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Autotoxicity in Vegetables and Ornamentals and Its Control

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1. Introduction

Allelopathy comes from the Latin words allelon 'of each other' and pathos 'to suffer' refers to the chemical inhibition of one species by another. The 'inhibitory' chemical is released into the environment where it affects the development and growth of neighboring plants. Allelopathic chemicals can be present in any parts of an allelopathic plant. They can be found in leaves, flowers, roots, fruits, or stems and also in the surrounding soil. Around 300 BC, the Greek botanist Theophrastus was possibly the first person to recognize the allelopathic properties of plants when he observed and recorded that chickpea plants exhausted the soil and destroyed weeds. Later, Pliny the Elder, a Roman scholar and naturalist, noted that walnut trees were toxic to other plants, and that both chickpea and barley ruined crop lands for maize. The term allelopathy was first introduced by a German scientist Molisch in 1937 to include both harmful and beneficial biochemical interactions between all types of plants including microorganisms. Rice (1984) reinforced this definition in the first monograph on allelopathy. Research on the recognition and understanding of allelopathy has been well documented over the past few decades (Rice, 1984; Rizvi & Rizvi, 1992). These include the symptoms and severity of adverse effects of living plants or their residues upon growth of higher plants and crop yields, interactions among organisms, ecological significance of allelopathy in plant communities, replanting problems, problems with crop rotations, autotoxicity, and the production, isolation and identification of allelochemicals in agro ecosystem.

Autotoxicity is a phenomenon of intraspecific allelopathy that occur when a plant species releases chemical substances which inhibit or delay germination and growth of the same plant species (Putnam, 1985; Singh et al., 1999). It been reported to occur in a number of crop plants in agro ecosystem causing serious problems such as growth reduction, yield decline and replant failures (Singh et al., 1999; Pramanik et al., 2000; Asao et al., 2003). Plants when experiences autotoxicity it releases chemicals to its rhizosphere (Singh et al., 1999) through various mechanisms such as leachation (Overland, 1966), volatilization (Petrova, 1977), root exudation (Tang & Young, 1982), and crop residue decomposition (Rice, 1984). Autotoxicity was found to be pronounced if the plants were cultivated consecutively for years on the same land or grown by hydroponic culture without renewal of nutrient solution. One of the

principal causes of this autotoxic growth inhibition in the successive culture of plants has been attributed to the effect of exuded chemicals from plant roots. Root extracts and exudations are the common sources of allelochemicals with potent biological activity and are produced by numerous plant species, with great variation in chemical components (Inderjit & Weston, 2003). It represents one of the largest direct inputs of plant chemicals into the rhizosphere environment. The synthesis and exudation of allelochemicals, along with increased overall production of root exudates, is typically enhanced by stress conditions that the plant encounters such as extreme temperature, drought and UV exposure (Inderjit & Weston, 2003; Pramanik et al., 2000). Accumulations of these allelochemicals are immense in reused nutrient solution during hydroponic culture.

Vegetable and ornamental plants generally cultured through hydroponics in Japan and other developed countries and recently closed type hydroponic systems gained popularity for the production of these crops on a commercial basis. However, this managed and viable technique has the autotoxicity constraint. Therefore, we have studied autotoxicity phenomenon in several vegetables crops such as cucumber (*Cucumis sativus*), taro (*Colocasia esculenta*), strawberry (*Fragaria* × *ananassa* Duch.), some leafy vegetables, and many ornamentals at the glasshouse of Experimental Research Center for Biological Resources Science, Shimane University, Matsue, Japan using hydroponic culture. We have also investigated the isolation, identification, phytotoxicity evaluation of the allelochemicals and means to recover growth inhibition. In this chapter we illustrate autotoxicity of the above crops in hydroponics, its occurrence, autotoxic substances isolation and phytotoxicity evaluation, and control methods.

2. Materials and methods

2.1 Plant materials

In our laboratory we have investigated the autotoxicity from root exudates of several vegetable crops such as cucumber, taro, strawberry, some leafy vegetables, and many ornamentals following hydroponic systems. Uniform seedlings or plantlets of similar growth stage produced through seeds or tissue culture means were used as the test plant materials.

2.2 Plant cultivation in hydroponics

Plant materials under investigation were planted into plastic containers (34 cm × 54 cm × 20 cm) and three containers were used for each treatment (plants with or without Activated charcoal, AC). The containers were filled with 'Enshi' nutrient solution (Table 1) for each crop (Hori, 1966). The nutrient solution concentration employed for each crop was 75% for cucumber, taro and strawberry, and 50% for several ornamentals. Nutrient solution in the container was continuously aerated (3.8 liter/min.) using air pumps with two small air filters each packed with 100 g of AC (Type Y-4P, 4-8 mesh, Ajinomoto Fine Techno Co., Kawasaki, Japan). 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 ^{o}C for later extraction. $FeSO_4.7H_2O$ (0.75 g) was added to each

Chemicals	Concentration (μM/l)
$Ca(NO_3)_2 \cdot 4H_2O$	4.03
KNO_3	8.02
$MgSO_4 \cdot 7H_2O$	2.03
$NH_4H_2PO_4$	1.35
H_3BO_3	0.05
$ZnSO_4 \cdot 7H_2O$	7.64×10^{-4}
$MnSO_4 \cdot 5H_2O$	8.30×10^{-3}
$CuSO_4 \cdot 5H_2O$	2.00×10^{-4}
Na_2MoO_4	9.71×10^{-5}
NaFe-EDTA	0.06

