

Phytotoxic effect of aqueous extracts and essential oils from southern marigold (*Tagetes minuta*)

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Abstract The phytotoxic effects of aqueous extracts and essential oils derived from southern marigold (*Tagetes minuta* L.), were studied using test species bioassays. The phytotoxic potential of the chemical substances was evaluated on callus induction and growth of four receptor plant species: *Oryza sativa* (Dongjinbyeon), *Brassica campestris* subsp. *napus* var. *pekinensis*, *Raphanus sativus* var. *acanthiformis*, and *Sesamum indicum* (Ansanggae). The receptor plants were grown on MS (Murashige & Skoog) media supplemented with 2 mg/litre 2,4-D and 0.5 mg/litre kinetin containing aqueous extract and essential oil extracted from *T. minuta*.

The induction of calluses was significantly inhibited proportional to the concentrations of aqueous extract and essential oil used. The callus induction and growth were rapidly decreased in 30 and 50% of original aqueous extract, and in 10 and 20 µl essential oil. The allelopathic potential of aqueous extract from *T. minuta* may be more than that of essential oil. It was concluded that the inhibitory allelopathic effects of marigold exhibited species-specific inhibitory trends.

Keywords phytotoxic effect; *Tagetes minuta*; aqueous extract; callus induction; essential oil; inhibitory potential; species-specificity

INTRODUCTION

Secretion of phytotoxic natural products by plant roots could have competitive or interfering effects on neighbouring plants. Chemical interference is one of various stresses which plants must cope with in nature, and has been studied for several plant processes including its chemically-mediated interaction among plants. Allelochemic natural products have been implicated in the patterning of vegetation and weed growth in agricultural systems (Liebl & Worsham 1983). It has been suggested that the release of allelochemicals to the environment may decrease crop yields (Chou 1993; Cruz-Ortega et al. 1998). Alternatively, these allelochemicals offer potential as natural herbicides.

Various allelochemicals secreted from some plant species are water soluble and non-phytotoxic substances, but may be gradually degraded into toxic components by soil micro-organisms and interactions with other soil factors (Balke et al. 1987; Jose & Gillespie 1998; Tongma et al. 1998). There are many examples of naturally occurring chemical inhibitors that seriously interfere with one or more of the vital processes of plants. Chemical substances from some plant species may inhibit the seedling growth and seed germination of selected test species (Elakovich & Yang 1996; Haugland & Brandsaeter

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H01050; published 25 September 2002

Received 30 October 2001; accepted 2 July 2002

1996; Yun & Kil 1997; Kil 1999). Aqueous extracts and essential oils of certain plant species have antimicrobial activities (Dellar et al. 1996; Kil et al. 1999a,b).

The development of a plant is sequential, and most growth-promoting compounds are involved in such development including mitotic activity. The plant growth-promoting compounds would produce significant changes in plant growth. It was reported that the damage of growth by some allelopathic chemicals was the result of interruptions in mitotic processes or to nuclear aberrations (Kil et al. 1999a). The effect of allelopathic agents can be explored by a cytological procedure or growth analysis. Since the bioassay by tissue-culture techniques provides a promising method of determining the role of allelochemicals in developmental processes of plants, the use of tissue culture to study allelopathic effects has been previously reported (Hogan & Manners 1990; Kil et al. 1992, 1993, 1999a). The composition of essential oils of plants of the genus *Tagetes* have been analysed (Hethelyi et al. 1986; Chalchat et al. 1995; Singh et al. 1995; Gil et al. 2000), and anti-microbial activities of have been studied (Hethelyi et al. 1986; Weaver et al. 1994; Zygadlo et al. 1994), but phytotoxic effects of *Tagetes minuta* L. essential oil has not been studied in depth as of yet. The purpose of the present study was to analyse allelopathic effects of aqueous extract and essential oil from *T. minuta* on the induction and growth of callus of some plant species.

