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Autotoxic effects of Chrysanthemum (*Chrysanthemum morifolium* (Ramat) Tzvel.)

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ABSTRACT

We evaluated the autotoxic effects of aqueous extracts of leaves, stem, root, rhizosphere soil, litter and root exudates from field grown chrysanthemum on the seed germination and seedling growth of the same plant specie. The aqueous extracts, from above-ground parts were highly autotoxic to seed germination and seedling growth and autotoxicity increased with the increasing concentrations. The aqueous extracts inhibited the enzymatic activities of isocitrate dehydrogenase (IDH) in roots and nitrate reductase (NR) in leaves and significantly stimulated the formation of malonylodialdehyde (MDA) in leaves. We conclude that autotoxicity observed in laboratory conditions was partly responsible for negative effects observed in field or greenhouse, or for problem of continuous cropping of chrysanthemum.

Keywords: Allelopathy, autotoxicity, *Chrysanthemum morifolium* (Ramat) Tzvel, isocitrate dehydrogenase (IDH), malonylodialdehyde (MDA), nitrate reductase (NR)

INTRODUCTION

The continuous growing of the same crop in the same field leads to problem of poor establishment and stunted growth and it has been partly attributed to allelopathy. Allelopathy is defined as the direct or indirect harmful or beneficial effects of one plant on another through the production of chemical compounds that escape into the environment (29). Autotoxicity is an intraspecific form of allelopathy that occurs when a plant species releases chemical substances that inhibit or delay the germination and growth of the same plant species (28). A plant species inhibits the growth of its own kind through the release of toxic chemicals into the environment making the soil unproductive in both managed and natural ecosystems. The allelopathic soil sickness occurs, when flowers, crops and fruits are cultivated in the same soil over extended periods of time, which reduces the growth and yield of same plant species (14,26,36).

Chrysanthemum (*Chrysanthemum morifolium* (Ramat) Tzvel.) is a major ornamental crop and is known to Chinese since 1000 years B.C. (7). It has ornamental, medicinal and industrial uses, which has lead to its protected cultivation and specialized production in China. Under protected cultivation and specialized production, the

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agricultural soil may contain autotoxic levels of allelochemicals, due to their accumulation after the harvest of chrysanthemum and then replanting of the same crop seedlings in the same land in consecutive years. Autotoxicity caused by continuous cropping is the major problem which must be solved for stable production of chrysanthemum and other crops in protected and specialized production. Soybean autotoxicity due to continuous cropping in Northeast China has been studied (25), but no information is available about the ecological role of chrysanthemum autotoxicity. To evaluate the autotoxic potential and to avoid the soil sickness from the continuous cropping under protected and specialized production, more intensive studies are required. The allelopathic potential of chrysanthemum and other plants of Compositae family has been well investigated in natural and agricultural ecosystem (3,4,16,17,19,40). The chrysanthemum cultivars have poor establishment and less productivity, when replanted to the same land in consecutive years (19) and this is the major problems of this crop (30). However, autotoxic effects of chrysanthemum have not yet been investigated. In this study, we did laboratory and greenhouse experiments to determine the possible autotoxic effect of different plant parts from field grown chrysanthemum on seed germination and seedling growth of the same plant species. Different parts of chrysanthemum, (roots, stems and leaves), litter, root exudates and rhizosphere soil were extracted with distilled water, to detect possible autotoxic activities.

Nitrate reductase (NR; EC 1.6.6.1) is a key enzyme in nitrate assimilation in crops. Isocitrate dehydrogenase (IDH; EC 1.1.1.42) is an important enzyme which participates in the citric acid cycle. The measurements of NADH-nitrate reductase and isocitrate dehydrogenase activities can be used as indices of the biological activity and natural biochemical processes in plant. Malonylodialdehyde (MDA) is the product of lipoxygenase; its levels were measured as a marker of oxidative stress. Therefore, MDA in leaves was also determined to evaluate the possible autotoxic stress against seedling growth of the same plant species.

MATERIALS AND METHODS

Fresh leaves, stems, roots, litter and rhizosphere soil from field grown chrysanthemum cultivar 'Gaoyataizi' and its seeds were also collected from the Youbang Chrysanthemum Limited Corporation in Nanjing, China in November and December, 2002 respectively. The seeds were stored in dark at 4^{o}C.

