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Autotoxicity in Some Ornamentals with the Means to Overcome It

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Abstract. **Autotoxicity in some ornamentals was investigated. The plants were grown by hydroponics with or without the addition of activated charcoal (AC) to the nutrient solution. The AC was used to trap the exuded organics from roots. Among the 37 plants under study, growth of lily, prairie gentian, corn poppy, farewell-to-spring, rocket larkspur, and carnation was drastically reduced in the absence of AC compared with those in the presence of AC in the nutrient solution. Root exudates of some plants were analyzed and several organic compounds were detected. The strong growth inhibitors such as lactic acid in pot marigold, benzoic and *p*-hydroxybenzoic acid in lily, *o*-hydroxyphenylacetic acid in rocket larkspur, benzoic and *p*-hydroxybenzoic acid in sweet pea, and maleic and benzoic acid in prairie gentian were detected in the root exudates. The reduced growth of prairie gentian after prolonged cultivation in a field might be avoided by amending the soil with AC at a rate of 60 kg·10a^{-1}.**

Plants synthesize, store, and exude various kinds of organic compounds in their surroundings as exudates, volatiles, or residues of decomposition (Hale and Orcutt, 1987). Some of the released compounds (allelochemicals) inhibit the growth of the source plants (autotoxicity) or the other species grown in the vicinity of source plants (heterotoxicity). This autotoxicity or heterotoxicity can be treated as allelopathy and the autotoxicity was found to be increased if the plants were cultivated consecutively for years on the same land (Rice, 1984) or grown by hydroponic culture without renewal of nutrient solution (Asao et al., 1998a, 2001). One of the principal causes of this growth inhibition in the successive culture of plants has been attributed to the effect of exuded chemicals from plants (Pramanik et al., 2000). Growth of some vegetables such as asparagus, taro, cucumber, and tomato was inhibited by allelochemicals found in their root exudates (Asao et al., 1998a, 2003, 2004; Shafer and Garrison, 1986; Yang, 1982; Yu and Matsui, 1993a). Inhibition in growth of apple, peach, rice, strawberry, and sugarcane has been documented for the autotoxicity (Kitazawa et al., 2005; Mizutani et al., 1988; Rice, 1984). This autotoxicity in tomato (Yu and Matsui, 1993a) and cucumber (Asao et al., 1998a; Pramanik et al., 2000) has been recovered by addition of activated charcoal (AC) to the nutrient solution, because the added AC adsorbed the phytotoxic root exudates and thus favored plant growth. However, research on autotoxicity in ornamentals is limited. Tukey (1969) showed that when chrysanthemum was grown repeatedly in the same place for several years, growth was reduced owing to accumulation of toxic substances in the soil. Kaul (2000) reported on autotoxicity in African marigold, but did not identify the allelochemicals involved. So, in this study, we attempted to investigate autotoxicity, if any, in selected ornamentals along with a possible remedial measure to overcome the growth inhibition from autotoxicity.

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Materials and Methods

Planting materials. Thirty-seven different ornamentals belonging to 16 different families were chosen for this experiment (Table 1).

Plant cultivation with or without activated charcoal. Plant cultivation was carried out according to Pramanik et al. (2000). Seedlings, scions, germinated bulbs, and corms of the plants under study were transplanted to plastic containers (34 cm × 54 cm × 20 cm) in the greenhouse of Shimane University. The container was filled with 30 L of continuously aerated (3.8 L·min^{-1}) 50% Enshi nutrient solution with electrical conductivity (EC) of 1.3 dS·m^{-1} (Table 2; Hori, 1966). Two small air filters, each packed with 100 g of AC (Type GH2C, 4–8 mesh; Takeda Chemical Industry Co., Osaka, Japan), were immersed into the nutrient solution of the container and were attached to the top of tubes with an air pump. The same aeration system was maintained for the nutrient solution without AC. The AC was used to trap the chemicals exuded from the plants and was replaced by fresh AC at 2-week intervals until the end of the experiment for efficient adsorption of the chemicals. The used AC was either immediately extracted with alkaline methanol or stored at 4 °C for later extraction. $FeSO_4 \cdot 7H_2O$ (0.75 g) was added to each solution container at 2-d intervals because the AC-absorbed Fe-EDTA and Fe^{2+} was rapidly oxidized to Fe^{3+} and less available for plants. During cultivation, the water level of the solution containers was kept constant by regularly adding tap water. Nutrient concentrations (NO_3^-, PO_4^{2-}, K^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}) in the solution were adjusted as close as possible to the initial concentration at 2-week intervals on the basis of chemical analyses with an atomic absorption spectrometer (Shimadzu AA-630, Kyoto, Japan) and ion meter (Horiba D-23, Kyoto, Japan). Twelve plants were planted in each container and three containers were used for each treatment (plants with or without AC). The pH of the nutrient solutions ranged from 5.5 to 6.9 irrespective of whether AC was added to the containers. At the end of the experiment, plant length, number of leaves per plant, maximum root length, flesh and dry weight of shoot and dry weight of root, and number of flowers per plant were recorded.