MATERIALS AND METHODS

Preparation of allelochemic substances

Tagetes minuta was used as a donor species of the aqueous extract and the volatile compounds. The donor species was collected in the area surrounding Buan, Chollabuk-do, Republic of Korea in 1999. Aqueous extract of *T. minuta* was made by the following procedures: within 24 h after being harvested, 1 litre of distilled water was added to 2-litre Erlenmeyer flask containing 200 g fresh *T. minuta* material. The aqueous mixture of dried *T. minuta* was incubated at 25°C for 24 h and filtrated first through a 0.053 mm sieve, and then through Whatman No. 40 filter paper. Filtrates were diluted to 10, 30, 50, and 70% of the original extract by adding distilled water, whereas undiluted filtrate was defined as 100% extract. Osmotic potentials below

143 mOsm/kg had no influence (Elakovich & Wooten 1991); the extracts in this study were diluted to osmotic potentials no greater than 90 mOsm.

Volatile essential oil extracted from *T. minuta* was obtained by the following procedure: crushed fresh leaves of *T. minuta* were placed in a 2-litre round-bottomed flask, which was steam-distilled in a Karlruker's apparatus (Stahl 1973), and was then extracted with ether. The ether extract was concentrated in rotary evaporator, and resultant extract was kept at -70°C in a deep freezer to minimise escape of volatile compounds.

Preparation of tissue explants

Receptor plants were *Oryza sativa* (Dongjinbyeo), *Brassica campestris* subsp. *napus* var. *pekinensis* (Hwangkeumbaechu), *Raphanus sativus* var. *acanthiformis*, and *Sesamum indicum* (Ansanggae). Seeds of the receptor species were sterilised with 75% ethanol (v/v) for 30 s and then in 3% sodium hypochlorite solution for 15 min, before being rinsed 3 times with sterile distilled water. The sterilised seeds, except *O. sativa*, were germinated in petri dishes (87 × 15 mm) containing MS (Murashige & Skoog) medium supplemented with 3% sucrose and 1.0% agar (Murashige & Skoog 1962). The medium was adjusted to pH 5.8 and autoclaved at 1.4 kg cm⁻² pressure and 121°C for 20 min. Petri dishes were incubated at 25°C for 30 days in 16 h day and 8 h night photoperiod with a light intensity of 2000 Lux.

Bioassay of aqueous extract and essential oil

At Day 5 after germination of the three receptor species (*B. campestris* var. *pekinensis*, *R. sativus* var. *acanthiformis*, and *S. indicum*), hypocotyl explants (5 mm length) from the seedlings and the sterilised seeds of *O. sativa* (brown rice) were inoculated to induce callus on MS medium. The basal MS medium was supplemented with 2 mg/litre 2,4-D and 0.5 mg /litre kinetin containing different concentrations (0, 10, 30, 50, and 70%) of the aqueous extracts. Twenty explants per petri dish of four receptor species were inoculated, sealed with parafilm, and incubated at 25°C for 30 days in the dark. The experiment was replicated 4 times.

After the inoculation of explants on the basal medium as described above the explants were exposed to the essential oil according to the following procedure: two sheets of filter paper discs (diam. 8 mm, No. 2, TOYO Ltd) were placed in the centre of petri dish containing the solid basal

medium, and were impregnated with different concentrations (0, 5, 10, and 20 μ l) of the essential oil in the refrigerator (at 4°C). The petri dishes were immediately sealed with parafilm and incubated at 25°C for 30 days in the dark.

Callus induction was estimated according to callus condition (i.e., excellent, good, and bad) (Arya et al. 1993; Kil et al. 1993) 30 days after inoculation. To investigate callus growth rate of the receptor species, the gross fresh weight and dry weight of calluses per replication were measured. Calluses were oven-dried at 70°C for 72 h. The rates of callus growth and induction were estimated using the following equations:

$$(1) \text{ Relative growth rate of callus (RGR, \%)} = \frac{\text{Gross fresh/dry weight of calluses in the treatment}}{\text{Gross fresh/dry weight of calluses in the control}} \times 100$$

$$(2) \text{ Relative induction rate of callus (RIR, \%)} = \frac{\% \text{ of calluses induced in the treatment}}{\% \text{ of calluses induced in the control}} \times 100$$

Statistical data analysis

Data were analysed statistically using SPSS Windows program. Statistical differences among the treatments and controls were tested using Duncan's multiple range test.