Table 1. Mineral nutrient concentrations in full strength 'Enshi` nutrient solution (Hori, 1966).

solution container at 2-day intervals since the AC that absorbed Fe-EDTA and Fe^{2+} was rapidly oxidized to Fe^{3+} and less available for the plants (Yu et al., 1993). 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 (AA-630, Shimadzu Co., Kyoto, Japan), a spectrophotometer (UVmini-1240, Shimadzu Co., Kyoto, Japan), and an ion meter (D-23, Horiba, Kyoto, Japan). At the end of the experiment growth parameters, yield and yield components were compared with untreated control.

2.3 Gas chromatography-mass spectrometry (GC-MS) analysis of root exudates

The AC used to trap the exudates (organics) were desorbed three-times using 200 ml 1:1 (v/v) methanol (100 ml):0.4 M aqueous NaOH (100 ml) (Pramanik et al., 2001). Each batch of AC (200 g) was gently shaken with the mixture for 12 h at room temperature (25 ^oC) with an electric shaker (20 rpm). The three extracts (600 ml) were combined and filtered through Whatman (No. 6) filter paper. The filtrates were neutralized with 6 M HCl and concentrated to 25 ml in a rotary vacuum evaporator at 40 ^oC. Organic compounds in the concentrate were then extracted according to Yu & Matsui (1993). The concentrated AC-extract was adjusted to pH 2.0 with 4 M HCl, extracted three times with 35 ml of refined diethyl ether (DE), and a further three times with 35 ml of ethyl acetate (EA). DE2 and EA2 were the pooled DE and EA extract fractions (105 ml), respectively at pH 2.0. DE2 and EA2 fractions were dried over anhydrous $CaSO_4$ and concentrated to 5 ml each in a rotary evaporator at 40 ^oC. Both concentrated fractions (DE2 and EA2) extracted from the AC were analyzed using a gas chromatograph coupled to a mass spectrometer (GC-MS, Hitachi M-80B, Hitachi, Tokyo, Japan) before or after methylation with diazomethane from *N*-methyl-*N*-nitoso-*p*-toluene sulfonamide. An aliquot of each concentrated fraction (1 or 2 ml) was diluted in 50 ml ether, treated with diazomethane and concentrated to 5 μl in a rotary evaporator then in a N_2 stream in a water bath at 35 ^oC. One microliter of the concentrated sample was injected into a GC-MS with a capillary column (0.25 mm × 60 m) of TC-5 (GL Science, Tokyo, Japan). Helium was used as the carrier gas at a pressure of 78.4 kN/m^2. The column was held

initially at 100 °C for 2 minutes and then raised at 5 °C/min. to a final temperature of 260 °C for 10 minutes. The injector temperature was held at 270 °C. The ionization voltage and temperature in the electron impact (EI) mode were 70 eV and 250 °C, respectively.

2.4 Bioassay with the identified chemicals

The bioassays with identified chemicals were carried out according to Asao et al., (1998b). Inhibitions of the test solution were assayed by their effects on seedling growth of the source plant species. Aqueous solutions of the identified compounds at several concentrations between 0 (control) and 400 μM were prepared with nutrient solutions for each crops studied. These test solutions were added to glass flasks (approx. 420 ml) wrapped in black polythene to exclude light from the roots (Asao et al., 1999a). The selected plants were transplanted into each flask with urethane foam as a support. The planted flasks were then placed in a growth chamber at 25°C with a light intensity of 74-81 μM/m²/s and required photoperiod under fluorescence lights. To minimize the effects of aeration and microbial degradation of the organic acids (Sundin & Waechter-Kristensen, 1994) on the bioassay, the test solutions in the flasks were renewed every 3-4 d. The plantlets were grown for 3 weeks then growth parameters were measured in terms of fresh weight (FW) and dry weight (DW) of the shoots, DW of roots, and the longest root per plant.

2.5 Statistical analysis

A randomized complete block design with three replicates was used for growth chamber bioassay and hydroponic culture in the greenhouse as described above. These experiments were not repeated over time due to the consistent result. Multiple-comparison test were performed by SPSS 11.0J for Windows (SPSS Japan Inc., Tokyo, Japan) as Tukey's test at a level of significance of P=0.05. Regression analyses were performed by Statcel2 (Add-in soft for Microsoft Excel, OMS Publishing Inc., Saitama, Japan) at a level of significance of P=0.05.