Gas chromatography-mass spectroscopy analysis of root exudates adsorbed in activated charcoal. The ACs used to trap the exuded organics were desorbed three times using a mixture of methanol (100 mL) and 0.4 M aqueous NaOH (100 mL). Each batch of AC (200 g) was gently shaken with the mixture for 12 h at room temperature with an electric shaker. The extracts were combined and filtered. The filtrates were neutralized and concentrated to 25 mL by a rotary vacuum evaporator at 40 °C. Organic compounds in the concentrate were extracted according to Yu and Matsui (1993b). The concentrated solution was adjusted to pH 2.0 with 4 M HCl, extracted three times with 35 mL of refined diethyl ether (DE), and another three times with 35 mL of ethyl acetate (EA). DE2 and EA2 were the ether and ethyl acetate-soluble fractions at pH 2.0, respectively. The DE2 and EA2 fractions were dried over anhydrous $CaSO_4$ and concentrated to 5 mL each in a rotary evaporator at 40 °C.

All the fractions (DE2 and EA2) extracted from AC were analyzed with a gas chromatograph coupled to a mass spectrometer (GC-MS; Hitachi M-80B, Tokyo) before or after methylation. Fraction DE2 gave a number of peaks in the gas chromatogram, whereas the EA2 fraction gave only a few detectable peaks. An aliquot of the concentrated DE2 fraction (1 or 2 mL) was diluted in a 50-mL ether solution, treated with diazomethane, and concentrated in a rotary evaporator before being bubbled with a N_2 stream in a water bath at 35 °C. One microliter of the concentrated sample was injected into a GC-MS unit coupled with a capillary column (TC-5, 60 m; GL Science, Tokyo). Helium was used as the carrier gas at a pressure of 0.8 kg·cm^{-2}. The initial column temperature was held at 100 °C for 2 min and then raised at 5 °C·min^{-1} to a final temperature of 260 °C with isotherm for 10 min. The injector temperature was held at 270 °C. The ionization voltage and temperature in the electron impact mode were 70 eV and 250 °C, respectively.

Table 1. Planting materials.