RESULTS

Effect of aqueous extract

Effects of *T. minuta* aqueous extract on callus induction of brown rice of *O. sativa* and hypocotyle of *B. campestris* var. *pekinensis* cultured on MS media containing different concentrations of the aqueous extracts are presented in Table 1. Total callus induction was significantly inhibited in proportional to the concentrations of aqueous extract of *T. minuta*, and especially, callus induction of the two receptor species was rapidly decreased in 30% and 50% of the aqueous extract respectively. The number of excellent callus in the treatment except in 10% aqueous extract was significantly different from that of the control. Relative induction rate (RIR) of callus in for each *T. minuta* extract

Table 1 Effects of the aqueous extracts of *Tagetes minuta* added to growth media on callus induction of *Oryza sativa* and *Brassica campestris* var. *pekinensis* 30 days after inoculation. (RIR = relative induction rate of callus to the control.)

Receptor species	Conc. aqueous extracts (%) [*]	Callus induction (%) [†]				
		Excellent	Good	Bad	Total	RIR
<i>O. sativa</i>	Control	41.3 ^a	50.0 ^a	7.5 ^b	98.8 ^a	100.0
	10	45.0 ^a	30.0 ^{bc}	17.5 ^{ab}	92.5 ^a	93.6
	30	18.8 ^{bc}	37.5 ^{ab}	27.5 ^{ab}	83.8 ^a	84.8
	50	5.0 ^c	15.0 ^{cd}	36.3 ^a	56.3 ^b	57.0
	70	0.0 ^c	1.3 ^d	22.5 ^a	22.8 ^c	24.1
<i>B. campestris</i> var. <i>pekinensis</i>	Control	37.5 ^a	36.3 ^a	22.5 ^a	92.5 ^a	100.0
	10	25.0 ^{ab}	36.3 ^a	26.3 ^a	83.8 ^a	90.6
	30	10.0 ^{bc}	22.5 ^{ab}	26.3 ^a	56.3 ^b	60.9
	50	0.0 ^c	8.8 ^{bc}	26.3 ^a	35.1 ^c	37.9
	70	0.0 ^c	0.0 ^c	12.5 ^a	12.5 ^d	13.5

^{*}Basal medium was MS (Murashige & Skoog) medium supplemented with 2 mg/litre 2,4-D and 0.5 mg/litre kinetin. Aqueous extract was made by soaking 200 g fresh shoot per 1 litre distilled water for 24 h at 25°C.

[†]Homogeneous subsets are displayed by Duncan's multiple range test ($P < 0.05$).

concentrations *O. sativa* was higher than that *B. campestris* var. *pekinensis*. *O. sativa* appeared to be a more resistant species to *T. minuta* than *B. campestris* var. *pekinensis*.

The callus growth of *O. sativa* and *B. campestris* var. *pekinensis* was compared in various concentrations of the *T. minuta* aqueous extracts (Table 2). Fresh weight, dry weight, and RGR of the calluses were significantly inhibited proportion to the concentrations of the *T. minuta* aqueous extract used. The inhibitory effect of *T. minuta* appeared to be more severe on *B. campestris* var. *pekinensis* than on *O. sativa*. In the media containing 70% aqueous extracts, callus of *B. campestris* var. *pekinensis* hardly developed and the explant died 2 weeks after.