3. Autotoxicity from the root exudates of vegetables and ornamentals in hydroponics

Successive culture of the same crop on the same land for years cause soil sickness or replanting injuries (Bonner & Galson, 1944; Davis, 1928; Hirano, 1940; Rice, 1984; Tsuchiya, 1990) resulting reduction in both crop yield and quality. This phenomenon is evidenced in agricultural production especially in the production of horticultural crops (Grodzinsky, 1992; Tsuchiya, 1990; Young, 1984). It leads to resurgence of disease pest, exhaustion of soil fertility, and developing chemical interference in the rhizosphere referring allelopathy (Hegde & Miller, 1990; Komada, 1988; Takahashi, 1984; Young, 1984). Allelopathic effects from crop residues and root exudates have extensively studied in vegetable crops such as in alfalfa (Miller, 1983; Nakahisa et al., 1993, 1994; Chon et al., 2002, Chung et al., 2011), asparagus (Young, 1984; Young & Chou, 1985; Hartung et al., 1990), cucumber (Yu & Matsui, 1994, 1997), watermelon (Kushima et al., 1998; Hao et al., 2007), taro (Asao et al., 2003), strawberry (Kitazawa et al., 2005), tomato (Yu & Matsui, 1993b), lettuce (Lee et al., 2006) and so on. Therefore, growth of these plants found to be inhibited by the released allelochemicals. So far several methods has been found to be effective in removing or degradating the phytotoxic substances released from plant roots during autotoxicity such as

adsorption by activated charcoal (Asao et al., 1998a), degradation by microbial strain (Asao et al., 2004a) or auxin (2,4-D and NAA) supplementation (Kitazawa et al., 2007), electro-degradation of root exudates (Asao et al., 2008) and TiO_2 photocatalysis (Sunada et al., 2008).

Similar to successive culture, in closed hydroponics accumulation of phytotoxic chemical leads to the occurrence of autotoxicity. In our lab we have investigated the autotoxic potentials of several vegetables and ornamentals and suggested means to overcome it. Our research history started with the selection of cucumber cultivars suitable for a closed hydroponics system using bioassay with cucumber seedlings (Asao at al., 1998b). Experiments were conducted to clarify why fruit yield decrease during the late growing period of cucumber cultured in non-renewed hydroponic nutrient solution and results were suggested that root exudates had induced the decrease in fruit yield, especially by affecting young fruits, the decrease was reversible through removal of root exudates by AC (Asao et al., 1998a). We found extended harvesting period in a closed nutrient flow system by grafting 'Shogoin-aonaga-fushinari' on 'Hokushin' or 'Aodai' (Asao et al., 1999b) and increased number of harvested fruits by adding AC in the nutrient solution (Asao et al., 2000). Growth inhibiting substances of unknown origin found in the growing nutrient solution of cucumber plants were isolated and identified. The growth inhibitors were adsorbed on AC and extracted by organic solvent (Asao et al., 1999a). We developed a bioassay technique to evaluate toxicity of aromatic acids to cucumber seedlings and to select cucumber cultivars that release little or no 2,4-dichlorobenzoic acid (an autotoxic chemical found in cucumber root exudates), thereby avoiding cucumber autotoxicity in the closed hydroponics system (Asao et al., 1999a). Root exudates, which are detrimental to vegetative growth and yield of cucumber plants, were adsorbed by the AC irrespective of dissolved oxygen levels (Asao et al., 1999d).

The number of organic acids and their exudation rates were higher in high temperatures and long photoperiods than that in low temperature with short photoperiod condition and caused higher cucumber autotoxicity in the former conditions (Pramanik et al., 2000). Species differences in the susceptibility to autotoxicity among leaf vegetables were also investigated in hydroponics (Asao et al., 2001a). Autotoxicity of root exudates from taro were showed benzoic acid as the potent growth inhibitor (Asao et al., 2003). A number of aromatic organic acids were identified in several leaf vegetables (Asao et al., 2004b). 2,4-dichlorobenzoic acid (DCBA)-degrading microbial strains, may degrade DCBA including other growth inhibitors exuded from cucumber roots and avoid autotoxicity in cucumber resulting increased fruit yield (Asao et al., 2004a). In strawberry we found vegetative and reproductive growth inhibition due to autotoxicity developed in non-renewed nutrient solution through accumulation of autotoxic root exudates, and the most potent inhibitor was benzoic acid (Kitazawa et al., 2005). Foliar application of auxin such as 1-naphthaleneacetic acid (NAA) avoided the growth reduction of strawberry caused by autotoxicity. NAA at 5.4 μM found to be the most effective for alleviating autotoxicity of strawberry and increasing the yield (Kitazawa, et al., 2007). Autotoxicity in some ornamentals were investigated in hydroponics with or without the addition of AC to the nutrient solution and several organic compounds were detected (Asao et al., 2007). Benzoic acid being the strongest growth inhibitor, its removal from the nutrient solution is imperative for sustainable production of taro and strawberry or other crops exudates containing it. Therefore, electro-degradation method have been tried to degrade benzoic acid and it was found to be recovered strawberry yield up to 71% from non-renewed nutrient solution (Asao et al., 2008).

