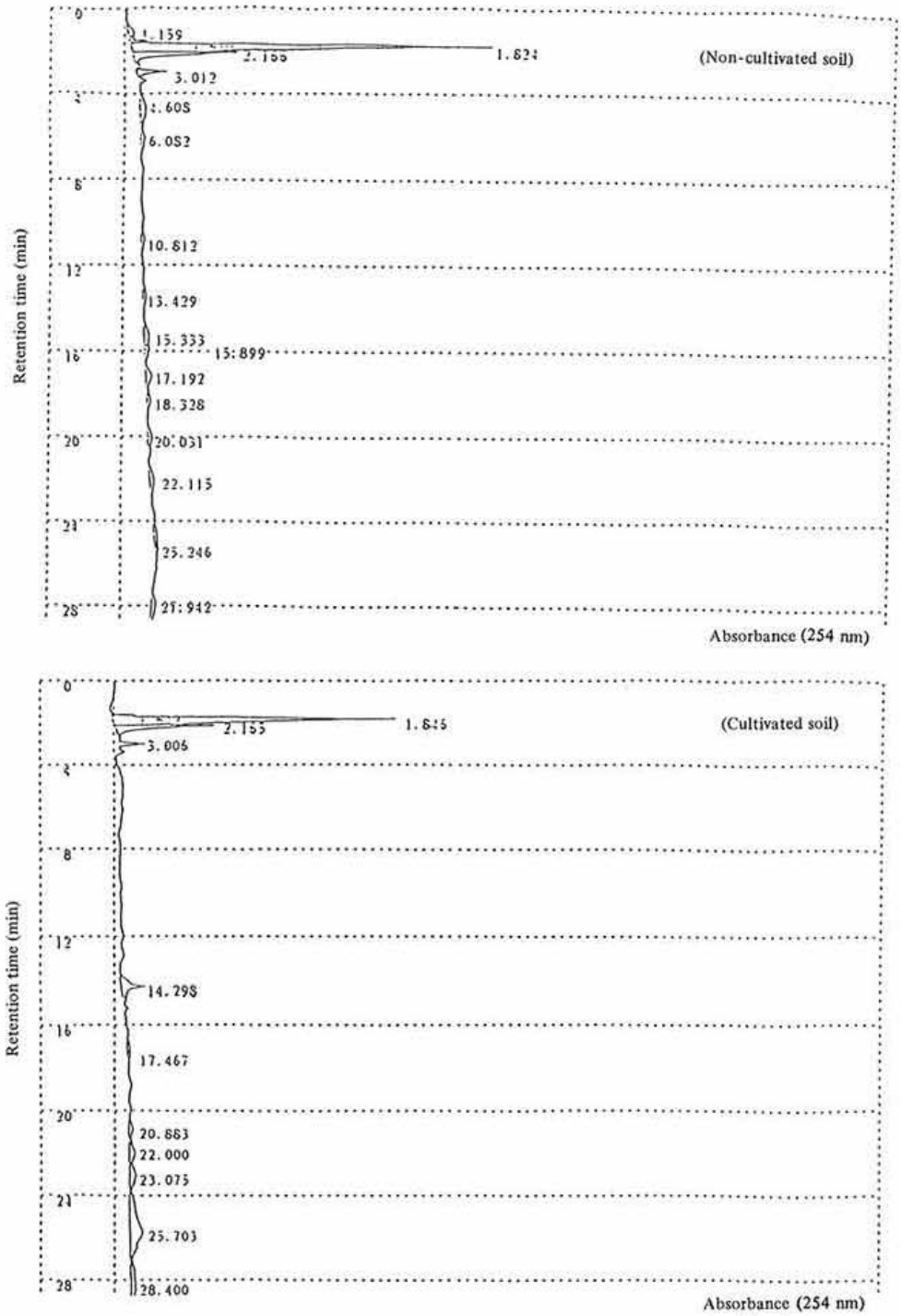

Fig. 6. HPLC analysis of methanol extracts of non-cultivated and cultivated soil of red pepper

Table 6. Effect of root exudate of red pepper on the germination and growth of red pepper

Donor plant	XAD-4 column	Germination rate (%)	Plant height (cm)	Leaf number	Leaf area (cm^2)	Top dry matter (mg)	Root dry matter (mg)
Red pepper	−	96.7	26.9	13.5	176	523	123
	+	90.0	29.1	15.0	248	619	140
None	−	90.0	33.8	14.8	303	818	159
	+	93.3	35.5	15.3	343	941	183

Table 7. Effect of exudate of red pepper root using XAD-4 resin on the germination of red pepper

Conc. (ppm)	Germination rate (%)	Radicle length (cm)	Hypocotyl length (cm)
1,000	93.3 a*	1.01 c	0.74 c
500	93.7 a	1.31 b	1.02 b
100	95.3 a	1.71 a	1.26 a
D.W.	93.0 a	1.64 a	1.24 a

*See the footnote of Table 1.

NO_3^- in the plot with Amberlite XAD-4 resin and the plot without resin was almost the same.

The root exudate of red pepper did not affect the germination rate of red pepper seed (Table 6). In the absence of the XAD-4 resin column, the growth of red pepper was inhibited by the root exudate by 15% as compared with the presence of the column (Plate 1).

Germination rate of red pepper was not affected by 100–1,000 ppm of absorbent onto XAD-4 resin (Table 7). Radicle and hypocotyl growth was inhibited by 40% at 1,000 ppm, 20% at 500 ppm, respectively, but was not inhibited at 100 ppm.

The exudate adsorbed onto the Amberlite XAD-4 resin showed a characteristic absorption peak as in the case of the methanol extract of cultivated soil at 254 nm (Fig. 7). The retention time of this peak was 14.5 min. However, the substances responsible for this peak have not been identified.