To estimate the inhibitory effect of aqueous extract of *T. minuta* on the callus induction among the four receptor plants (*O. sativa*, *B. campestris* var. *pekinensis*, *R. sativus* var. *acanthiformis*, and *S. indicum*) explants were grown on MS medium containing 50% aqueous extract of *T. minuta* (Table 3). The percentage of total callus induction of the four receptor plants and the number of excellent callus decreased proportionally to the concentrations of the aqueous extract. Aqueous extract of *T. minuta* exhibited a more allelopathic effect on callus induction of *R. sativus* var. *acanthiformis* than other the receptor plants.

The callus growth of the four receptor species (*O. sativa*, *B. campestris*, *R. sativus*, and *S. indicum*)

Table 2 Effects of the aqueous extracts of *Tagetes minuta* added to growth media on the callus growth of *Oryza sativa* and *Brassica campestris* var. *pekinensis* 30 days after inoculation.

Conc. aqueous extracts (%)*	<i>O. sativa</i> †				<i>B. campestris</i> var. <i>pekinensis</i>			
	FW (mg)	FW-RGR (%)	DW (mg)	DW-RGR (%)	FW (mg)	FW-RGR (%)	DW (mg)	DW-RGR (%)
Control	1723.3 ^a	100.0	240.5 ^a	100.0	1060.0 ^a	100.0	117.0 ^a	100.0
10	1625.8 ^a	94.3	232.0 ^a	96.5	589.5 ^b	55.6	70.0 ^b	59.3
30	1104.8 ^b	64.1	171.8 ^b	71.4	337.3 ^c	31.8	43.5 ^c	36.9
50	974.0 ^b	56.5	154.3 ^b	64.1	204.5 ^{cd}	19.3	27.5 ^{cd}	23.3
70	799.5 ^b	46.4	128.8 ^c	53.5	155.5 ^d	14.7	19.5 ^d	16.5

*Basal medium was MS (Murashige & Skoog) medium supplemented with 2 mg/litre 2,4-D and 0.5 mg/litre kinetin. Aqueous extract was made by soaking 200 g fresh shoot per 1 litre distilled water for 24 h at 25°C.

†Fresh weight (FW) and dry weight (DW) were estimated for 20 explants. Relative growth rate of callus (RGR) was FW/DW of treated callus to the control. Homogeneous subsets are displayed by Duncan's multiple range test ($P < 0.05$).

Table 3 Callus induction of receptor species on the media added aqueous extracts of *Tagetes minuta* 30 days after inoculation. (RIR = relative induction rate of callus to the control. NS = not significantly different at the 0.05 level.)

Receptor species	Aqueous extract*	Callus induction (%)				RIR
		Excellent	Good	Bad	Total	
<i>Oryza sativa</i>	Control	41.3	50.0	7.5	98.8	100.0
	50%	5.0 $P < 0.01$	15.0 $P < 0.01$	36.3 $P < 0.01$	56.3 $P < 0.01$	57.0
<i>Brassica campestris</i> var. <i>pekinensis</i>	Control	37.5	36.3	22.5	96.3	100.0
	50%	0.0 $P < 0.01$	8.8 $P < 0.01$	26.3 NS	35.1 $P < 0.01$	36.4
<i>Raphanus sativus</i> var. <i>acanthiformis</i>	Control	16.3	57.5	22.5	96.3	100.0
	50%	0.0 $P < 0.01$	0.0 $P < 0.01$	18.8 NS	18.8 $P < 0.01$	19.5
<i>Sesamum indicum</i>	Control	98.7	1.3	0.0	100.0	100.0
	50%	0.0 $P < 0.01$	0.0 NS	36.3 $P < 0.01$	36.3 $P < 0.01$	36.3

*Basal medium was MS (Murashige & Skoog) medium supplemented with 2 mg/litre 2,4-D and 0.5 mg/litre kinetin. Aqueous extract was made by soaking 200 g fresh shoot per 1 litre distilled water for 24 h at 25°C.

on the MS medium containing 50% aqueous extract is presented in Table 4. Fresh weight and dry weight of four receptor species significantly decreased compared with the control. The RGR of the callus was inhibited in the following order: *B. campestris*, *S. indicum*, *R. sativus*, and *O. sativa*. The result showed different trends for callus induction in the receptor species. Callus induction of *R. sativus* was the most inhibited of the receptor species, but callus growth was least decreased compared with *B. campestris* and *S. indicum* respectively. The explants of *R. sativus* also showed hypertrophic development with a small portion being the callus.

