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Inhibitory effect of marine green algal extracts on germination of *Lactuca sativa* seeds

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Abstract

The allelopathic potential of nine green seaweed species was examined based on germination and seedling growth of lettuce (*Lactuca sativa* L.). Out of nine methanol extracts, *Capsosiphon fulvescens* and *Monostroma nitidum* extracts completely inhibited germination of *L. sativa* at 4 mg/filter paper after 24 hr of treatment. Water extracts of these seaweeds generally showed low anti-germination activities than methanol extracts. Of the nine water extracts, *Enteromorpha linza* extract completely inhibited *L. sativa* germination at 16 mg/filter paper after 24 hrs. To identify the primary active compounds, *C. fulvescens* powder was successively fractionated according to polarity, and the main active agents against *L. sativa* were determined to be lipids (0.0% germination at 0.5 mg of lipids/paper disc). According to these results, extracts of *C. fulvescens* can be used to develop natural herbicidal agents and manage terrestrial weeds.

Key words

Anti-germination effect, *Capsosiphon fulvescen*, Green seaweed, *Lactuca sativa*

Introduction

Due to increase in the number of herbicide-resistant weeds and environmental concern regarding the use of synthetic herbicides, alternative weed management strategies are required (Bich and Kato-Noguchi, 2012; Sodaeizadeh *et al*., 2009). Allelopathy is defined as any effect (direct or indirect, positive or negative) of one species on growth of another, through release of chemical compounds into the environment (Rice, 1984). In previous reports, some allelochemicals have been shown to act as herbicides (Bhowmik and Inderjit, 2003).

Allelopathy has been documented in a wide range of plant species, including weeds, forest trees (Kohli *et al*., 2001), aquatic plants (Gross, 2003), and seaweeds (Kim *et al*., 2004). The allelopathic potential of fresh water plants on terrestrial plant species (Bich and Kato-Noguchi, 2012) and blue-green algae (Xian *et al*., 2006), and of water lettuce on some microalgae and algae (Aliotta *et al*., 1991), has been reported. However, to the best of our knowledge, the allelopathic potential of seaweeds against terrestrial plant species has not been studied.

Eutrophic coasts are subject to blooms of green macroalgae, commonly referred to as 'green tides', which consist of *Chaetomorpha* sp., *Cladophora* sp., *Enteromorpha* sp., *Percursaria* sp., *Rhizoclonium* sp., and *Ulva* sp. (predominantly *Ulva* and *Enteromorpha*) (Taylor *et al*., 2001; Liu *et al*., 2010). Although green tides are major global environmental problem, these biomasses can be used as resources. *Lactuca sativa* has been reported to be sensitive to allelopathic chemical (Dandelot *et al*., 2008), heavy metal (Lamb *et al*., 2010) and toxic chemical (Valerio *et al*., 2007) and *L. sativa* as a model plant is available in the standard protocol for pollutants.

Thus, allelopathic properties and natural herbicidal potential of green seaweed collected from the Korean coast were examined on lettuce seedlings.

Materials and Methods

Seaweed extracts : Nine species of green seaweed were collected from the coast of South Korea from September, 2008, to August 2009. The seaweed tissues were washed with tap water to remove salt, epiphytes, sand, and then dried for 1 day at room temperature. They were then ground to powder for 5 min using coffee grinder. For methanol extraction, 1 l of methanol was added per 20 g of powder and incubated for 1 day at room temperature. This was repeated three times and the combined extracts were evaporated to dryness. Stock solutions were prepared by adding 1 ml of methanol per 100 mg of the dried extract. Stock solutions were filtered through 0.22-μm filters and stored at -20°C until required.

***Lactuca sativa* seeds :** Lettuce seeds (*L. sativa* L.; Korean cultivar, Hongbitjeokchimasangchu) was purchased from Danong Co. Ltd. (Namyangju, Korea). Seeds of uniform size were selected. Prior to germination tests, seeds were surface-sterilized with 10:1 water/NaOCl (425044; Sigma-Aldrich, St. Louis, MO) solution for 5 min and then washed five times with distilled water.