Family	Ornamental	Scientific name	Cultivar
Compositae	Pot marigold	*Callendula officinalis* L.	Gold-star
	Cornflower	*Centaurea cyanus* L.	Echo-sultan
	Chrysanthemum	*Chrysanthemum morifolium* Ramat.	Shuhou-no-chikara
	Cosmos	*Cosmos bipinnatus* Cav.	Dearboro
	Zinnia	*Zinnia elegans* Jacq.	Sunbow-orange
	Thistle	*Cirsium japanicum* DC.	Rakuonzi-Azami
	Sunflower	*Helianthus annuus* L.	Big-smile
	Safflower	*Carthamus tinctorius* L.	—[z]
	African marigold	*Tagetes erecta* L.	Orange-isis
	China aster	*Callistephus chinensis* Nees	Kurenai
	Coneflower	*Rudbeckia hirta* L.	Gloriosa-daisy
Liliaceae	Tulip	*Tulipa gesneriana* L.	Blue-champion
	Thunberg lily	*Lilium* × *elegans* Thunb.	Iberu-flora
	Toritelia	*Tritelelia laxa* Benth	Bridgesii
	Lily	*Lilium* × *formolongi* Hort.	Hananomai
Labiatae	Rocket larkspur	*Delphinium ajacis* L.	Lilac
	Love-in-a-mist	*Nigella damascena* L.	Transformer
	Scarlet sage	*Salvia splendens* Ker.	Lavender
	Fan columbine	*Aquilegia flabellate* Sieb. et Zucc.	Macana-giant
Caryophyllaceae	Corn cockl	*Agrostemma githago* L.	Purple queen
	Gypsophilla	*Gypsophila elegans* M.B	Covent-garden
	Carnation	*Dianthus caryophyllus* L.	Feminist
Leguminosae	Sweet pea	*Lathyrus odoratus* L.	Rolay-lavender
	Lupine	*Lupine luteus* L.	Lassell
Cruciferae	Rape blossoms	*Brassica rapa* L.	Wase-fushimi-kanzaki
	Stock	*Matthiola incana* R. Br.	Love-me rose
Onagraceae	Farewell-to-spring	*Godetia amoena* G. Don	Kyokuhai
Umbelliferae	Bishop's weed	*Ammi majus* L.	—[z]
Scrophulariaceae	Snapdragon	*Antirrhinum majus* L.	F1-butterfly-bronze
Papaveraceae	Corn poppy	*Papaver rhoeas* L.	Red-sales
Amaryllidaceae	Narcissus	*Narcissus tazetta* L.	Fernandesii
Amaranthaceae	Feather cockscomb	*Celosia argentea* L.	Red-cupid
	Globe amaranth	*Gomphrena globosa* L.	Strawberryfields
Gentianaceae	Prairie gentian	*Eustoma grandiflorum* (Raf.) Shinn.	Blue line 1
Campanulaceae	Balloon flower	*Platycodon grandiflorum* A. DC.	Samidare-murasaki
Plumbaginaceae	Statice	*Limonium sinuatum* Mill.	Marine-blue
Solanaceae	Chinese-lantern plant	*Physalis alkekengi* L. var. franchetii	Tanba houzuki.

[z]Unknown.

Table 2. Enshi nutrient solution.[z]

Chemicals	Amounts[y] (g/1000 L)
$Ca(NO_3)_2 \cdot 4H_2O$	950
KNO_3	810
$MgSO_4 \cdot 7H_2O$	500
$NH_4H_2PO_4$	155
H_3BO_3	3
$ZnSO_4 \cdot 7H_2O$	0.22
$MnSO_4 \cdot 4H_2O$	2
$CuSO_4 \cdot 5H_2O$	0.05
$Na_2MoO_4 \cdot 2H_2O$	0.02

[z]Full strength.
[y]Amounts of salts per 1000 L of tap water (Hori, 1966).

Bioassay with identified chemicals. The bioassay was carried out according to Asao et al. (1998b). Aqueous solutions of the identified compounds at concentrations of 0 (control), 50, 100, 200, and 400 μM were prepared with a 50% Enshi nutrient solution (EC 1.3 $dS \cdot m^{-1}$). The test solutions were added to flasks (capacity ≈420 mL) wrapped with black polyethylene to avoid direct light to the roots. Some selected test plants were transplanted to each flask with urethane foam as support. The planted flasks were placed in a growth chamber at 25 °C with a light intensity of 74 to 81 $\mu mol \cdot s^{-1} \mu m^{-2}$ and a 16-h photoperiod. To minimize the effect of aeration and the microbial degradation of organic compounds (Sundin and Waechter-Kristensen, 1994) on the bioassay, we renewed the test solutions in the planted flask every 3- or 4-d interval. The plants were grown for 2 weeks and then the fresh and dry weights of shoots were measured. Each treatment was replicated 10 times.

Bioassay in soils amended with activated charcoal. Some soil was collected from a field successively cultivated with prairie gentian for over 10 years in Nagano prefecture, Japan, and was used as medium of growth for the bioassay. Three kilograms of the soil was pulverized and placed in each plastic container (17 cm × 29 cm × 9.5 cm) after amending with AC corresponding to the rate of 0 (control), 30, 60, 120, 240, and 480 $kg \cdot 10a^{-1}$. Soil collected outside the prairie gentian field was also used as a reference to compare the growth performance of the test plants growth with or without AC (control). The physical and chemical properties of the reference soil were essentially similar to the soil in the prairie gentian field (data not shown). Ten prairie gentian seedlings were planted into the treated containers and were placed in the Shimane University greenhouse. Irrigation (500 mL water) was applied to each container at 2-week intervals and 500 mL Enshi nutrient solution (50%) with EC of 1.3 $dS \cdot m^{-1}$ was applied to each container at 2-week intervals. The cultivation was continued for 8 weeks.