Effect of essential oil

The effect of essential oil of *T. minuta* on callus induction was investigated using *O. sativa* and *B. campestris* var. *pekinensis* as assay plants (Table 5). For *O. sativa*, the number of excellent callus significantly decreased in 20 μ l essential oil. The percentage of total callus induction slightly decreased in proportional to the concentrations of essential oil used, but it was not significantly different to the control. For *B. campestris* var. *pekinensis*, the number of excellent callus and the percentage of total callus induction were significantly inhibited by 10 and 20 μ l essential oil respectively.

Table 4 Callus growth of receptor species on the medium added 50% aqueous extracts of *Tagetes minuta* 30 days after inoculation.

Receptor species	Conc. of aqueous extract (%) [*]	Callus growth [†]			
		FW (mg)	FW-RGR (%)	DW (mg)	DW-RGR (%)
<i>Oryza sativa</i>	Control	1723.3	100.0	240.5	100.0
	50%	974.0 <i>P</i> <0.01	56.5	154.3 <i>P</i> <0.01	64.1
<i>Brassica campestris</i> var. <i>pekinensis</i>	Control	1060.0	100.0	118.0	100.0
	50%	204.5 <i>P</i> <0.01	19.3	27.5 <i>P</i> <0.01	23.3
<i>Raphanus sativus</i> var. <i>acanthiformis</i>	Control	1214.3	100.0	123.5	100.0
	50%	383.5 <i>P</i> <0.01	31.6	49.8 <i>P</i> <0.01	40.3
<i>Sesamum indicum</i>	Control	1280.3	100.0	88.8	100.0
	50%	254.5 <i>P</i> <0.01	19.9	26.3 <i>P</i> <0.01	29.6

^{*}Basal medium was MS (Murashige & Skoog) medium supplemented with 2 mg/litre 2,4-D and 0.5 mg/litre kinetin. Aqueous extract was made by soaking 200 g fresh shoot per 1 litre distilled water for 24h at 25°C.

[†]Fresh weight (FW) and dry weight (DW) were estimated for 20 explants, and relative callus growth rate (RGR) was fresh/dry weight of treated callus to the control.

Table 5 Effect of the essential oils of *Tagetes minuta* on callus induction of *Oryza sativa* and *Brassica campestris* var. *pekinensis* 30 days after inoculation. (RIR = relative induction rate of callus to control.)

Receptor species	Conc. of essential oil (ml/80 ml) [*]	Callus induction (%) [†]				
		Excellent	Good	Bad	Total	RIR
<i>O. sativa</i>	Control	60.0 ^a	28.8 ^a	8.8 ^b	97.6 ^a	100
	5	51.3 ^{ab}	31.3 ^a	15.0 ^{ab}	97.6 ^a	100
	10	38.8 ^{ab}	33.8 ^a	20.0 ^{ab}	92.6 ^a	94.9
	20	23.8 ^b	37.5 ^a	30.0 ^a	91.3 ^a	93.5
<i>B. campestris</i> var. <i>pekinensis</i>	Control	30.0 ^a	52.5 ^a	13.8 ^b	97.6 ^a	100
	5	18.8 ^{ab}	48.8 ^a	26.3 ^{ab}	92.6 ^{ab}	94.9
	10	15.0 ^b	37.5 ^a	36.3 ^a	91.3 ^b	93.5
	20	10.0 ^b	42.5 ^a	36.3 ^a	88.8 ^b	91.0

^{*}Basal medium was MS (Murashige & Skoog) medium supplemented with 2 mg/litre 2,4-D and 0.5 mg/litre kinetin.