Extraction procedure

Fresh leaves, stems, roots, litter and rhizosphere soil were air-dried, and stems, leaves and root were then separated into small pieces. These were soaked in deionised water in 1:10 ratio (g dry weight/ml) at room temperature for 24 h on a shaker. Extracts were filtered to remove the fibre debris, rotary evaporated and condensed at 51^{o}C under reduced pressure of 0.095 atm (ZFQ-85A, Shanghai Medical Instruments Co., Ltd, China).

Air-dried, grinded and sieved (2 mm mesh) rhizosphere soil were soaked in deionised water in 8:10 ratio (g dry weight/ml) at room temperature for 24 h on a shaker. The extracts were centrifuged at 1811×*g* for 10 min, then filtered, rotary evaporated and condensed under reduced pressure of 0.095 atm at 51^{o}C. Air-dried and chopped litter was treated as described above but soaked for 72 h. The extracts were stored in a refrigerator at 4^{o}C.

Root exudates

The rooting of cuttings (10 cm in length) of 'Gaoyataizi' was done in greenhouse (Photoperiod: 12 h, temperature 25°C and 15°C) at Nanjing Agricultural University, China in March, 2003 with quartz sand as the substrate for rooting. After 15 days, cuttings that had rooted were transferred into half-strength Hoagland nutrient solution (replaced once every 3 days and aerated continuously). After 120 days, chrysanthemum was blotted and weighed, then incubated in half-strength Hoagland solution replaced once every 3 days in 1:10 ratio (g fresh weight/ml) at 25°C. The remnant of solutions were collected and condensed with the same method as described above.

Seed germination, seedling growth and data conversion

The experimental treatments consisted of two factors: (i). Aqueous extracts: 6 (Leaf, litter, stem, root, rhizosphere soil, root exudates) and (ii). Concentrations: 4 (Control, 4:10,8:10,16:10g dry weight/ml). The treatment was replicated three times in a randomized block design. Bioassay method was designed as per Leather and Einhellig (23). Prior to germination, seeds were surface sterilized with 0.1% (w/v) mercuric chloride for 1 min. The seeds were washed several times with distilled water to remove its residues. Each treatment had 50 sterilized seeds, which were evenly placed for germination on filter paper (9-cm dia Petri dish) containing 5 mL of different concentrations of aqueous extract (4:10, 8:10 and 16:10, g dry weight/ml, the same below) or deionised water as control. The dishes were covered and placed in a growth chamber at 25°C for 24 h in dark. During seed germination, the losses of original extracts or deionised water by evaporation were compensated. Numbers of seeds germinated were counted daily till 8th day. The seeds with 1 cm radicle were considered as germinated.

The seeds after surface sterilization [0.1% (w/v) mercuric chloride, for 1 minute], were germinated in sand before being treated. 10 uniform seeds each were placed on filter paper glass pots (5.5 cm dia and 9.5 cm high) containing 5 mL of aqueous extract or deionised water as control. These were sealed with polyethylene foil and kept in growth chamber (25°C during the 14 h light and 22°C during the 10 h darkness). After 11-days growth, the plant height, root length and fresh weight of seedlings were measured. All treatments consisted of at least three replications under identical conditions. From seed germination, data germination index and germination rate were calculated. The data of germination index, germination rate, plant height, root length and fresh weight of the seedlings were then transferred into response index (*RI*) as per by Williamson and Richardson (33), respectively as under:

$$RI = 1\text{-}C/T \quad \text{if } T \geq C \quad \text{or} \quad RI = T/C\text{-}1 \quad \text{if } T < C$$

Where, C : control data, T : treatment data. $RI \geq 0$ or $RI < 0$ indicates stimulation or inhibition over control, respectively. The absolute value of *RI* represents the autotoxic intensity of aqueous extracts.

Activity of NR, IDH and MDA content evaluation

NR activities in leaves were assayed as per Alsam *et al* (2). The reaction medium contained 100 µmol potassium phosphate buffer (pH 7.5), 20 µmol KNO_3, 0.3 µmol NADH and an appropriate amount of crude extract (*in vitro* assay). The reaction was

initiated by adding NADH. The amount of nitrite formed was determined after 30 min at 30°C. The NO_2^{-1} formed was determined by spectrophotometer at 485 nm (38).