Germination bioassay : All the germination bioassay was performed from October 2012 to February 2013. Each extract (1 ml) was added to filter paper (55 mm in diameter; Toyo Roshi Kaisha, Ltd., Tokyo, Japan) in sterile Petri dishes (6 cm in diameter; SPL Life Science, Pocheon, Korea), and filter paper was then completely dried on a clean bench. Next, ten sterilized seeds (of similar sizes) of *L. sativa* were evenly placed on filter paper containing seaweed extracts in each Petri dish and moistened with 1.2 ml of sterilized distilled water (for water extraction) or differenct concentration of polyoxyethylene sorbitan monolaurate (Tween 20) (P2287; Sigma-Aldrich) aqueous solution (for methanol extraction), which was used as surfactant and did not cause any toxic effects (Kato-Noguchi *et al*., 2009; Kang *et al*., 2012). Sterilized distilled water was used as control. All Petri dishes were then kept in an incubator in dark at 25 ± 1ºC. Germination rate was determined by counting the number of germinated seeds at 24 hr interval for 2 days. Root length was also recorded. Germination was considered to have occurred only after radicle had protruded by at least 1 mm (Gallardo *et al*., 2002). Percentage germination for each treatment was calculated and compared with that of control, which had been treated with distilled water without seaweed extract. Percentage germination was calculated by the following equation: Germination (%) = n/N × 100 where, n is number of seeds germinated and N is the number of seeds sown.

Effect of Tween 20 on seed germination : Tween 20 was used as a surfactant and did not cause any toxic effect on several plant seed within appropriate concentration (Kato-Noguchi *et al*., 2009; Kang *et al*., 2012). Further experiments were performed to support surface-active effects. Using 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, and 2.0% (v/v) aqueous solutions of Tween 20, germination bioassays were performed as described above.

Constituent separation : For constituent separation, seaweed powders (20 g) were extracted in 1 l of methanol-water (4:1) three times. Crude extracts were evaporated under vacuum and then fractionated according to their polarity to isolate saccharides, lipids, phenolics, alkaloids, and nitrogenous compounds (Harborne, 1998).

Statistical analysis : All the experiments were performed at least three times. The significance of the results was calculated using Student's *t*-test. Results were considered statistically significant at $p < 0.01$ vs. control.

Results and Discussion

From 0.05-1.0% (v/v), aqueous solution of Tween 20 had no effect on *L. sativa* seed germination after 24 hr (100% germination rate and 5.18-mm radicle length) (Fig. 1). At 1.25, 1.5, and 2.0% (v/v) aqueous Tween 20 solution-treated groups, germination rates were 92.5, 85.0, and 82.5%, respectively, after 24 hr. After 48 hrs of incubation, germination rates increased in all the tested groups to 98.5 and 97.0% in 1.5 and 2.0% (v/v) aqueous Tween 20 solution-treated groups, respectively. Additional experiments were performed using 0.5% Tween 20 in water.

The allelopathic potential of nine green seaweed species was examined based on germination and seedling growth of *L. sativa*. Out of the nine methanol extracts, *Capsosiphon fulvescens* and *Monostroma nitidum* extracts

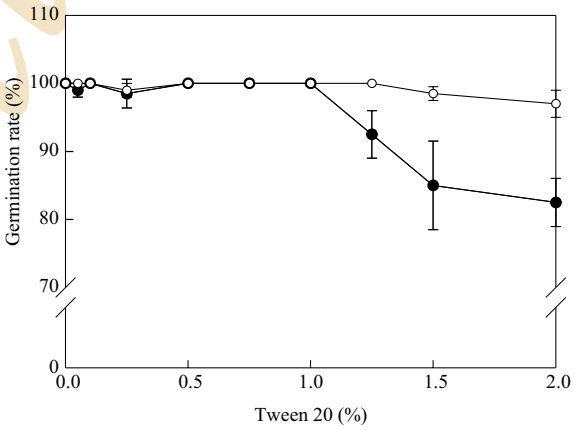

Fig. 1 : Effect of Tween 20 on seed germination after 1 (●) and 2 (○) days, All measurements were performed in triplicate, and values were mean of three replicates. Statistical significance was calculated using Student's *t*-test and deemed statistically significant at $p < 0.01$ as compared to control

completely inhibited *L. sativa* germination at 4 mg per filter paper after 24 hrs. Methanol extracts of *Codium arabicum*, *Enteromorpha compressa*, *Monostroma nitidum*, *Ulva pertusa*, and *Ulva* sp. completely inhibited *L. sativa* germination at 8 mg per filter paper after 24 hrs. At 16 mg per filter paper, all of the tested methanol extracts (excluding *Cladophora sakaii*) completely inhibited (or reduced to 5%) the germination rate of *L. sativa* seeds after 24 hrs (Table 1).