[†]Homogeneous subsets are displayed by Duncan's multiple range test (*P* < 0.05).

Table 6 Effect of essential oil of *Tagetes minuta* on the callus growth of *Oryza sativa* and *Brassica campestris* var. *pekinensis* 30 days after inoculation.

Conc. of essential oils (ml/80 ml)*	<i>O. sativa</i> †				<i>B. campestris</i> var. <i>pekinensis</i>			
	FW (mg)	FW-RGR (%)	DW (mg)	DW-RGR (%)	FW (mg)	FW-RGR (%)	DW (mg)	DW-RGR (%)
Control	1185.3 ^a	100.0	114.3 ^a	100.0	1273.0 ^a	100.0	117.0 ^a	100.0
5	1155.5 ^a	97.5	112.5 ^a	98.4	1234.0 ^a	96.9	114.8 ^a	98.1
10	1037.3 ^{ab}	87.5	103.0 ^{ab}	90.1	1020.5 ^b	80.2	101.0 ^b	85.9
20	952.0 ^b	80.3	96.5 ^b	84.4	940.3 ^b	73.9	94.0 ^b	80.3

*Basal medium was MS (Murashige & Skoog) medium supplemented with 2 mg/litre 2,4-D and 0.5 mg/litre kinetin.
 †Fresh weight (FW) and dry weight (DW) were estimated for 20 explants. Relative growth rate of callus (RGR) was fresh/dry weight of treated callus to the control. Homogeneous subsets are displayed by Duncan's multiple range test ($P < 0.05$).

Table 7 Callus induction of receptor species tested in essential oil of *Tagetes minuta* 30 days after inoculation. (RIR = relative induction rate of callus to the control. NS = not significantly different at the 0.05 level.)

Receptor species	Conc. of essential oil (ml/80 ml)*	Callus induction (%)				
		Excellent	Good	Bad	Total	RIR
<i>Oryza sativa</i>	Control	60.0	28.8	8.8	97.6	100.0
	20	23.8 $P < 0.01$	37.5 NS	30.0 $P < 0.01$	91.3 NS	95.4
<i>Brassica campestris</i> var. <i>pekinensis</i>	Control	30.0	52.5	13.8	96.3	100.0
	20	10.0 $P < 0.01$	42.5 NS	36.3 $P < 0.01$	88.8 $P < 0.01$	91.7
<i>Raphanus sativus</i> var. <i>acanthiformis</i>	Control	16.3	57.5	23.8	97.6	100.0
	20	12.5 NS	62.5 NS	20.0 NS	95.0 NS	97.3
<i>Sesamum indicum</i>	Control	98.7	1.3	0.0	100.0	100.0
	20	0.03 $P < 0.01$	26.3 $P < 0.05$	68.8 $P < 0.01$	95.1 NS	95.1

*Basal medium was MS (Murashige & Skoog) medium supplemented with 2 mg/litre 2,4-D and 0.5 mg/litre kinetin.

Table 8 Callus growth of receptor species tested in essential oil of *Tagetes minuta* in 30 days after inoculation. (NS = not significantly different at the 0.05 level.)

Receptor species	Conc. of essential oil (ml/80 ml)*	Callus growth†			
		FW (mg)	RGR (%)	DW (mg)	RGR (%)
<i>Oryza sativa</i>	Control	1185.0	100.0	114.3	100.0
	20	952.0 $P < 0.01$	80.3	96.5 $P < 0.01$	84.4
<i>Brassica campestris</i> var. <i>pekinensis</i>	Control	1273.0	100.0	117.0	100.0
	20	940.3 $P < 0.01$	73.9	94.0 $P < 0.01$	80.3
<i>Raphanus sativus</i> var. <i>acanthiformis</i>	Control	1214.3	100.0	115.0	100.0
	20	1098.0 NS	90.4	104.8 NS	91.1
<i>Sesamum indicum</i>	Control	838.8	100.0	65.5	100.0
	20	602.8 $P < 0.01$	71.9	48.8 $P < 0.01$	74.4

*Basal medium was MS (Murashige & Skoog) medium supplemented with 2 mg/litre 2,4-D and 0.5 mg/litre kinetin. Aqueous extract was made by soaking 200 g fresh shoot per 1 litre distilled water for 24 h at 25°C.

