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The Interaction of Volatile Chemicals and Phytohormones on Seed Germination and Seedling Growth of Lettuce

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상치의 發芽와 幼苗生長에 미치는 揮發性 化學物質과 식물호르몬의 相互作用

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ABSTRACT

To evaluate allelopathic effect of volatile chemicals and phytohormones, seed germination and seedling growth test of *Lactuca sativa* have performed in laboratory experiments. Among used chemicals terpienen-4-ol was the most inhibitory to seed germination of lettuce. ABA and GA inhibited seed germination at 5×10^{-6}M concentration but promoted germination at 2.5×10^{-5}M and 5×10^{-5}M. ABA and GA alleviated volatile chemical-induced inhibition of seed germination and seedling elongation of lettuce (Table 5 and Table 6).

Key words: Volatile chemicals, Phytohormones, Allelopathic activity, Chemical-induced inhibition, Antagonist.

INTRODUCTION

Terpenoids are well-known allelochemicals. Allelochemicals decreased the germination rate, enzyme activity, hormone-induced growth, membrane permeability and mineral uptake(Rice 1984). The effect of hormones and allelochemicals on plants have been cited in the literature. For example, the action of abscisic acid (ABA) in inhibiting seed germination is the control of water uptake of the embryo tissue of *Sinapsis alba* (Schopfer *et al.* 1972, Green and Corcoran 1975). But there are few research on interaction of volatile chemicals and phytohormones.

Phenolic compounds in low concentrations when present together with GA and ABA favour GA-induced growth by antagonizing the inhibitory effect of ABA(Ray and Laloraya 1984).

To estimate interactions between naturally occurring volatile chemicals from plants and phytohormones, it is necessary to obtain information about influence on seed germination and seedling growth at different concentrations of phytohormones and volatile chemicals.

Thus the aims of the present study were to evaluate the effects of four volatile chemicals, ABA and GA on seed germination and seedling growth of lettuce.

MATERIALS AND METHODS

Allelopathic activity of volatile chemicals

Allelopathic activity of volatile chemicals identified from *Artemisia princeps* var. *orientalis* were assayed on lettuce (Yun 1994). In the Petri dish (210 ml), fifty seeds of lettuce were placed on the filter paper which was layered on absorbent cotton with sufficient moisture. Chemicals applied in different quantities (1, 3, 5, 7, 10 μl/ 210 ml) were placed at foil container standing in the Petri dish. The Petri dish without chemicals was used as a control. The Petri dish was covered with vinyl wrap. The chemical compounds were terpinen-4-ol, cineole, (−)-thujone, and α-humulene. The other procedure was made according to Kil and Yun (1992) and Yun *et al.* (1993).

Interaction of allelochemicals and phytohormones

1. Germination and seedling elongation of lettuce in hormone solution

Abscisic acid (ABA) and gibberellic acid (GA) were purchased from Sigma Co. (U.S.A.). The hormones were diluted such concentrations as 5×10^{-6}M, 2.5×10^{-5}M and 5×10^{-5}M. Fifty seeds of lettuce were placed on filter papers in Petri dish containing either distilled water (control) or solution of ABA and GA.

2. Germination and seedling elongation of lettuce in volatile chemicals and plant hormones

In the Pertri dish, fifty seeds of lettuce were placed on filter paper which was layered on absorbent cotton with sufficient 2.5×10^{-5}M ABA and GA sloution. Chemicals applied in different quantities (1, 5, 10 μl/ 210 ml) were placed in foil container standing in Petri dish. The same Petri dish was covered with vinyl wrap.

The Petri dishes were kept at 25℃ by daytime and 20℃ at night. The experiment extended over a period of 10 days to allow maximum seed germination. The results were determined by counting the number of germinated seeds and measuring the whole length of seedlings in millimeters. Four replications were used. From these data the relative germination ratio and relative elongation ratio were calculated according to Kil and Yun (1992) by following equation:

$$\text{Relative germination ratio} = \frac{\text{Germination percentage of tested plant}}{\text{Germination percentage of control}} \times 100$$

$$\text{Relative elongation ratio} = \frac{\text{Mean length of tested seedlings}}{\text{Mean length of control seedlings}} \times 100$$

RESULTS

Allelopathic activity of volatile chemicals

Four chemical compounds were used for the bioassay. Seed germination of lettuce responded differently to each of these compounds. α-Humulene was not particularly toxic to lettuce even at higher concentrations, whereas terpinen-4-ol, cineole and (−)-thujone exhibited inhibitory effects heavily. Of the compounds, terpinen-4-ol was the most inhibitory to seed germination of lettuce, that is, germination percentage of lettuce at different concentrations of the chemicals was inversely proportional (Table 1). The germination ratio to the control at different concentrations was shown as followed : 81.2% at 1 μl/ 210 ml, 61.7% at 5 μl/ 210 ml and 7.6% at 10 μl/ 210 ml, respectively (Table 1).

Those compounds affected also the seedling elongation of lettuce. General tendency was similar with germination test. In other words terpinen-4-ol, cineole and (−)-thujone have a toxic effect to seedling growth of lettuce against concentration increment (Table 2).