At the end of the experiment plant length, number of leaves per plant, maximum root length, shoot dry weight and root dry weight, and number of flowers per plant were recorded.

Results and Discussion

Thirty-seven ornamentals were grown through hydroponic culture with or without addition of AC in the nutrient solution. Plant growth was significantly affected by the added AC. Performances of the plants were evaluated as percent comparing the growth of the plants grown without AC (control) with those grown with AC. Different plants responded differently to the addition of AC (Table 3). Growth in lily was the most severely retarded. Plant length, number of leaves and flowers per plant, root length, and plant dry weight almost all declined significantly in most of the plants grown without AC compared with those grown with AC. However, root growth was found to be more responsive to AC than the other studied parameters possibly for being the roots in direct contract with the exuded chemicals (Pramanik et al., 2000). Root dry weight of lily and rocket larkspur was reduced to ≈85% and 74%, respectively, followed by prairie gentian with growth reduced to 55%. Root length of lily was reduced to ≈58%, whereas that in prairie-gentian was reduced to ≈49%. It appears that lily, prairie gentian, corn poppy, pot marigold, toritelia, and farewell-to-spring were the most sensitive to autotoxicity. Autotoxicity in plants from their own exuded chemicals is also observed in natural ecosystems (Rice, 1984) and was well documented in many crops (Asao et al., 1998a; Kitazawa et al., 2005; Mizutani et al., 1988; Pramanik et al., 2000; Yu and Matsui, 1993a). Asao et al. (2001) detected autotoxicity in some species of Umbellifeae, Compositiae, and Cruciferae. So, autotoxicity in the ornamentals might be incited by the exuded chemicals from their roots. Stimulated growth was observed in the plants such as African marigold, love-in-a-mist, and rape blossoms grown in nonrenewal nutrient solution, however. The exact reasons for this growth stimulation in the latter plants were not discovered. However, it is well known that a chemical at low concentration acts as a growth stimulant to a plant and the same chemical at high concentration becomes toxic or growth-retardant to the same plant (Rizvi and Rizvi, 1992). Functional activity of an allelochemical depends on its concentration and time exposure to the test plants. So, it is possible that the quality and quantity of root exudates in the nutrient solution in absence of AC might not be sufficient to inhibit growth in the latter ornamental plants, but rather their growth was stimulated.

Root exudates from the ornamentals were analyzed and some compounds were detected. The identified chemicals were mainly some small chain aliphatic acids and some simple phenolic acids or phenolic compounds and those varied from extract to extract in the

ornamentals that experienced autotoxicity. Eleven organic compounds were detected in the root exudates of toritelia roots and seven in prairie gentian (Table 4). Many compounds in the root exudates of the plants are yet to be identified. However, at least one aliphatic acid or phenolic compound has been detected in the root exudates of the studied plants. A bioassay was carried out to evaluate the inhibition potential of some identified compounds. Different test concentrations were made with the compounds and a bioassay was furnished with some test plants. Almost all the compounds inhibited the growth of tested plants in a concentration-dependent manner. Lactic acid significantly reduced fresh shoot weight (FSW) and root dry weight (RDW) in pot marigold to 79% and 66% of control, respectively, even at low concentration (50 μM) (Table 5). Benzoic and *p*-hydroxybenzoic acid in lily, even at 50

Table 3. Growth performances of some ornamental plants grown through hydroponic culture in the presence or absence of activated charcoal (AC) in the nutrient solution (%).[z]