IDH was determined by colorimetric method (27). Triplicates of 1 g fresh root samples of chrysanthemum were mixed with 10 ml reaction set [50 µmol potassium phosphate buffer (pH 7.5), 4 µmol D,L-isocitrate, substrate 0.4% 2,3,5-triphenyltetrazolium chloride (TTC) (w/v)]. In control sample, instead of substrate an appropriate amount of potassium phosphate buffer was added. These samples were incubated at 30°C for 4 h in darkness, as trizolium dyes are light sensitive. After incubation triphenyl formazan (TPF) was formed, it was extracted with 25 mL ethyl acetate and measured spectrophotometrically at 485 nm. Concentrations of TPF in the filtrate were determined from calibration standards. All chemical used in these experiments were of analytic grade.

The MDA content was determined by the 2-thiobarbituric acid (TBA) reaction with minor modification of Dhindsa *et al* (8) method. A 0.50 g crushed sample of chrysanthemum was homogenized in 5 ml trichloroacetic acid (TCA) (5%, w/v). The homogenate was centrifuged at 1811×*g* for 10 min. To 2 mL aliquot of the supernatant was added 2 mL 0.67% (w/v) TBA. The mixture was heated at 100°C for 30 min, cooled off and then centrifuged at 1811×*g* for 10 min. The absorbance of aliquot of supernatant was recorded spectrophotometrically at 450 nm, 532 nm and 600 nm, respectively. The concentration of MDA was calculated using the extinction coefficient of 155 $mM^{-1}cm^{-1}$. as Li *et al.* (24) formula as under:

$$C = 6.45(A_{532} - A_{600}) - 0.56A_{450}$$

Where *C* is concentration of MDA, A is absorbance of aliquot of the supernatant.

Statistical analysis

Differences among the means were analyzed with LSD test. Statistical analysis was performed in SPSS 11.5 software package for Windows (SPSS Inc. USA).

RESULTS

Seed germination

Aqueous extracts from different parts of chrysanthemum had significant autotoxic effects on seed germination of the same plant species (Table 1). Under laboratory conditions, the inhibitory effects of aqueous extracts from different parts increased with their increasing concentrations (Table 1). The extracts from above-ground parts (shoot) of chrysanthemum inhibited the seed germination and growth of the same plant species. Compared to control, significant differences were especially observed in litter, leaf extracts and stem extract treatment. However, lower concentrations of underground parts had weak inhibitory or stimulatory effects on seed germination.

Table 1. Autotoxic effects of aqueous extracts of various plant organs on the seed germination of 'Gaoyataizi'

Aqueous extracts	Concentration (g dry weight/ml)	Germination index *RI*	Germination rate *RI*	Average of *RI*
Litter	Control	0.000	0.000	0.000
	4:10	-0.182**	-0.174**	-0.178
	8:10	-0.273**	-0.248**	-0.261
	16:10	-0.428**	-0.313**	-0.371
Leaf	Control	0.000	0.000	0.000
	4:10	-0.162**	-0.041	-0.101
	8:10	-0.218**	-0.246**	-0.232
	16:10	-0.393**	-0.364**	-0.379
Stem	Control	0.000	0.000	0.000
	4:10	+0.084*	+0.050*	+0.067
	8:10	-0.208**	-0.153*	-0.181
	16:10	-0.325**	-0.204**	-0.265
Rhizosphere soil	Control	0.000	0.000	0.000
	4:10	-0.050	-0.026	-0.038
	8:10	-0.064	-0.058*	-0.056
	16:10	-0.070*	-0.105*	-0.088
Root	Control	0.000	0.000	0.000
	4:10	+0.105**	+0.057*	+0.081
	8:10	-0.092**	-0.055	-0.074
	16:10	-0.141**	-0.110*	-0.125
Root exudates	Control	0.000	0.000	0.000
	4:10	+0.044	+0.025	+0.035
	8:10	-0.106*	-0.090	-0.098
	16:10	-0.120**	-0.106*	-0.113

Note: Total time for germination: 8 days; *RI* data are the mean of three replicates in the same treatment; + and – indicate effect of increase and decrease respectively; Germination index =$\sum$Gi/I (%d). Where, *Gi* is germination rate at *i* day (%), *I* is time of seed germination. Asterisks represent significant differences among means respect to control at 0.05(*) and 0.01 (**) level (LSD test), and so does the following tables.