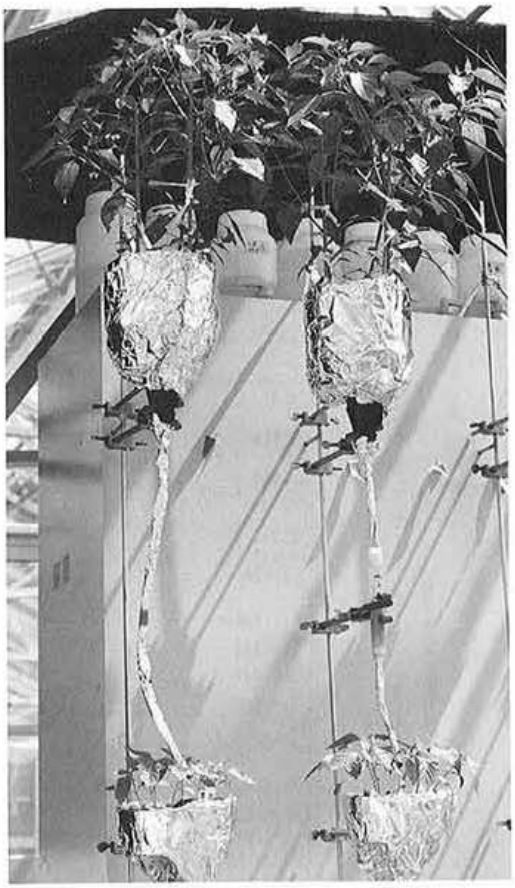

Plate 1. Effect of root exudate of red pepper on the growth of seedling of red pepper with (right) or without (left) XAD-4 column

Further studies should be carried out to identify the inhibiting substances and determine whether soil toxicity persists.

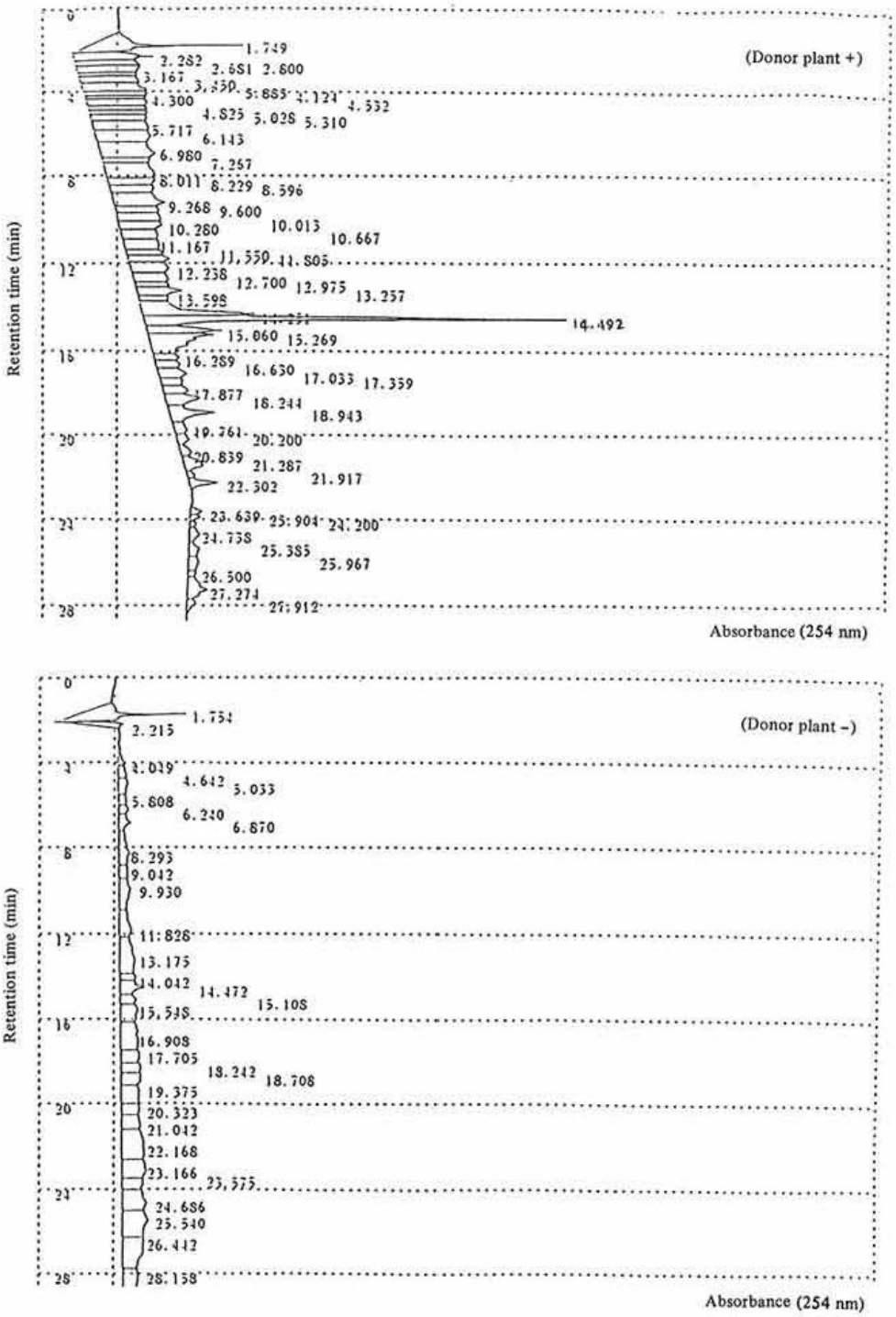

Fig. 7. HPLC analysis of exudate of red pepper root using XAD-4 resin

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