After 48 hr of incubation, germination of *L. sativa* was completely inhibited in *C. fulvescens* extract-treated group at 4 mg per filter paper. Otherwise, *L. sativa* germination was completely inhibited in *M. nitidum* extract-treated group at 8 mg per filter paper and was reduced to 5% germination rate at 4 mg per filter paper.

In all the tested groups, anti-germination effect of water extracts were lower than those of methanol extracts. Of the nine water extracts, *Enteromorpha linza* extract completely inhibited *L. sativa* germination at 16 mg per filter paper after 24 and 48 hr of incubation. In *U. pertusa* and *Ulva* sp. water extract-treated groups, germination rates were 6.67 and 10.0%, respectively, at 16 mg per filter paper after 24 hrs of incubation (Table 2).

Table 1 : Inhibitory effect of methanol extracts of marine green alga on *Lactuca sativa* seed germination

Scientific name	Collection site	Incubation time (hr)	Methanol extract (mg)			
			2	4	8	16
Capsosiphon fulvescens	Gokumdo, Wando	24	5.00±5.00 % (2.50±0.28 mm)	0.00±0.00 % (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)
		48	5.00±5.00 % (11.95±1.34 mm)	0.00±0.00 % (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)
Cladophora sakaii	Cheongsapo, Busan	24	86.67±5.77 % (2.68±0.16 mm)	96.67±5.77 % (2.44±0.42 mm)	70.00±30.00 % (2.29±0.06 mm)	53.33±15.28 % (1.99±0.52 mm)
		48	90.00±10.00 % (10.87±0.34 mm)	96.67±5.77 % (10.47±1.07 mm)	80.00±10.00% (10.20±1.98 mm)	70.00±10.00 % (9.94±0.91 mm)
Codium arabicum	Cheongsapo, Busan	24	60.00±10.00 % (1.68±0.17 mm)	6.67±5.78% (1.35±0.21 mm)	0.00±0.00% (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)
		48	63.33±15.28 % (7.13±0.52 mm)	26.67±15.28% (3.65±0.92 mm)	20.00±10.00% (5.64±1.93 mm)	0.00±0.00 % (0.00±0.00 mm)
Codium fragile	Cheongsapo, Busan	24	73.33±5.78 % (2.06±0.42 mm)	36.67±15.28 % (1.48±0.11 mm)	6.67±5.77 % (1.00±1.41 mm)	0.00±0.00 % (0.90± mm)
		48	96.67±5.77 % (7.62±1.16 mm)	46.67±25.17 % (6.97±1.81 mm)	16.67±11.55 % (3.75±2.59 mm)	0.00±0.00 % (0.00±0.00 mm)
Enteromorpha compressa	Cheongsapo, Busan	24	73.33±11.54 % (2.43±0.18 mm)	43.33±15.28 % (0.82±0.45 mm)	0.00±0.00 % (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)
		48	90.00±10.00 % (8.07±2.64 mm)	80.00±10.00 % (4.79±3.84 mm)	3.33±5.78 % (1.00±1.41 mm)	0.00±0.00 % (0.00±0.00 mm)
Enteromorpha linza	Cheongsapo, Busan	24	73.33±5.78 % (2.21±0.30 mm)	86.67±5.78 % (2.02±0.03 mm)	40.00±10.00 % (1.39±0.44 mm)	3.33±5.78% (0.65±0.92 mm)
		48	86.67±5.78 % (6.44±0.01 mm)	96.67±5.78 % (6.19±0.05 mm)	90.00±0.00% (3.27±0.01 mm)	6.67±±5.77 % (1.03±1.45 mm)
Monostroma nitidum	Galmoonri, Wando	24	16.67±15.27 % (0.84±1.18 mm)	0.00±0.00% (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)
		48	30.00± 10.00 % (5.13±1.24 mm)	6.67±5.77 % (0.75±1.06 mm)	0.00±0.00 % (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)
Ulva pertusa	Cheongsapo, Busan	24	40.00±10.00 % (1.80±0.42 mm)	40.00±10.00 % (2.00±0.05 mm)	0.00±0.00 % (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)
		48	70.00±10.00 % (5.24±1.08 mm)	56.67±15.28 % (5.57±0.71 mm)	10.00±0.00 % (1.50±2.12 mm)	0.00±0.00 % (0.00±0.00 mm)
Ulva sp.	Sinyang, Jeju	24	23.33±5.77 % (1.62±0.54 mm)	13.33±5.77 % (1.88±0.53 mm)	0.00±0.00 % (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)
		48	73.33±15.28 % (5.72±0.16 mm)	30.00±20.00 % (6.80±2.40 mm)	6.67±5.78 % (2.00±2.83 mm)	0.00±0.00 % (0.00±0.00 mm)