Family	Ornamental	Plant length	No. of leaves	Maximum root length	Flesh wt of shoot	Dry wt of shoot	Dry wt of root	No. of flowersper plant
Compositae	Pot marigold	89.9^{*y}	95.8^{NS}	101.9^{NS}	55.9**	79.9*	70.4**	—
	Cornflower	102.9^{NS}	115.5**	102.1^{NS}	—	111.3^{NS}	86.8^{NS}	—
	Chrysanthemum	103.8^{NS}	—	—	99.9^{NS}	98.9^{NS}	126.6**	—
	Cosmos	—^{x}	—	—	119.9^{NS}	120.1^{NS}	111.2^{NS}	—
	Zinnia	93.7^{NS}	—	—	88.6^{NS}	91.7^{NS}	96.8^{NS}	—
	Thistle	114.8^{NS}	—	114.6*	99.9^{NS}	118.1^{NS}	120.8^{NS}	142.9^{NS}
	Sunflower	106.1^{NS}	96.8^{NS}	84.4^{NS}	113.3^{NS}	—	95.8^{NS}	100.0^{NS}
	Safflower	104.8^{NS}	89.7**	79.4**	91.6^{NS}	100.2^{NS}	84.6^{NS}	100.0^{NS}
	African marigold	146.1**	95.5^{NS}	—	146.7**	176.2**	—	100.0^{NS}
	China aster	103.2^{NS}	97.3^{NS}	79.1**	80.7*	82.4*	70.6**	68.4*
	Coneflower	93.7^{NS}	87.2^{NS}	102.8^{NS}	79.2*	84.2*	119.4^{NS}	80.3^{NS}
Liliaceae	Tulip	110.6^{NS}	102.6^{NS}	86.2^{NS}	104.4^{NS}	110.5^{NS}	69.7^{NS}	100.0^{NS}
	Thunberg lily	88.2*	96.0^{NS}	118.2^{NS}	107.3^{NS}	97.1^{NS}	155.3^{NS}	—
	Toritelia	93.1*	100.0^{NS}	55.9**	77.2**	80.2**	74.8**	71.5**
	Lily	37.2**	64.6**	42.1**	13.5**	13.2**	15.6**	—
Labiatae	Rocket larkspur	71.5**	93.8^{NS}	51.4**	25.5**	38.1**	26.3**	88.3^{NS}
	Love-in-a-mist	181.4**	110.3^{NS}	122.7^{NS}	151.6**	127.1*	162.5**	100.0^{NS}
	Scarlet sage	99.5^{NS}	101.0^{NS}	91.6^{NS}	103.6^{NS}	106.1^{NS}	112.5^{NS}	—
	Fan columbine	104.4^{NS}	—	68.1**	74.6*	74.2*	80.3^{NS}	—
Caryophyllaceae	Corn cockl	74.1**	85.4**	62.1**	27.9**	33.1**	83.7^{NS}	—
	Gypsophilla	105.3^{NS}	102.6^{NS}	83.9**	99.9^{NS}	118.1^{NS}	121.8^{NS}	100.0^{NS}
	Carnation	42.4**	75.0**	61.2**	34.6**	46.5**	58.5**	—
Leguminosae	Sweet pea	85.1*	105.8^{NS}	—	78.5*	82.2*	79.8^{NS}	—
	Lupine	98.1^{NS}	106.5^{NS}	—	120.3^{NS}	107.2^{NS}	96.3^{NS}	71.9^{NS}
Cruciferae	Rape blossoms	106.1*	100.0^{NS}	95.6^{NS}	121.2**	113.3*	50.2*	—
	Stock	60.3*	89.9^{NS}	101.5^{NS}	62.9**	78.3**	100.0^{NS}	95.3^{NS}
Onagraceae	Farewell-to-spring	78.4**	92.1*	75.1**	44.7**	51.4**	28.3**	56.3**
Umbelliferae	Bishop's weed	91.3*	97.5^{NS}	—	66.3**	69.4*	—	91.1^{NS}
Scrophulariaceae	Snapdragon	72.8**	96.7^{NS}	100.7^{NS}	46.1**	56.3**	79.5^{NS}	73.1*
Papaveraceae	Corn poppy	50.4*	75.3^{NS}	98.1^{NS}	32.1**	52.5*	52.6*	—
Amaryllidaceae	Narcissus	97.1^{NS}	102.0^{NS}	78.8**	96.3^{NS}	89.2^{NS}	97.7^{NS}	100.0^{NS}
Amaranthaceae	Feather cockscomb	92.9^{NS}	80.7*	85.7^{NS}	100.5^{NS}	—	—	100.0^{NS}
	Globe amaranth	102.8^{NS}	100.0^{NS}	102.8^{NS}	84.5**	83.2**	100.0^{NS}	82.7**
Gentianaceae	Prairie gentian	83.8**	107.9*	51.1**	50.8**	60.2**	45.4**	62.2**
Campanulaceae	Balloon flower	117.5*	102.5^{NS}	78.8**	95.7^{NS}	89.4^{NS}	112.5^{NS}	113.2^{NS}
Plumbaginaceae	Statice	109.2^{NS}	94.2^{NS}	98.1^{NS}	94.7^{NS}	97.8^{NS}	68.5*	114.2^{NS}
Solanaceae	Chinese-lantern plant	105.3^{NS}	104.7^{NS}	—	67.6**	64.8**	74.8**	114.7^{NS}