Autotoxic effects of aqueous extracts from different parts on seed germination followed the order of *RI* at 16:10 (g dry weight/ml): leaf (0.379) > litter (0.371) > stem (0.265) > root (0.125) > root exudates (0.113) > rhizosphere soil (0.088), which suggested that autotoxic effects of aqueous extracts mainly depended on above-ground parts (shoot) of chrysanthemum.

Seedling growth

Aqueous extracts from underground parts of chrysanthemum slightly stimulated the seedling growth at low concentration (4:10), but inhibited at higher concentrations of 8:10 and 16:10. The effects of aqueous extracts from above-ground parts (shoots) on seedling growth caused growth abnormalities (Table 2). It could be concluded that aqueous extracts were more inhibitory to root length than plant height and fresh weight of the same plant species.

Table 2. Autotoxic effects of aqueous extracts various plant organs on the early growth of seedlings

Aqueous extracts	Concentrations (g dry weight/ml)	Plant height *RI*	Root length *RI*	Fresh weight *RI*	Average of *RI*
Litter	Control	0.000	0.000	0.000	0.000
	4:10	-0.104**	-0.200**	-0.017	-0.161
	8:10	-0.140**	-0.309**	-0.038*	-0.244
	16:10	-0.166**	-0.357**	-0.082**	-0.303
Leaf	Control	0.000	0.000	0.000	0.000
	4:10	-0.129**	-0.200**	-0.073*	-0.201
	8:10	-0.132**	-0.250**	-0.070*	-0.226
	16:10	-0.241**	-0.314**	-0.098**	-0.326
Stem	Control	0.000	0.000	0.000	0.000
	4:10	-0.101*	-0.165*	-0.024	-0.145
	8:10	-0.094*	-0.197**	-0.059*	-0.175
	16:10	-0.120**	-0.224**	-0.093**	-0.219
Rhizosphere soil	Control	0.000	0.000	0.000	0.000
	4:10	+0.055*	+0.120**	+0.036*	+0.111
	8:10	-0.073*	-0.096*	-0.054*	-0.112
	16:10	-0.108**	-0.147**	-0.069**	-0.162
Root	Control	0.000	0.000	0.000	0.000
	4:10	+0.062*	+0.070	+0.071*	+0.101
	8:10	-0.109*	-0.109*	-0.090*	-0.154
	16:10	-0.124**	-0.103*	-0.082*	-0.155
Root exudates	Control	0.000	0.000	0.000	0.000
	4:10	+0.046	+0.061*	+0.080*	+0.094
	8:10	-0.070*	-0.077*	-0.061*	-0.104
	16:10	-0.103**	-0.092**	-0.095**	-0.145

NR activity in leaves and IDH activity in roots

The aqueous extracts of above-ground parts (shoots) were more autotoxic to NR activity than extracts of rhizosphere soil, root and root exudates (Fig. 1). At 11th day, the activities of NR in leaves were high (8.16 to 9.12 µg NO_2^{-1} g^{-1} FW h^{-1}) at lower concentration (4:10 in w/v) for all the extracts. It suggested that lower concentrations of aqueous extracts from different parts of chrysanthemum were slightly stimulatory to NR activities in leaves. In control, the activities of NR in leaves were 8.05 µg $NO_2^{-1}g^{-1}$ FW h^{-1}. Thus there were no significant differences for NR activity between control and lower concentration (4:10 in w/v) of aqueous extracts from different parts. Aqueous extracts inhibited the NR activity as the concentrations were increased to 8:10 or 16:10. At 8:10 concentration, the NR activities decreased from 6.25 to 7.12 µg NO_2^{-1} g^{-1} FW h^{-1}, while at 16:10 concentration the decrease was from 4.31 to 7.16 µg $NO_2^{-1}g^{-1}$FW h^{-1} (in Fig. 1).