Values are mean of three replicates ±SD. Statistical significance was calculated using Student's *t*-test and deemed statistically significant at $p < 0.01$ as compared to control

To characterize active compound(s) in the extracts, methanol-water (4:1) extracts of powdered *C. fulvescens* were fractionated according to their polarity. This led to isolation of saccharides, lipids, phenolics, alkaloids and nitrogenous compounds (Table 3). Seaweed powder (20 g) was extracted in 1 l of methanol-water (4:1) and mixed three times, after which the crude extract was evaporated to yield a dark greenish-brown gummy residue. The fraction that was acidified to pH 2 and extracted with chloroform yielded a moderately polar mixture of phenolic compounds (0.63 g), which showed only minor anti-germination activity. Seaweed powder residue that remained after extraction with methanol-water (4:1) mix was extracted three times in 1 l of ethyl acetate, and the crude extract was evaporated to yield dark green crude lipids (0.44 g), which contained majority of anti-germination activity. Anti-germination rate of lipid fractions of *C. fulvescens* extracts against *L. sativa* seeds was 100.0% at 0.5 mg per paper disc after 48 hrs of incubation. Anti-germination rates of phenolic fractions of *C. fulvescens* extracts against *L. sativa* seeds at 0.5, 1, and 2 mg per filter paper were 30.0, 13.3, and 0.0%, respectively, after 24 hrs of incubation (Table 3).

Some of the seaweeds that belong to Chlorophyceae are fast-growing and opportunistic for space and nutrients in eutrophic coastal waters, and can proliferate in the form of

Table 2 : Inhibitory effect of water extracts of a marine green alga on *Lactuca sativa* seed germination