[z]Growth performance (%) = growth in absence of AC/growth in presence of AC × 100.
[y]Significant at 5% level (*), 1% level (**) and not significant (^{NS}) by *t* test (n = 36).
[x]No data.

Table 4. The compounds identified in the exudates of some ornamentals adsorbed on activated charcoal added in the nutrient solution.

Allelochemicals	Pot marigold	Toritelia	Lily	Rocket larkspur	Sweet pea	Stock	Farewell-to-spring	Bishop's week	Snapdragon	Prairie gentian
Lactic acid	+[z]	+	–	+	–	+	–	+	–	–
Valeric acid	–	+	–	–	–	–	–	–	–	–
Malonic acid	–	–	–	–	+	+	–	–	–	+
Fumaric acid	–	+	–	–	–	–	–	–	–	–
Maleic acid	–	+	–	–	–	–	–	–	–	+
n-Caproic acid	–	+	+	–	–	–	–	–	+	+
Succinic acid	+	+	–	+	–	+	–	–	–	–
Benzoic acid	+	–	+	–	+	–	–	–	–	+
Malic acid	–	+	–	–	–	–	–	–	–	+
m-Hydroxybenzoic acid	–	–	–	–	–	–	+	–	–	+
p-Hydroxybenzoic acid	–	–	+	–	+	–	–	–	–	+
Adipic acid	–	+	+	–	–	–	–	–	–	–
o-Hydroxyphenylacetic acid	–	–	–	+	–	–	–	–	–	–
p-Hydroxyphenylacetic acid	–	+	–	–	–	–	–	–	–	–
Vanillin	–	–	+	–	–	–	–	–	–	–
3,4-Dihydroxybenzoic acid	–	+	–	–	–	–	–	–	–	–
Vanillic acid	–	–	–	+	+	–	–	–	–	–
n-Capric acid	–	+	–	–	–	–	–	–	–	–

[z]Detected (+) and not detected (–).

Table 5. Effects of the identified compounds at different concentrations on the fresh (FW) and dry (DW) weights (mg) of shoot and root of some ornamental plants.