3.1 Autotoxicity in cucumber (*Cucumis sativus*)

In closed hydroponic culture without renew of the nutrient solution, we found that the fruit yield of cucumber plants decreased significantly in the late reproductive stage (2 weeks ahead of final harvest) and the growth was recovered by the biweekly renewal of nutrients or supplementation of AC to the nutrient solution (Asao et al., 1998a). Shrunken fruits were harvested from the plant grown in non-renewed culture solution (Fig. 1). This inhibition has been attributed due to the autotoxicity from root exudates (Yu et al., 1994). The autotoxicity of cucumber also differs among cultivars (Asao et al., 1998b). Fruit harvesting of a susceptible cucumber cultivar grown in a closed nutrient flow system was prolonged by grafting onto a non-autotoxic cultivar (Asao et al., 1999b). Thus, cucumber root exudates from a closed hydroponic system were analyzed and among a number of growth inhibitors detected (Asao et al., 1999c; Pramanik et al., 2000), 2,4-dichlorobenzoic acid (DCBA) was the strongest inhibitor. Microorganisms can degrade chemical substances in soil and water (Markus et al., 1984; Nanbu, 1990; Sundin & Waechter-Kristensen, 1994). Van den Tweel et al. (1987) reported that 2,4-dichlorobenzoate was degraded through reductive dechlorination by microorganisms. Recently, we found that the inhibitory effect of DCBA on cucumber seedlings could be reversed using strains of microorganisms (Asao et al., 2001b). However, the effects of such strains on cucumber reproductive growth in the presence or absence of DCBA have yet to be elucidated. Therefore, in this study we investigated the effects of microbial strains on the autotoxicity of cucumber plants grown with or without DCBA in the nutrient solution.

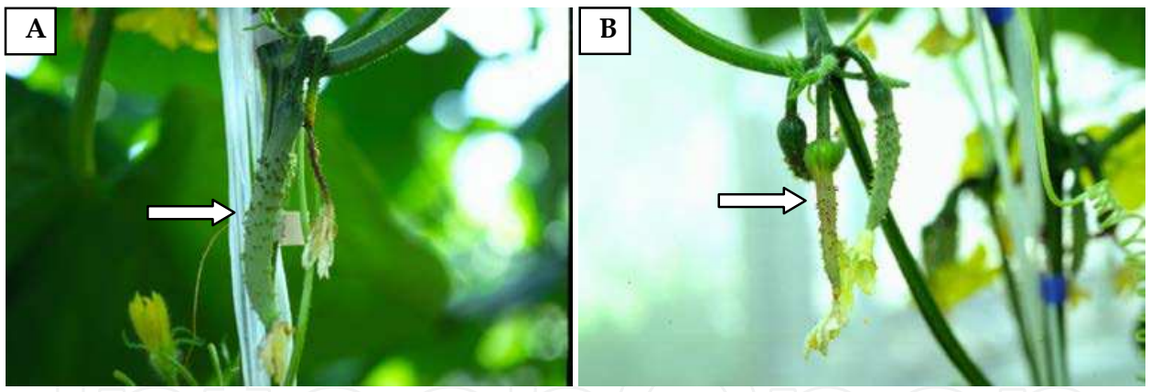


Fig. 1. Fruits of cucumber cv. 'Shogin-aonaga-fushinari', white arrow indicating (A) a normal developing fruit at ten days after anthesis; (B) shrunken fruit.

3.1.1 Cultivation of cucumber plants with or without microbial strain in presence of DCBA

The DCBA-degrading microorganism (microbial strain) was isolated and screened from soil in Aichi prefecture (Asao et al., 2001b). Nutrient solutions with DCBA (10 mg/l) and sucrose (1 g/l) were prepared and sterilized by autoclave. A 200 ml volume of sterile nutrient solution was inoculated with the DCBA-degrading microorganism and shaken continuously by machine at 25 ^{o}C for 9 days to have stock microbial suspension. Cucumber (*Cucumis sativus* L. var. Shougoin-aonaga-fushinari) plants were grown in a greenhouse by hydroponics at different concentrations of DCBA with or without addition of DCBA-degrading microorganisms to the nutrient solution. One-week-old cucumber seedlings

raised in vermiculite were transplanted into plastic containers containing 50 l of continuously aerated (3.8 l/min) 75% Enshi nutrient solution having an electrical conductivity (EC) of 2.0 dS/m. Three seedlings with four leaves were transplanted to each container with three replications. The solutions were prepared at concentrations of 0 (control), 2 or 10 μM/l of DCBA with or without bacterial suspension in the nutrient solution. The solutions were renewed biweekly. Three plants were planted in each container with three repetitions. At the 15-leaf stage, the apical buds of cucumber plants were plucked to maintain 15 leaves on the main stem. The terminal buds of all the developing primary and secondary branches were removed keeping only one node in each branch. The mean air and water temperature during the experiment ranged from 23.0 to 25.5 ^{o}C and from 23.3 to 34.4 ^{o}C, respectively. At the end of the experiment, data were recorded on plant growth, dates of anthesis in male and female flowers, number of healthy female flowers, and harvested fruit number.

3.1.2 Cucumber cultivation with microbial suspension in absence of DCBA

Similar cultivation procedure was followed without addition of DCBA to the nutrient solution in another set of experiments. Three cucumber seedlings with four leaves were transplanted to each container containing the nutrient solution without DCBA and three containers were used for each treatment. During culture, the water level of containers was kept constant by regularly adding tap water. Nutrient contents of the solutions were adjusted to the initial concentrations following procedures described earlier. In all the treatments, the EC and pH in the nutrient solution ranged from 1.4 to 2.8 dS/m and 6.4-7.9, respectively. The microbial suspension was supplied to the nutrient solution added (a) at planting, at the plucking of apical buds and at 2 weeks after initial harvest, (b) at the plucking of apical buds and at 2 weeks after initial harvest, and (c) at 2 weeks after initial harvest. No DCBA was added. An additional cultivation with biweekly renewal of the nutrient solution in the absence of the microbial suspension or DCBA was set up to serve as a control. At the 15-leaf stage, the apical buds of cucumber plants were removed to maintain 15 leaves on the main stem. The terminal buds of the branches were removed keeping only one node on each branch. The mean air and water temperature during the experiment ranged from 24.9 to 31.3 ^{o}C and from 25.8 to 32.1 ^{o}C, respectively. Data were recorded as mentioned for the preceding experiment.