IDH activity in roots was also high at lower concentrations (4:10) of litter, leaf, stem and rhizosphere soil extracts, whereas, extracts significantly inhibited the activity as concentrations were increased to 8:10 and 16:10 (Fig. 2). At 11th day, the IDH activity in control was 4.12 µg $TPFg^{-1}FWh^{-1}$, but at 8:10 concentration it decreased from 1.28 to 3.44 µg TPF g^{-1} FW h^{-1} and at 16:10 concentration from 0.29 to 3.38 µg $TPF^{-1}g^{-1}$ FW h^{-1}. The aqueous extracts of above-ground parts (shoots) were more inhibitory to root IDH activity than of rhizosphere soil, root and root exudates. Compared to controls, the

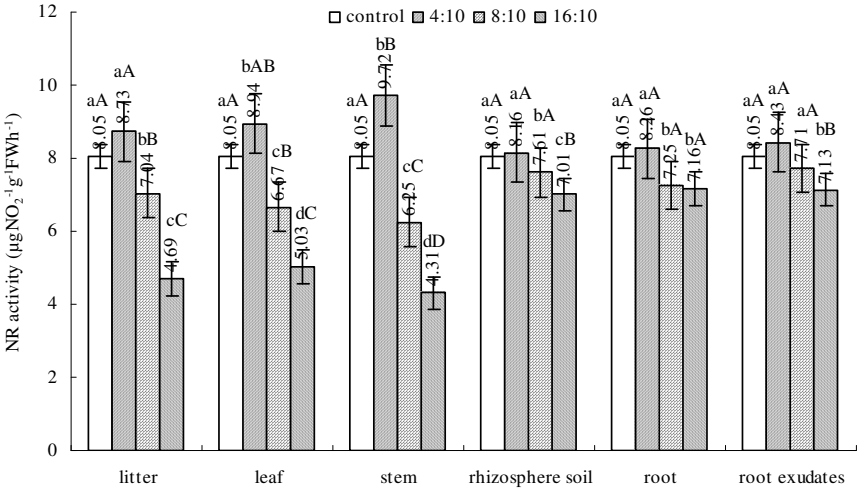

Figure 1. Autotoxic effects of aqueous extracts on NR activities in leaves of chrysanthemum seedlings.

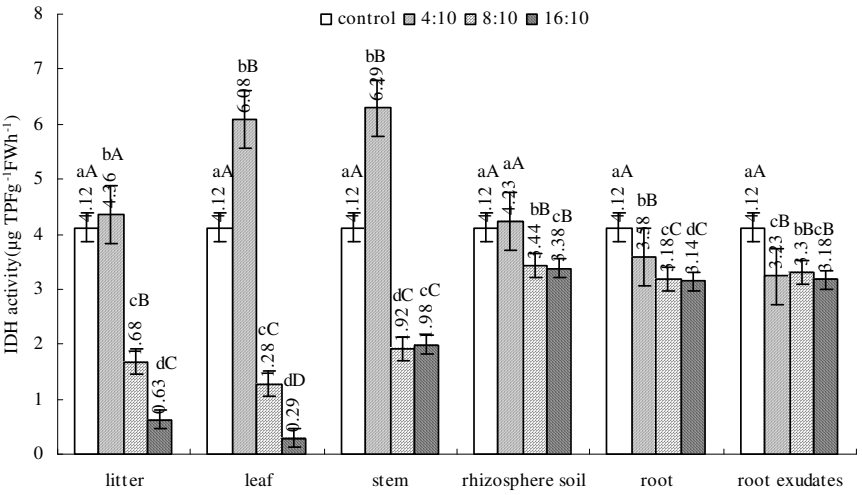

Figure 2. Autotoxic effects of aqueous extracts on IDH activities in roots of chrysanthemum seedlings.

decrease in IDH activity at 16:10 concentration was 93, 85 and 52 % for aqueous extracts of leaf, litter and stem, respectively. The IDH activity was similar between the control and aqueous extracts at lower concentration, but the differences were significant at higher concentration extracts of various parts.

MDA content

MDA content in leaves were significantly stimulated with increasing concentrations of aqueous extracts from different parts of chrysanthemum (Fig. 3). In control MDA content was 8.45 nmol g^{-1} FW, whereas at 16:10 concentration it increased to 12.57 nmol g^{-1}FW for leaf extracts, 12.14 for litter and 10.51 nmol g^{-1} FW for stem. This increase in MDA content in leaves at 16:10 concentration was 49, 44 and 24 % for leaf, litter and stem, respectively compared to controls. The extracts from rhizosphere soil, root and root exudates stimulated the formation of MDA, it implied that autotoxic stress of the aqueous extracts plays important role in the formation of MDA in chrysanthemum.