		Pot Marigold		Lily		Rocket larkspur		Sweet pea		Stock		Praire gentian	
Allelochemicals	Concn (μM)	F W of shoot	D W of root	F W of shoot	D W of root	F W of shoot	D W of root	F W of shoot	D W of root	F W of shoot	D W of root	F W of shoot	D W of root
None(control)	0	530 b[z]	4.7 b	1640 a	63 a	140 a	4.4 a	1210 a	18 a	130 a	1.1 a	560 a	23 a
Lactic acid	50	420 c	3.1 c	–	–	130 a	3.5 a	–	–	150 a	1.1 a	–	–
	100	420 c	3.3 c	–	–	140 a	3.7 a	–	–	120 a	0.9 a	–	–
	200	430 c	3.4 c	–	–	160 a	4.5 a	–	–	120 a	1.1 a	–	–
	400	420 c	3.4 c	–	–	150 a	4.6 a	–	–	110 a	0.8 a	–	–
Malonic acid	50	–	–	–	–	–	–	1340 a	23 a	110 a	1.1 a	530 a	24 a
	100	–	–	–	–	–	–	1320 a	32 a	140 a	1.2 a	510 a	24 a
	200	–	–	–	–	–	–	1330 a	21 a	140 a	1.1 a	510 a	25 a
	400	–	–	–	–	–	–	1070 b	17 b	110 a	1.5 a	490 b	29 a
Maleic acid	50	–	–	–	–	–	–	–	–	–	–	490 b	17 b
	100	–	–	–	–	–	–	–	–	–	–	460 b	17 b
	200	–	–	–	–	–	–	–	–	–	–	420 b	18 b
	400	–	–	–	–	–	–	–	–	–	–	390 c	18 b
n-Caproic acid	50	–	–	1410 a	43 b	–	–	–	–	–	–	530 a	22 a
	100	–	–	1380 a	41 b	–	–	–	–	–	–	580 a	25 a
	200	–	–	940 b	34 b	–	–	–	–	–	–	550 a	23 a
	400	–	–	850 b	35 b	–	–	–	–	–	–	530 a	25 a
Succinic acid	50	510 b	4.7 b	–	–	120 a	4.3 a	–	–	130 a	1.1 a	–	–
	100	490 b	4.3 b	–	–	160 a	4.7 a	–	–	120 a	1.7 a	–	–
	200	510 b	3.7 b	–	–	140 a	3.9 a	–	–	120 a	1.2 a	–	–
	400	490 b	4.1 b	–	–	140 a	3.9 a	–	–	110 a	1.3 a	–	–
Benzoic acid	50	470 b	4.2 b	810 b	34 b	–	–	1150 b	18 a	–	–	460 b	19 b
	100	750 a	6.2 a	810 b	34 b	–	–	1110 b	18 a	–	–	470 b	18 b
	200	530 b	4.3 b	800 b	34 b	–	–	1090 b	21 a	–	–	480 b	17 b
	400	440 c	3.1 c	890 b	42 b	–	–	1110 b	21 a	–	–	470 b	16 b
Malic acid	50	–	–	–	–	–	–	–	–	–	–	510 a	22 a
	100	–	–	–	–	–	–	–	–	–	–	480 b	22 a
	200	–	–	–	–	–	–	–	–	–	–	380 c	22 a
	400	–	–	–	–	–	–	–	–	–	–	390 c	22 a
m-Hydroxybenzoic acid	50	–	–	–	–	–	–	–	–	–	–	520 a	19 b
	100	–	–	–	–	–	–	–	–	–	–	510 a	18 b
	200	–	–	–	–	–	–	–	–	–	–	510 a	18 b
	400	–	–	–	–	–	–	–	–	–	–	420 b	16 b
p-Hydroxybenzoic acid	50	–	–	990 b	28 b	–	–	1330 a	21 a	–	–	510 a	22 a
	100	–	–	1170 b	35 b	–	–	1310 a	34 a	–	–	550 a	23 a
	200	–	–	1010 b	31 b	–	–	980 b	15 b	–	–	610 a	26 a
	400	–	–	1010 b	35 b	–	–	870 b	16 b	–	–	470 b	25 a
Adipic acid	50	–	–	1370 a	36 b	–	–	–	–	–	–	–	–
	100	–	–	1210 a	28 b	–	–	–	–	–	–	–	–
	200	–	–	910 b	29 b	–	–	–	–	–	–	–	–
	400	–	–	970 b	22 c	–	–	–	–	–	–	–	–
o-Hydroxyphenylacetic acid	50	–	–	–	–	110 a	3.0 b	–	–	–	–	–	–
	100	–	–	–	–	110 a	2.8 b	–	–	–	–	–	–
	200	–	–	–	–	110 a	2.4 b	–	–	–	–	–	–
	400	–	–	–	–	60 b	2.2 b	–	–	–	–	–	–
Vanillin	50	–	–	1340 a	38 b	–	–	–	–	–	–	–	–
	100	–	–	1310 a	34 b	–	–	–	–	–	–	–	–
	200	–	–	1030 b	27 b	–	–	–	–	–	–	–	–
	400	–	–	1010 b	26 b	–	–	–	–	–	–	–	–
Vanillic acid	50	–	–	–	–	140 a	4.6 a	1230 a	19 a	–	–	–	–
	100	–	–	–	–	140 a	4.6 a	1190 a	19 a	–	–	–	–
	200	–	–	–	–	120 a	2.8 b	1010 b	23 a	–	–	–	–
	400	–	–	–	–	110 a	2.4 b	1110 b	21 a	–	–	–	–

[z]Values in a column followed by a different letter differ significant by Tukey's test ($P = 0.05$; n = 10).