Scientific name	Collection site	Incubation time (h)	Water extract (mg)			
			2	4	8	16
Capsosiphon fulvescens	Gokumdo, Wando	24	96.67±5.78 % (3.03±0.56 mm)	96.67±5.78 % (3.80±0.26 mm)	90.00±10.00 % (3.16±0.71 mm)	83.33±15.28 % (2.60±0.62 mm)
		48	96.67±5.78 % (14.03±2.61 mm)	100.00±0.00 % (16.73±0.49 mm)	100.00±0.00 % (14.03±1.71 mm)	90.00±10.00 % (14.00±1.87 mm)
Cladophora sakaii	Cheongsapo, Busan	24	100.00±0.00 % (3.30±0.10 mm)	93.33±5.77 % (2.87±1.07 mm)	40.00±20.00 % (1.57±1.52 mm)	3.33±5.77 % (0.67±1.15 mm)
		48	100.00±0.00 % (22.13±1.15 mm)	100.00±0.00 % (18.97±1.06 mm)	93.33±5.77 % (10.67±2.16 mm)	16.67±20.82 % (4.33±4.04 mm)
Codium arabicum	Cheongsapo, Busan	24	93.33±11.55 % (2.67±0.15 mm)	100.00±0.00 % (2.63±0.76 mm)	80.00±34.64 % (1.33±0.57 mm)	10.00±10.00 % (0.47±0.50 mm)
		48	93.33±11.55 % (14.60±0.46 mm)	100.00±0.00 % (10.63±1.16 mm)	83.33±28.87 % (8.43±0.47 mm)	53.33±32.15 % (3.87±0.86 mm)
Codium fragile	Cheongsapo, Busan	24	93.33±5.77 % (3.03±0.21 mm)	100.00±0.00 % (2.77±0.40 mm)	96.67±5.78 % (2.53±0.64 mm)	26.67±30.55 % (0.63±0.78 mm)
		48	93.33±5.77 % (16.30±0.70 mm)	100.00±0.00 % (14.67±0.40 mm)	100.00±0.00 % (13.10±3.46 mm)	73.33±23.09 % (7.37±3.30 mm)
Enteromorpha compressa	Cheongsapo, Busan	24	96.67±5.78 % (3.70±0.26 mm)	100.00±0.00 % (3.63±0.25 mm)	93.33±5.77 % (2.87±0.25 mm)	86.67±5.77 % (2.97±0.35 mm)
		48	93.33±5.77 % (15.87±2.48 mm)	100.00±0.00 % (12.90±0.46 mm)	93.33±5.77 % (12.47±1.22 mm)	90.00±10.00 % (11.57±1.42 mm)
Enteromorpha linza	Cheongsapo, Busan	24	83.33±15.28 % (2.53±0.15 mm)	53.33±15.28 % (2.22±0.77 mm)	13.33±23.09 % (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)
		48	100.00±0.00 % (9.08±2.05 mm)	76.67±23.09 % (7.29±1.67 mm)	30.00±20.22 % (5.16±3.86 mm)	0.00±0.00 % (0.00±0.00 mm)
Monostroma nitidum	Galmoonri, Wando	24	100.00±0.00 % (3.13±0.51 mm)	90.00±10.00 % (3.30±0.26 mm)	80.00±17.32 % (2.43±0.25 mm)	30.00±10.00 % (0.80±0.61 mm)
		48	100.00±0.00 % (10.70±10.4 mm)	100.00±0.00 % (10.53±1.46 mm)	80.00±17.32 % (8.80±1.04 mm)	70.00±26.46 % (6.53±1.47 mm)
Ulva pertusa	Cheongsapo, Busan	24	76.67±32.15 % (3.21±0.98 mm)	86.67±15.28 % (2.87±0.51 mm)	46.67±20.81 % (1.92±0.29 mm)	6.67±11.54 % (0.00±0.00 mm)
		48	96.67±5.78 % (9.12±3.20 mm)	100.00±0.00 % (9.00±2.60 mm)	93.33±11.55 % (5.93±3.19 mm)	46.64±40.41 % (5.50±3.27 mm)
Ulva sp.	Sinyang, Jeju	24	83.33±20.82 % (2.76±0.55 mm)	80.00±10.00 % (2.46±0.57 mm)	60.00±36.06 % (1.97±0.49 mm)	10.00±10.00 % (0.70±0.42 mm)
		48	100.00±0.00 % (9.33±2.47 mm)	80.00±10.00 % (8.58±2.38 mm)	80.00±20.00 % (7.46±4.35 mm)	40.00±36.06 % (6.22±4.78 mm)

Values are mean of three replicates ±SD. Statistical significance was calculated using Student's *t*-test and deemed statistically significant at $p < 0.01$ as compared control

green tides (Hong *et al*., 2011; Lee *et al*., 2011). This phenomenon often results in mass production and harmful accumulation of seaweed (Taylor *et al*., 2001; Liu *et al*., 2010).

Till date, these biomasses have been applied as soil fertilizers (Oh *et al*., 1990), for foodstuff production (Hong *et al*., 2011), and for bioethanol production (Lee *et al*., 2011). In the present study, germination of *L. sativa* was inhibited completely by *C. fulvescens* methanol extract at 4 mg filter paper after 48 hrs of incubation, showing strongest anti-germination effects.

Capsosiphon fulvescens, which contains 7.6% moisture, 10.3% protein, 0.47% fat, and 51.1% carbohydrate in its powder, is traditionally used in Korea as foodstuff because of its unique flavor and soft texture (Sohn, 2003; Hwang *et al*., 2008a). In addition, it has various beneficial health properties, including protective effects against alcohol-induced gastric injury (Hwang *et al*., 2008b), immunomodulatory activities (Karnjanapratum *et al*., 2012), immunostimulating activity (Na *et al*., 2012; Park *et al*., 2006), cholesterol lowering effects (Lee *et al*., 2006), antidiabetic and antioxidant activities (Lee *et al*., 2013), and anticancer effects (Kwon *et al*., 2007; Kim *et al*., 2012; Park *et al*., 2006). Although many human health-related studies on *C. fulvescens* have been published, to the best of our knowledge this is the first report on the allelopathic potential and natural herbicidal properties of *C. fulvescens*.