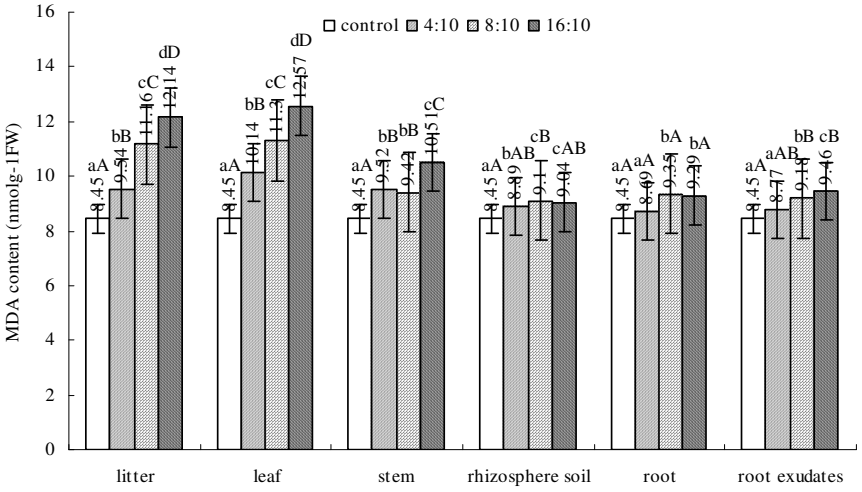

Figure 3. Autotoxic effects of aqueous extracts on MDA content in leaves of chrysanthemum seedlings.

DISCUSSION

The chrysanthemum had strong autotoxic effects on its own seed germination and seedling growth. The aqueous extracts of chrysanthemum (i). substantially suppressed the seed germination and seedling growth of the same plant species and (ii) inhibited the activities of nitrate reductase in leaves and isocitrate dehydrogenase in roots but stimulated the formation of MDA in plants. The inhibitory effect on activity of isocitrate dehydrogenase in roots may inhibit the nutrients uptake, as the root is the first organ to come into

contact with autotoxins in the rhizosphere (5). Under the autotoxic stress, aqueous extracts accelerated the membrane lipid peroxidation and increased the MDA content significantly and the inhibition was concentration dependent. The allelochemicals obtained from different Compositae family plants inhibited the root growth and germination and injured the plasma membrane and caused electrolyte leakage in receptor plants (13). The autotoxic stress stimulates the peroxidation of membrane lipids, decreases the level of soluble protein in leaves and inhibits the photosynthesis in leaves of receptor chrysanthemum (39). In this study, aqueous extracts increased the MDA content in chrysanthemum leaves, especially in leaves treated with extracts of above-ground parts (shoot), which disturbed the balance between the activity of anti-oxidative enzymes and peroxidation of membrane lipids and accordingly affected the structure and functions of membranes, the main mechanisms of allelopathy (11,28,32). Autotoxicity is a chemical interaction between the intra-specific individual plants. Inhibition in growth of the same species will alter the plant response to population density (15). This mechanism will result in avoiding the future competitions that were acquired through long time adaptation to the environment and selection (6). Autotoxicity may be a common phenomenon in agriculture and natural plant communities and it (i). inhibits the germination and early growth of seedlings (1,10,18,21,22,31,35), (ii). inhibits the activities of examined enzymes (34,37) and (iii). stimulates the formation of MDA (37). Our results indicated that aqueous extracts of chrysanthemum reduced the growth of the same species, inhibited the activities of nitrate reductase and isocitrate dehydrogenase but stimulates the MDA formation in plants exposed to autotoxic stress of chrysanthemum. Autotoxic potential varied with extracted plant part and enhanced with the increase in aqueous extract concentration (1,12). Autotoxicity observed in laboratory conditions is partially responsible for harmful effects observed in field or greenhouse, or in continuous cropping problem of chrysanthemum.