3.1.3 Effects of DCBA with or without microbial suspension on the growth and yield of cucumber

Cucumber plants were grown in hydroponics using different concentrations of DCBA with or without the addition of microorganisms to the nutrient solution. Results reveal that the length of the main stem and primary branches decreased with the increase in DCBA concentration (Table 2). The dry weight of stem, leaf, root and primary branches of plants grown with DCBA (10 μM/l) was also decreased by about 60, 30, 26 and 32% of that without DCBA, respectively. This growth inhibition was significantly recovered by the addition of soil microorganisms to the nutrient solution. This indicates that the microbes efficiently degraded the added DCBA in the nutrient solution and thus restored the inhibitory effect of DCBA on the plants. The date of male flower anthesis was unaffected by the addition of DCBA and the microbial suspension. However, the presence of DCBA at a concentration of 10 μM/l shifted the date of female flower anthesis and harvesting time by about 5 and 16

DCBA (µmol/liter)	Microbial suspension	Stem length (cm)	Lateral branch (cm)	DW of stem (g)	DW/ leaf (g)	DW of lateral branch/ plant[z]	DW of root (g)	Date of anthesis (month/day)		Beginning of harvest (month/day)	No. of female flowers/plant	Harvested fruits/plant
								male flower	female flower			
0	–	177.2	57.0	11.0	5.3	70.7	85.9	9/19	9/26	10/5	36.2	20.2
	+	158.3	54.3	11.0	5.2	70.7	77.7	9/19	9/27	10/7	26.3	18.0
		NS[y]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2	–	165.1	49.1	11.3	5.7	69.2	69.2	9/19	9/25	10/9	31.2	14.9
	+	178.2	56.5	12.0	5.4	80.0	80.0	9/20	9/26	10/7	24.6	17.0
		*	**	NS	NS	NS	NS	NS	NS	NS	NS	NS
10	–	136.9	18.9	6.6	1.6	22.5	22.5	9/20	10/1	10/21	12.8	2.3
	+	147.2	37.9	8.8	3.4	69	69.0	9/20	9/27	10/16	31.1	9.2
		NS	**	**	**	**	**	NS	**	**	**	**
Significance												
Non-microorganism												
Linear		**[x]	**	**	**	**	**	NS	**	**	**	**
Quadratic		NS	NS	NS	**	NS	NS	NS	NS	NS	NS	NS
Microorganism												
Linear		**	**	**	**	NS	NS	NS	NS	**	NS	**
Quadratic		NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS

[z]stem and leaf; [y]significant at the 5 % level (*), 1 % level(**), and not significant (NS) by T-test;
[x]significant at the 1 % level (**), and not significant (NS) by regression analysis of the concentrations.

Table 2. Effects of DCBA with or without microbial suspension on the growth and yield of cucumber plants grown in hydroponics.

days, respectively. Addition of the microbial suspension to the nutrient solution enhanced early flowering and fruit setting in the cucumber plants treated with DCBA. The number of healthy female flowers and the harvested fruit number per plant also decreased as the DCBA concentration increased, and this decrease was significantly compensated by the microbial suspension.

3.1.4 Effects of microbial suspension on cucumber growth in the absence of DCBA

The suspension of DCBA-degrading microorganisms was added once at 2 weeks after the initial harvest, twice upon plucking the apical buds and at 2 weeks after the initial harvest, and three times at the beginning of the culture, on plucking the apical buds and at 2 weeks after the initial harvest. There was no significant difference in the growth of cucumber except in the dry weight of roots and fruit number (Table 3). Root dry weight increased by about 43% with the addition of the suspension of DCBA-degrading microorganisms once at 2 weeks after the initial harvest. The treatments did not affect the dates of anthesis in male and female flower, the beginning of harvest, or the number of flowering female flowers per plant. The harvested fruit number per plant was the lowest (14.2 per plant) in non-renewed nutrient solution. The number of fruits recovered increased from 14.2 to 17.4 on addition of the suspension to the nutrient solution once at 2 weeks after the initial harvest.

DCBA is one of the growth inhibitors found in cucumber root exudates (Pramanik et al., 2000) and we found it as the most effective inhibitor of the growth of cucumber plants (Asao et al., 1999c). In these experiments we also found that DCBA strongly retarded the growth of cucumber plants (Table 2). However, this inhibition was significantly recovered by the addition of DCBA-degrading microbes (Asao et al., 2001b) into the nutrient solution. This result reveals that the microbial strain appreciably deactivated the inhibitory action of DCBA including the other inhibitors in cucumber root exudates in the nutrient solution and thus, the cucumber plant growth was enhanced. The recovery of growth, especially the dry weight of roots and branches, in the plants grown with the microbial suspension, was about three times higher than that of cucumber grown with DCBA alone at a concentration of 10 μM/l. Consequently, dates of male and female flower anthesis, and initial harvest were several days earlier. The number of healthy female flowers as well as fruits also significantly increased on the addition of the microbial suspension.