Interaction of allelochemicals and phytohormones

Table 1. Germination percentage of *Lactuca sativa* tested at different concentrations of chemicals[a]

Chemicals	Control	Concentration (μl/210 ml)				
		1	3	5	7	10
α-Humulene	74.7a	74.7a	76.0a	74.7a	74.0a	68.0a
Terpinen-4-ol	88.7a	72.0a	62.7b	54.7b	23.3c	6.7c
Cineole	82.0a	67.3ab	58.7abc	54.0abc	43.3bc	32.0c
(−)-Thujone	86.7a	76.7ab	53.3abc	43.3bc	43.3bc	34.7c

[a]Means within rows followed by same letters are not significantly different at the 5% level by Duncan's multiple-range test.

Table 2. Seedling length(mm) of *Lactuca sativa* tested at different concentrations of chemicals[a]

Chemicals	Control	Concentration (μl/210 ml)				
		1	3	5	7	10
α-Humulene	71.2a	70.2a	59.9a	66.7a	65.1a	68.3a
Terpinen-4-ol	64.7a	50.5ab	38.1bc	23.7cd	14.8de	0.0e
Cineole	71.6a	45.6ab	35.7b	31.3b	26.1b	14.7d
(−)-Thujone	65.3a	36.5b	27.7b	21.5bc	17.9bc	7.3c

[a]Means within rows followed by same letters are not significantly different at the 5% level by Duncan's multiple-range test.

1. Germination and seedling growth of lettuce in phytohormone solution

Relative germination ratio of lettuce is more than 100% 25×10^{-5}M and 5×10^{-5}M of ABA. That means ABA hastened seed germination of lettuce at 2.5×10^{-5}M and $5\times^{-5}$M (Fig. 1) GA inhibited seed germination at 5×10^{-5}M.

But, seedling growth of lettuce at each concentration (5×10^{-6}, 2.5×10^{-5} and $5\times^{-5}$M) of ABA was inhibited and the inhibition shows a concentration response. GA hastened seedling growth at all tested concentration (Fig. 2).

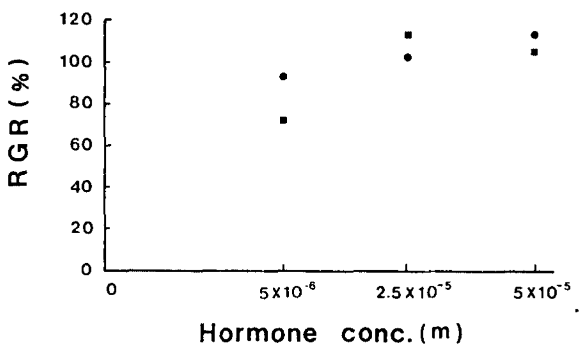

Fig. 1. Relative germination ratio of *Lactuca sativa* in different concentration of phytohormones. ●, GA ; ■, ABA.

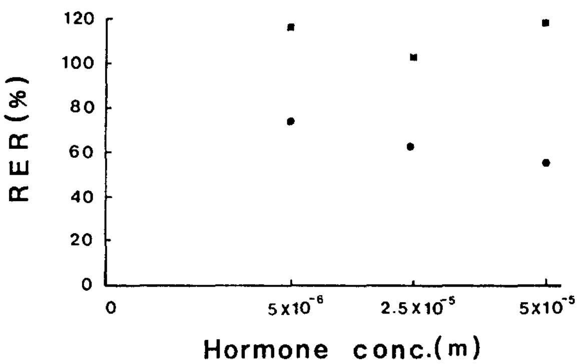

Fig. 2. Relative elongation ratio of *Lactuca sativa* tested in different concentrations of phytohormones. ●, GA ; ■, ABA.

2. Seed germination and seedling growth of lettuce in volatile chemicals and plant hormone

ABA were applied(2.5×10^{-5}M) with volatile chemicals in seed germination (Table 3) and seedling growth (Table 4). ABA alleviated volatile chemical-induced inhibition of seed germination and seedling growth. The concentration of ABA solution was same in each case(2.5×10^{-5}M) and the concentration

Table 3. Germination percentage of *Lactuca sativa* tested in ABA and different concentration of chemicals[a]

Chemicals	Control	2.5×10^{-5}M	Concentration(μl /210 ml)		
			+1	+5	+10
α-Humulene	75.5b	91.0a	77.0ab	65.5b	62.5b
Terpinen-4-ol	75.5a	91.0a	57.5b	46.5b	43.5b
Cineole	75.5a	74.5a	75.5a	81.5a	64.5b
(−)-Thujone	75.5a	74.5a	63.5ab	58.5b	25.5c

[a]Means within rows followed by same letters are not significantly different at the 5% level by Duncan's multiple -range test.