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REFERENCES

1. Asao, T., Hasegawa, K., Sueda, Y., Tomita, K., Taniguchi, K., Hosoki T., Pramanik, M. H. R. and Matsui, Y. (2003). Autotoxicity of root exudates from taro. *Scientia Horticulturae* **97**: 389-396.
2. Aslam, M., Rosichan, J.L. and Huffaker, R.C. (1987). Comparative induction of nitrate reductase by nitrate and nitrite in barley leaves. *Plant Physiology* **83**: 579-584.
3. Beninger, C.W. and Hall, J.C. (2005). Allelopathic activity of luteolin 7-O-β-glucuronide isolated from *Chrysanthemum morifolium* L. *Biochemical Systematics and Ecology* **33**: 103-111.
4. Beninger, C.W., Abou-Zaid, M.M., Kistner, A.L.E., Hallett, R.H., Iqbal, M.J., Grodzinski, B. and Hall, J.C. (2003). A flavanone and two phenolic acids from *Chrysanthemum morifolium* with phytoxic and

insect growth regulating activity. *Journal of Chemical Ecology* **30**: 579-596.

5. Blum, U., Shafer, R. and Lehmen, M.E. (1999). Evidence for inhibitory allelopathic interactions including phenolic acids in field soils: Concept vs. an experimental model. *Critical Reviews in Plant Sciences* **18**: 673–693.
6. Canals, R.M., Emeterio L.S. and Peralta, J. (2005). Autotoxicity in *Lolium rigidum*: analyzing the role of chemically mediated interactions in annual plant populations. *Journal of Theoretical Biology* **235**: 402-407.
7. Chen, J.Y. (1985). Studies on the origin of Chinese florist's chrysanthemum. *Acta Horticulturae* **167**: 349-361.
8. Dhindsa, R.S., Plumb-Dhindsa, P. and Thorpe, T.A. (1981). Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *Journal of Experimental Botany* **32**: 93-101.
9. Editorial Department of Agriculture Engineering Technology. (2006). Development status and trade of cutting flower industry in China. *Agriculture Engineering Technology* **10**: 30-31. (In Chinese).
10. Edwards, M.E., Harris, E.M., Wagner, F. H., Cross, M.C. and Miller, G.S. (1988). Seed germination of American pokeweed (*Phytolacca americana*). I. Laboratory techniques and autotoxicity. *American Journal of Botany* **75**: 1794-1802.
11. Einhellig, F.A. (1986). Mechanism and models of action allelochemicals. In: *The Science of Allelopathy,* (Eds., A.R. Putnam and C.S. Tang). pp. 171-188. Wiley Interscience, New York.
12. Ervin, G.N. and Wetzel, R.G. (2000). Allelochemical autotoxicity in the emergent wetland macrophyte *Juncus effusus* (Juncaceae). *American Journal of Botany* **87**: 853-860.
13. Galindo, J.C.G., Hernández, A., Dayan, F.E., Tellez, M.R., Macías, F.A., Paul, R.N. and Duke, S.O. (1999). Dehydrozaluzanin C, a natural sesquiterpenolide, causes rapid plasma membrane leakage. *Phytochemistry* **52**: 805-813.
14. Grodzinsky, A.M., Narwal, S.S. and Polytucka, B. (2006). *Allelopathy in Soil Sickness* (English Print). Scientific Publishers, Jodhpur, India.
15. Hirano, S. and Kira, T. (1965). Influence of autotoxic root exudation on the growth of higher plants grown at different densities. *Journal of Biology, Osaka City University* **16**: 27-44.
16. Hu, C.Q., Chen, K., Shi, Q., Kilkuskie, R.E., Cheng, Y.C. and Lee, K.H. (1994). Anti-aids agents: Acacetin-7-O-β-D-galactopyranoside, an anti-HIV principle from *Chrysanthemum morifolium* and a structure-activity correlation with some related flavonoids. *Journal of Natural Products* **57**: 42-51.
17. Hu, L.H. and Chen, Z.L. (1997). Sesquiterpenoid alcohols from *Chrysanthemum morifolium. Phytochemistry* **44**: 1287-1290.
18. Karin, R. and Kaul, K. (2000). Autotoxicity in *Tagetes erecta* L. on its own germination and seedling growth. *Allelopathy Journal* **7**: 109-113.
19. Kil, B.S. and Lee, S.Y. (1987). Allelopathic effects of *Chrysanthemum morifolium* on germination and growth of several herbaceous plants. *Journal of Chemical Ecology* **13**: 299-308.
20. Kil, B.S. and Yun, K.W. (1992). Allelopathic effects of water extracts of *Artemisia princeps* var. orientalis on selected plant species. *Journal of Chemical Ecology* **18**: 39-51.
21. Kitazawa, H., Asao, T., Ban, T., Pramanik, M.H.R. and Hosoki, T. (2005). Autotoxicity of root exudates from strawberry in hydroponic culture. *Journal of Horticultural Science & Biotechnology* **80**: 677-680.
22. Kraus, E., Voeten, M. and Lambers, H. (2002). Allelopathic and autotoxic interactions in selected populations of *Lolium perenne* grown in monoculture and mixed culture. *Functional Plant Biology* **29**: 1465-1473.
23. Leather, G.R. and Einhellig, F.A. (1988). Bioassay of naturally occurring allelochemicals for phytotoxicity. *Journal of Chemical Ecology* **14**: 1821-1843.
24. Li, H.S. (2000). Method for measurement of malonylodialdehyde in plant tissues. In: *Experimental Principle and Technique in Plant Physiology and Biochemistry*. pp. 260-261. Higher Education Press, Beijing. (In Chinese).
25. Ma, Y.Q. (2003). Allelopathy research in mainland China. *Allelopathy Journal* **12**: 163-178.