Experiments to clarify the influence of root exudates and microbes on cucumber plant growth were conducted with or without biweekly renew of the nutrient solutions (Table 3). Results revealed that the root growth and fruit number of the cucumber plants grown with biweekly renewed nutrient solution were significantly increased than those grown without renew of nutrient solution. The addition of the microbes to the nutrient solutions also increased the growth of cucumber plants compared to the non-renew nutrient solution. However, the microbial suspension added to the nutrient solutions in vegetative stage (at the start of the culture or the plucking of apical buds) did not make significant yield difference from non-renewed solution culture. Addition of DCBA-degrading microbial suspension applied once at 2 weeks after the initial harvest was effective enough to recover yield reduction of cucumber from autotoxicity.

DCBA causing autotoxicity in the cucumber was detected in their root exudates only in the reproductive stage (Pramanik et al., 2000). Apparently it indicates that the growth inhibitors

Nutrient solution[z]	Addition of microbial suspension			Stem length (cm)	Lateral branch (cm)	DW of stem (g)	DW /leaf (g)	DW of lateral branch/plant[w]	DW of root (g)	Date of anthesis (month/day)		Beginning of harvest (month/day)	No. of female flowers/plant	Harvested fruit number/plant
	at planting	at plucking[y]	at harvest[x]							male flower	female flower			
+	–	–	–	160.0	44.6	16.8	6.0	80.8	24.5b	7/5	7/9	7/17	29.9	16.2b
–	–	–	–	157.6	46.6	14.7	5.8	77.8	20.3b	7/5	7/9	7/18	31.5	14.2c
–	+	+	+	169.1	48.0	16.9	5.7	88.2	23.5b	7/5	7/9	7/19	33.5	15.6c
–	–	+	+	161.2	41.1	13.4	5.1	70.0	19.1b	7/5	7/10	7/19	30.6	14.4c
–	–	–	+	164.6	43.9	16.2	6.5	92.1	29.0a	7/5	7/9	7/20	26.5	17.4a
				NS[v]	NS	NS	NS	NS		NS	NS	NS	NS	

[z](+)= total renewal of the nutrient solution every other week; (−)= only supplement of the nutrient solution which decreased during culture; [y]at the plucking of apical buds; [x]at two weeks after the initial harvest; [w]stem and leaf; [v]different letters within a column indicate significance at the 5 % level and not significant (NS) by the Tukey test.

Table 3. Effects of addition of the microbial suspension at different growth phases on the growth and yield of cucumber plants grown in the absence of DCBA.

including DCBA would have sufficiently accumulated in the nutrient solution through cucumber root exudation at the reproductive stage. The degrader (applied two or three times) probably became a source of nutrients for other microorganisms. In this case, the degrader did not dominated more than other microorganisms. However, when supplied once at 2 weeks after the start of harvest, the degrader did not become a nutrient source for other microorganisms and degraded the DCBA exuded from cucumber. This was why the DCBA exuded from cucumber sustained the microbial activity. In conclusion, DCBA-degrading microorganisms, if added to the nutrient solution, may degrade DCBA including other growth inhibitors exuded from cucumber roots and avoid autotoxicity in cucumber resulting increase the fruit yield. Addition of the microbial suspension in the reproductive stage of cucumber plants appears to degrade the growth inhibitors efficiently. However, the timing of degrader addition to the nutrient solution for efficient mitigation of cucumber autotoxicity needs further study.

3.2 Autotoxicity in strawberry (*Fragaria ananassa* Duch.)

Closed hydroponics is a system used for plant cultivation in environmentally sensitive areas (Van Os, 1995) where the nutrient solution is not released into the surrounding environment, but recycled (Ruijs, 1994). However, in a closed hydroponic system, plants can suffer autotoxicity, due to the accumulation of toxic exudates from the roots themselves in the nutrient solution (Yu et al., 1993). Recently, closed hydroponics has been considered for strawberry cultivation (Takeuchi, 2000; Oka, 2002; Koshikawa & Yasuda, 2003). However, it was reported that a yield reduction, caused by unknown factors, occurred in closed hydroponic system for strawberry (Oka, 2002). Koda et al., (1977, 1980) reported that a growth reduction in mitsuba (*Cryptotaenia japonica* Hassk.) in hydroponic system was caused by root exudates such as organic acids. Some aromatic acids also accumulated in the nutrient solution during hydroponic cultivation of tomato and had inhibitory activity on growth (Yu & Matsui, 1993). Asao et al., (1998a, 1999c) demonstrated that a reduction in fruit yield in the late reproductive stage of cucumber was induced by root exudates and that the most potent inhibitor was 2,4-dichlorobenzoic acid. In a closed hydroponic culture of rose plantlets, root and shoot growth were reduced by root exudates (Sato, 2004). However, little is known about similar effects in strawberry culture. It was therefore felt necessary to examine the effects of root exudates on the growth of strawberry. Root exudates can be removed by adding activated charcoal (+AC) to the nutrient solution (Koda et al., 1977; Asao et al., 1998a, 1999c; Sato, 2004). In this study, we investigated the effects of non-renewal of the nutrient solution, and of adding AC on vegetative and reproductive growth of strawberry.