†Fresh weight (FW) and dry weight (DW) were estimated for 20 explants, and relative callus growth rate (RGR) was fresh/dry weight of treated callus to the control.

Fresh weight and dry weight of calluses of *O. sativa* and *B. campestris* var. *pekinensis* measured 30 days after inoculation are presented in Table 6. The callus growth of the two receptor species proportionally decreased with the concentration of the essential oil. *O. sativa* and *B. campestris* growth significantly decreased in 10 and 20 μ l essential oil, respectively.

The response of callus induction to 20 μ l essential oil in the four receptor plants (*O. sativa*, *B. campestris*, *R. sativus*, and *S. indicum*) is presented in Table 7. The percentage of total callus induction with the exception of *B. campestris* was not significantly different. Except for *R. sativus* the number of excellent callus significantly decreased as compared to the control. The condition of induced callus in *R. sativus* was hardly different when compared with the control.

When the calluses of the four receptor species were tested in 20 μ l essential oil, the growth of callus significantly decreased in all species when compared with the control (Table 8). Callus RGR in 20 μ l essential oil was inhibited in the following order: *S. indicum*, *B. campestris*, *O. sativa*, and *R. sativus*.

DISCUSSION

Allelopathic effects exist among plant species, in tissue culture, seed germination, and seedling growth bioassays. The inhibitory effect of the aqueous extract and essential oil has been reported on callus growth (Hogan & Manners 1990; Wolf & Earle 1990; Kil et al. 1992, 1993, 1994). Aqueous extract derived from leaves of *T. minuta* is known to inhibit the callus induction and growth of some receptor plants, and the degree of inhibition was proportional to the concentrations used (Kil et al. 1994; Yoo 2000). The response of callus growth of the receptor plants was divided into two groups: tolerant and sensitive species. The toxic effect of the aqueous extract and the essential oil is dependent on the receptor species. This result was similar to the inhibitory responses of other plant species previously studied (Kil et al. 1992, 1993; Yoo 2000). Species-specific allelopathic inhibitory effect on callus growth was reported earlier in a co-culture of rice and soybean (Yang & Futsuhara 1991).

However, the result of callus induction showed different trends from that of callus growth. Callus induction of *R. sativus* was the most inhibited among the receptor species, however callus growth

decreased to lesser extent than that in *B. campestris* and *S. indicum* cultures. The explants of *R. sativus* also showed the hypertrophic development with a small portion being the callus.

The phytotoxic effects of the aqueous extract were more pronounced on the receptor species than that of the essential oil. Especially, the inhibitory activity of essential oil derived from *T. minuta* was significantly lower when compared with essential oil derived from other plant species (Kil et al. 1992, 1993; Yoo 2000). This present result suggested that the content and composition of allelochemicals may have different effects depending on plant tissue or organ sources. Allelochemicals are contained in most plant tissues and organs including leaf, stem, root, fruit, flower, seed, and pollen (Kil & Lee 1987; Ortega et al. 1988). It seemed that the different inhibitory activity of the donor species on the receptor ones could be useful in evaluating the species-specific allelopathic inhibitory effect.

Recently, plant tissue culture techniques using suspension cells, protoplasts, or microplants, have been used to estimate the allelopathic effects among plant species (Hogan & Manners 1990, 1991, 1992; Preece et al. 1991; Kil et al. 1992, 1993, 1994). Furthermore, these methods could be profoundly useful for the bioassay of allelopathic effects.

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