Table 6. Effects of activated charcoal (AC) on the growth of prairie gentian, an ornamental plant, grown on the soil of prairie gentian field amended with different amount of the AC.

Soil	Addition of AC (kg/10a)	Plant length (cm)	No. of leaves	Dry wt of shoot (g)	Maximum root length (cm)	Dry wt of root (g)	No. of flowers per plant
New (control)	—	50.6 a[z]	11.4 b	2.06 a	19.2 a	0.18 b	6.7 a
Successive	—	39.9 c	11.1 bc	1.29 c	15.6 b	0.25 a	5.6 b
Successive	30	40.8 c	11.7 b	1.31 c	14.6 bc	0.18 b	5.2 c
Successive	60	48.4 a	12.2 a	1.85 a	18.1 a	0.19 b	6.8 a
Successive	120	44.0 b	11.4 b	1.60 b	16.5 b	0.19 b	6.7 a
Successive	240	42.2 bc	11.2 bc	1.54 b	14.5 bc	0.20 ab	5.8 b
Successive	480	40.3 c	10.9 c	1.35 c	10.1 c	0.11 c	5.4 c

[z]Values in a column followed by a different letter differ significant by Tukey's test ($P = 0.05$; n = 10).

μM, significantly reduced FSW to 49% and 60% of over control, *n*-caproic, benzoic, *p*-hydroxybenzoic, and adipic acid and vanillin decreased RDW to 68%, 54%, 44%, 57%, and 60% of control, respectively. *o*-Hydroxyphenylacetic acid at 50 μM reduced RDW in rocket larkspur to 68% of control (Table 5). Quantity and quality of exuded allelochemicals varied from plants to plants (Inderjit, 1996) and in cucumber plants, root exudation rate of different chemicals was found to range from 0.20 to 4.17 μg/d per plant (Pramanik et al., 2000). This low concentration is apparently not enough to cause autotoxicity in cucumber plants, but those cucumber plants experienced autotoxicity when grown in absence of AC in the nutrient solution plant (Pramanik et al., 2000). Actually, in natural conditions, occurrence of a chemical at high concentrations (100 μM or more) is rare or absent. However, under field conditions or hydroponic culture, the exuded compounds affect plant growth by additive or synergistic means (Inderjit, 1996) and thus, the compounds even at low concentrations could induce significant growth inhibition in plants, although their threshold inhibition at the individual level is quite high (Rice, 1984). Identical results were found in the experiment (Table 5). So, it appears that the identified compounds would be toxic enough to affect growth of the ornamental plants by additive or synergistic effects.

Performances of prairie gentian were very poor when successively grown for years in the same land. Significant growth inhibition was noticed in the plants grown in soils from a prairie gentian field without AC compared with those grown in reference soil (soil from outside the russell prairie gentian field) (Table 6). It suggests that soil from a prairie gentian field has some growth inhibitors. In hydroponic culture, we also detected some growth inhibitors in the root exudates of the test plant (Tables 4 and 5). Those inhibitors should have been adsorbed when the soil was amended with AC. Thus, the growth of the test plants was increased with an increase in amount of AC from 30 to 60 kg·10a^{-1} followed by a gradual decline at the highest dose of AC (480 kg·10a^{-1}). This high dose of AC might have affected other chemical properties in soil. Results revealed that the test plant length was increased by 96% over control as a result of the addition of AC (60 kg·10a^{-1}). Shoot dry weight and root length were increased by 90% and 94%, respectively, over control for the same concentration (60 kg·10a^{-1}). Flower setting was also increased at 60 kg AC per 10a. This indicated that the reduced growth of prairie gentian after prolonged cultivation in a field could be corrected by amending the soil with AC at the rate of 60 kg·10a^{-1}.

In conclusion, of the ornamentals experiencing autotoxicity owing to the chemicals exuded from their roots being more specific, this autotoxicity could be reduced, at least to some extent, using AC in the root media.

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