Studies of putative allelochemicals are complex because *C. fulvescens* synthesizes polysaccharides, glycoproteins, essential oils, fatty acids, amino acids (Yang *et al*., 2005; Hwang *et al*., 2008a; Sun *et al*., 2012), phenolics, nitrogenous compounds, and alkaloids that can act synergistically. In the present study, to chemically characterize active compound(s), methanol-water (4:1) extracts of *C. fulvescens* were fractionated according to their polarity. Lipid compounds of *C. fulvescens* methanol extracts showed potent anti-germination effects, showing 100% anti-germination rate at 0.5 mg per paper disc after 48 hrs of incubation. Phenolic compounds in *C. fulvescens* extracts also showed considerable anti-germination effect. Therefore, lipid and phenolic compounds may act synergistically.

It has been reported that oil and lipid compounds act as allelopathic agent(s) (Xian *et al*., 2006; Vilhena *et al*.,

Table 3 : Inhibitory effect of five different fractions of powdered *Capsosiphon fulvescens* (20 g) on *Lactuca sativa* germination

		Capsosiphon fulvescens				
Fractionate (mg)	**Incubation time (hr)**	**Saccharides**	**Lipids**	**Phenolics**	**Alkaloids**	**Nitrogen compound**
0.5	24	-	0.00±0.00 % (0.00±0.00 mm)	30.00±17.32 % (3.13±0.78 mm)	-	-
	48	-	0.00±0.00 % (0.00±0.00 mm)	73.33±15.28 % (6.47±0.40 mm)	-	-
1	24	-	0.00±0.00 % (0.00±0.00 mm)	13.33±5.77 % (2.77±1.12 mm)	-	-
	48	-	0.00±0.00 % (0.00±0.00 mm)	46.67±11.55 % (6.30±1.70 mm)	-	-
2	24	100.00±0.00 % (3.38±0.35 mm)	0.00±0.00 % (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)	10.00±0.33 % (2.50±0.10 mm)	70.00±10.00 % (2.71±0.56 mm)
	48	100.00±0.00 % (16.42±0.51 mm)	0.00±0.00 % (0.00±0.00 mm)	16.67±15.28 % (3.80±3.41 mm)	70.00±10.00 % (7.71±1.25 mm)	100.00±0.00 % (8.90±1.50 mm)
4	24	100.00±0.00 % (3.52±0.32 mm)	-	-	0.00±0.00 % (0.00±0.00 mm)	10.00±0.67 % (3.00±1..00 mm)
	48	100.00±0.00 % (16.0±0.10 mm)	-	-	0.00±0.00 % (0.00±0.00 mm)	90.00±10.00 % (0.00±0.00 mm)
8	24	90.00±0.00 % (2.85±0.39 mm)	-	-	0.00±0.00 % (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)
	48	100.00±0.00 % (12.90±1.71 mm)	-	-	0.00±0.00 % (0.00±0.00 mm)	0.00±0.00 % (0.00±0.00 mm)
16	24	66.67±15.28 % (2.73±0.52 mm)	-	-	-	0.00±0.00 % (0.00±0.00 mm)
	48	90.00±10.00 % (12.34±1.65 mm)	-	-	-	0.00±0.00 % (0.00±0.00 mm)

All measurements were performed in triplicate, and values are the mean of three replicates. Statistical significance was calculated using Student's *t*-test and deemed statistically significant at $p < 0.01$ as compared control

2009; Li *et al*., 2011). For example, essential oils from *Cyperus giganteus* showed inhibitory activity toward weed species from the Amazon (Vilhena *et al*., 2009), volatile oils from *Descurainia sophia* (L.) Webb ex Prantl exhibited allelopathic effects on wheat (Li *et al*., 2011), and essential oils from aquatic macrophytes had allelopathic effects on blue-green alga *Microcystin aeruginosa* (Xian *et al*., 2006).

Volatilization is a primary route for excretion of allelochemicals from terraneous plants and freshwater aquatic plants (Xian *et al*., 2006). However, further study is required to characterize volatile essential oils of *C. fulvescens*. These preliminary results indicate that *C. fulvescens* may be a source of anti-germination compounds; thus, future studies should isolate compound(s) responsible for these inhibitory effects.

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