Table 4. Seedling growth (mm) of *Lactuca sativa* tested in ABA and different concentration of chemicals[a]

Chemicals	Control	ABA	Concentration(μl /210 ml)		
		2.5×10^{-5}M	+1	+5	+10
α-Humulene	55.4ab	34.9c	48.9b	51.2b	52.9ab
Terpinen-4-ol	55.4a	34.9b	41.6b	39.5b	35.6b
Cineole	56.7a	35.7c	52.7ab	48.7b	36.1c
(−)-Thujone	56.7a	35.7c	47.2ab	40.7b	23.2c

[a]Means within rows followed by same letters are not significantly different at the 5% level by Duncan's multiple -range test.

of chemicals 1, 5, 10 μl/ 210 ml. When each chemicals treated with ABA, the inhibitory effect was alleviated. The inhibition was in proportion to concentrations of chemicals. ABA shows antagonizing volatile chemicals action.

To study the interation, volatile chemicals were applied (1, 5, 10 μl/ 210 ml) with 2.5×10^{-5}M GA. Of the four chemicals tested all but α-humulene in germination test alleviated chemical-induced inhibition of seed germination (Table 5) and seedling growth (Table 6) of lettuce.

Table 5. Germination percentage of *Lactuca sativa* tested in GA and different concentration of chemicals[a]

Chemicals	Control	GA	Concentration(μl /210 ml)		
		2.5×10^{-5}M	+1	+5	+10
α-Humulene	75.5a	78.0a	70.0ab	65.5b	63.0b
Terpinen-4-ol	75.5a	78.0a	69.5a	47.5b	17.5c
Cineole	75.5a	74.0a	75.0a	63.0b	48.5c
(−)-Thujone	75.5a	74.0a	74.0a	50.5b	45.5b

[a]Means within rows followed by same letters are not significantly different at the 5% level by Duncan's multiple -range test.

Table 6. Seedling growth (mm) of *Lactuca sativa* tested in GA and different concentration of chemicals[a]

Chemicals	Control	GA	Concentration(μl /210 ml)		
			+1	+5	+10
α-Humulene	56.9b	67.4a	60.2ab	56.6b	51.1b
Terpinen-4-ol	56.9b	67.4a	55.9b	36.2c	22.3d
Cineole	56.9b	64.4a	52.3bc	47.4cd	45.4d
(−)-Thujone	56.9b	64.4a	51.6bc	48.7cd	41.7d

[a]Means within rows followed by same letters are not significantly different at the 5% level by Duncan's multiple -range test.

DISCUSSION

Evidence presented in this study shows similarily that allelopathic effect of volatile chemicals identified from *Artemisia princeps* var. *orientalis* on seed germination and seedling growth (Yun 1994) and GA and ABA are antagonistic action against 4 terpenoids.

Among standard chemicals used in bioassay, terpinen-4-ol was shown the most inhibitory, and the inhibitory effect was proportional to concentration. This agrees with the result of Heisey and Delwiche (1984) that terpinen-4-ol is major inhibitor in *Trichostema*. Muller (1965) noted that root growth of *Cucumis* and *Avena* seedlings was inhibited by leaves of *Salvia leucophylla* and cineole and camphor were shown to be the most inhibited compound. Some other terpenes are also phytotoxic (Evenari 1949, Muller and Muller 1964, Asplund 1968, Halligan 1975, Vokou and Margaris 1986, Bradow and Connick 1988).

There are some report of terpenoids (Yun 1994) and hormones (Halloin 1976, Schopfer et al. 1979) affecting seed germination and seedling growth. Comparing with Table 1 and Table 5, 1 μl/ 210 ml terpinen-4-ol solution showed 72.0% (81.2% of control) of germination, but 10 μl/ 210 ml, 6.7% (7.6% of control) in Table 1, 1 μl/ 210 ml terpinen-4-ol added GA 2.5×10^{-5}M solution showed 69.5% germination, 17.5% (23.2% of control) in 10 μl/ 210 ml treatment. In other word these result represented alleviation of phytohormones. Furthermore bioassay with other volatile chemicals showed similar tendency. In seedling growth test, ABA inhibited growth of lettuce, this results are very similar to those of the Ray and Laloraya (1984) study. Combined action of phenolic compound and ABA and GA has been studied(Green and Corcornan 1975, Halloin 1976). Green and Corcoran (1975) reported that tannins are antagonists of a variety of chemically different gibberellins. Antagonism of GA by tannin is demonstrated by the inhibition of induced growth, lack of effect on endogenous growth, and most important by the reversibility of inhibition with additional GA. In the present study, the effectiveness of GA and ABA as antagonist 4 volatile chemicals is evident.

적 요

상치의 종자발아와 유묘생장에 미치는 휘발성 화학물질과 식물호르몬의 영향에 대하여 알레로파시효과를 실험하였다. 실험에 사용한 화학물질 즉, α-humulene, trpinen-4-ol, cineole, (−)-thujone은 종자발아와 유묘생장을 억제했으며, 억제정도는 농도에 비례하였고, 가장 심하게 억제한 화학물질은 terpinen-4-ol이었다. 식물호르몬 ABA와 GA는 5×10^{-10}M에서 종자발아를 억제했으며 2.5×10^{-5}M과 5×10^{-5}M에서는 촉진현상을 나타내었다. 휘발성 화학물질에 의한 종자발아와 유묘신장의 억제는 ABA와 GA 2.5×10^{-5}M에서 경감되었다(Table 5와 Table 6).

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