3.2.1 Cultivation of strawberry plants in hydroponics

Strawberry (*Fragaria ananassa* Duch. cv. 'Toyonoka') was used for these experiments following the cultivation method explain above (Section 2.2 & Fig. 2). Pollination was aided by vibrating the anthers over stigma with a soft brush at 2 d intervals. The fruits were collected when ripe. At harvest growth parameters were measured among the treatments. During cultivation, the number of flower clusters, flowers and fruits per plant were recorded. The allelochemicals in the root exudates of strawberry were identified by GC-MS (section 2.3) and their phytotoxicity were evaluated in growth chamber bioassay (section 2.4) using strawberry plantlets.

Fig. 2. Hydroponic system used for strawberry cultivation at the greenhouse of Shimane University, Matsue, Japan.

3.2.2 Effects of non-renewed nutrient solution on the growth and yield of strawberry

In the treatment '-AC', the number of leaves, FW of shoots, DW of shoots and roots per plant, and the root length decreased by 75%, 59%, 50%, 81% and 45% of control values, respectively. In the '+AC' treatment, each value was 103%, 83%, 75%, 102% and 98% of control values (Table 4). Although the number of flower clusters per plant was not significant by different between treatments, values in '-AC' and '+AC' treatment were 85% and 89% of control values, respectively (Table 4). In the '-AC' treatment, the number of flowers per plant decreased significantly to about 74% of control value. In the '+AC' treatment, each value was approx. 102% of the control value and there was no significant difference between the '+AC' treatment and control. In the '-AC' treatment, the number of harvested fruit per plant decreased significantly by approx. 49% of control values. In the '+AC' treatment, this value was about 107% of the control value, and there was no

Nutrient solution[z]	AC supplement	No. of leaves /plant	FW of shoots/ plant (g)	DW of shoots/ plant (g)	DW of root/ plant (g)	Root length (cm)	No. of flower cluster/ plant	No. of flower/ plant	No. of fruits/ plant
+	−AC	23.2a[y]	71.8a	15.5a	4.7ab	43.8a	4.6a[b]	21.0a	12.3a
−	−AC	17.5b	42.6b	7.8b	3.8b	19.8b	3.9a	15.6b	6.0b
−	+AC	24.0a	59.8a	11.6a	4.8a	42.8a	4.1a	21.4a	13.1a

[z](+)= complete renewal of the nutrient solution every second week, and (-)= only supplement of the nutrient solution which decreased during culture; [y]values in column followed by a different letter differ significantly by Tukey's test (P = 0.05; n = 9).

Table 4. Effects of non-renewed nutrient solution and activated charcoal on the growth, yield components and yield of strawberry in hydroponics.

significant difference between the '+AC' treatment and control (Table 4). In the '+AC' treatment with non-renewed nutrient solution, the growth and yield of strawberry plants were eventually equivalent to control plants.

3.2.3 Phytotoxicity of the identified chemicals in root exudates of strawberry

GC-MS analysis of strawberry root exudates (Fraction 'DE2') showed more than 20 peaks, whereas the 'EA2' fraction had only a few detectable peaks (Fig. 3). Based on a comparison of retention times and the mass spectra of standard samples, five peaks were identified as methyl esters of lactic acid, benzoic acid, succinic acid, adipic acid and p-hydroxybenzoic acid. The autotoxic effects by the five exudates compounds identified were evaluated by using micro-propagated the plantlet of the same species from which they originated. Lactic, succinic, adipic and p-hydroxybenzoic acid did not significantly reduce the FWs of shoot, or the DWs of shoots and roots (Table 5). No correlation was found between these growth parameters and increasing concentrations of these four exuded compounds. Benzoic acid, however, significantly reduced both FW and DW of shoots, even at 50 µM and, these growth parameters decreased further with increasing concentration. Benzoic acid also significantly reduced the DWs of roots at all concentrations. Root length was reduced by increasing concentrations of all five compounds, although lactic, succinic and adipic acids reduced root length significantly at concentrations of 100 µM or above, while benzoic and p-hydroxybenzoic acids significantly reduced root length even at 50 µM (Table 5).

The effects of non-renewed hydroponic nutrient solution and of adding AC ('+AC') on the vegetative and reproductive growth of strawberry were investigated. Non-renewed nutrient solution resulted in a significant decrease in the growth of strawberry plantlets compared to growth when the nutrient solution was renewed. The number of flower clusters, flowers and fruit harvested all decreased in non-renewed nutrient solution (Table 4). Growth and/or yield reductions in plants caused by non-renewed nutrient solution have been reported by many researchers (Koda et al., 1980; Yu & Matsui, 1993). Asao et al., (1998a) reported that fruit yields in cucumber plants decreased significantly at the late reproductive stage (2 weeks before final harvest) when nutrient solution was not renewed and that the reduction was caused by root exudates. In a closed hydroponic system for rose plantlets, the root and shoot growth were reduced by the root exudates (Sato, 2004). Thus, it was thought that vegetative and reproductive growth in strawberry was inhibited by root exudates during non-renewal of the nutrient solution. Growth and/or yield inhibition in cucumber (Asao et al., 1998a), mitsuba (Koda et al., 1980) and rose (Sato, 2004), caused their root exudates could be avoided by adding AC to the nutrient solution. AC added to the nutrient solution adsorbs the organic compounds exuded from plant roots and thereby removes the inhibitory effects of exudates.