26. Narwal, S.S., Dahiya, S.S. and Singh, J.P. (2004). *Research Methods in Plant Sciences: Allelopathy*. *Volume 1: Soil Analysis*. pp. ix-xiii. Scientific Publishers, Jodhpur, India.
27. Öhlinger, R. (1996). Dehydrogenase activity with the substrate TTC. In: *Methods in Soil Biology.*, (Eds., F. Schinner, R. Öhlinger, E. Kandeler and R. Margesin), pp. 241-243. Springer Verlag, Berlin.
28. Putnam, A.R. and Tang, C.S. (1986). *The Science of Allelopathy*. Wiley Interscience, Inc. New York,
29. Rice, E.L. (1984). *Allelopathy*. Academic Press, Inc., New York, USA.
30. Sakamoto, Y., Watanabe, S. and Okano, K. (2001). Growth and quality of chrysanthemum (*Dendranthema grandiflora*) grown in wet sheet culture and deep flow technique. *Acta Horticulturae* **548**: 459-468.
31. Sampietro, D.A. (2006). Sugarcane: soil sickness and autotoxicity. *Allelopathy Journal* **17**: 33-41.
32. Singh, H.P., Batish, D.R. and Kohli, R.K. (1999). Autotoxicity: Concept, organisms, and ecological significance. *Critical Reviews in Plant Sciences* **18**: 757-772.
33. Williamson, G.B. and Richardson, D. (1988). Bioassay for allelopathy: measuring treatment responses with independent controls. *Journal Chemical Ecology* **14**: 181-187.
34. Wu F.Z., Wang X.Z., Wang, S.M. and Zhou, X.G. (2007). Effects of cinnamic acid application on the contents in cucumber plants and soil. *Allelopathy Journal* **20**: 363-369.
35. Ye, S.F., Yu, J.Q., Peng, Y.H., Zheng, J.H. and Zou, L.Y. (2004). Incidence of Fusarium wilt in *Cucumis sativus* L. is promoted by cinnamic acid, an autotoxin in root exudates. *Plant and Soil* **263**: 143-150.
36. Yu, J.Q. and Du, Y.S. (2000). Soil-sickness problem in the sustainable development for the protected production of vegetables. *Journal of Shenyang Agricultural University* **2**: 124-126. (In Chinese).
37. Yu, J.Q., Ye, S.F., Zhang, M.F. and Hu, W.H. (2003). Effects of root exudates and aqueous root extracts of cucumber (*Cucumis sativus*) and allelochemicals on photosynthesis and antioxidant enzymes in cucumber. *Biochemical Systematics and Ecology* **31**: 129-139.
38. Zhang, Z.L. (1990). *Experiments and Methods in Plant Physiology.* pp. 65-68. Higher Education Press, Beijing, (In Chinese).
39. Zhou, K. and Guo, W.M. (2006). Autotoxication effects of aquatic extracts from different parts of Chrysanthemum 'Gaoyataizi' on qualities and physiological and biochemical characteristics. S05-P-3, 27th International Horticultural Congress (IHC 2006), Seoul, Korea.
40. Zhou, K., Guo, W.M. and Xu, Y.C. (2004). Advances in research on allelopathic potential in Compositae. *Acta Ecologica Sinica* **24**: 1780-1788. (In Chinese).