In this study, there was no significant difference in vegetative and reproductive growth of strawberry between addition of AC to non-renewed nutrient solution, and renewed the nutrient solution (Tables 4). Thus, our results suggest that the chemicals exuded from strawberry roots inhibit vegetative and reproductive growth, which can be avoided by adsorption of root exudates using AC. The substances adsorbed on the AC were extracted, analyzed and some identified as phenolic and aliphatic acids (Fig. 3). Growth and/or yield reduction in cucumber plant by root exudates had been confirmed to be due to (Pramanik et al., 2001) and alipathic acids (Yu & Matsui, 1997). In cucumber, 2,4-dichlorobenzoic acid was the most potent inhibitor of growth and yield (Asao et al., 1999c).

Compound[z]	(Concentration, µM)	FW of shoot/ plant (g)	DW of shoot/ plant (g)	DW of root/ plant (g)	Root length/ plant (cm)
Control (No compound)[y]	0	0.98a[b]	0.17a	0.07a	17.0a
Lactic acid	50	0.95a	0.16a	0.06a	16.2a
	100	0.88a	0.15a	0.06a	13.7b
	200	0.88a	0.16a	0.07a	11.9bc
	400	0.85a	0.15a	0.06a	11.4c
Benzoic acid	50	0.48b	0.09b	0.03b	12.1b
	100	0.42b	0.09b	0.03b	10.5b
	200	0.42b	0.08b	0.03b	10.3b
	400	0.36b	0.08b	0.03b	9.4c
Succinic acid	50	0.80a	0.14a	0.06a	16.2ab
	100	0.74a	0.13a	0.04a	13.6bc
	200	0.69a	0.19a	0.04a	12.5c
	400	0.87a	0.16a	0.05a	11.7c
Adipic acid	50	0.70a	0.12a	0.03a	14.1ab
	100	0.88a	0.15a	0.05a	12.0bc
	200	0.74a	0.13a	0.05a	10.6c
	400	0.76a	0.15a	0.05a	9.4c
p-hydroxybenzoic acid	50	0.77a	0.14a	0.04a	11.9b
	100	0.67a	0.13a	0.03a	10.8bc
	200	0.77a	0.15a	0.05a	9.1c
	400	0.65a	0.12a	0.04a	10.4bc

[z]compounds identified in the root exudates adsorbed onto the activated charcoal; [y]values in column followed by a different letter differ significantly by Tukey's test ($P = 0.05$; n = 9).

Table 5. Effects of purified exudate compounds at different concentrations on the growth of strawberry plantlets.

Benzoic acid was also the main inhibitor of growth and yield in taro plants (Asao et al., 2003). The phenolic and aliphatic acids identified in strawberry root exudates may have the potential to inhibit growth. The inhibitory potential of root exudates has been tested using germination test of lettuce seeds (Tsuchiya & Ohno, 1992). However, Asao et al. (2001a) suggested that lettuce plants suffer autotoxicity from their own root exudates. Vanillic acid was the most inhibitor of the growth of lettuce (Asao et al., 2004b). Thus, the potential of each compound identified in our study was evaluated using plantlets of the species which they originated. Five compounds including phenolic and aliphatic acid were found to affect plant growth but only benzoic acid significantly inhibited increases in the FW of shoots,

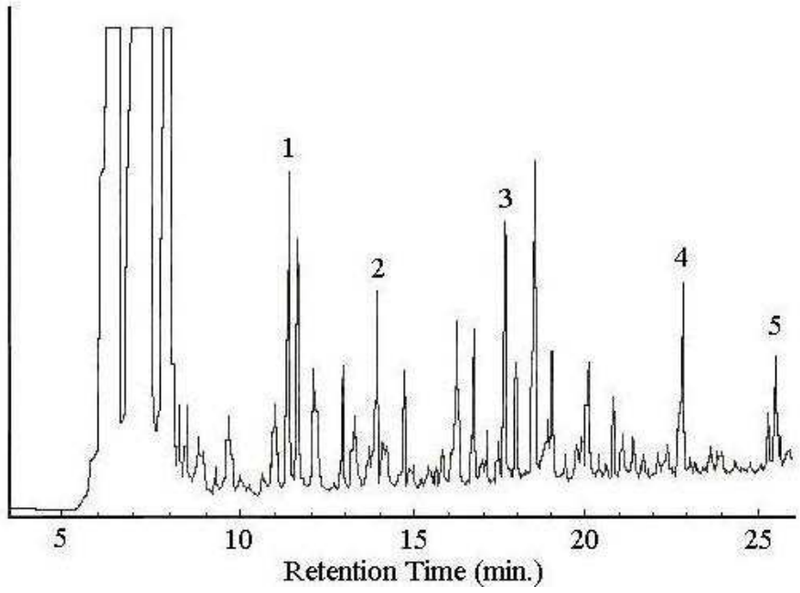


Fig. 3. Gas Chromatograms of all components from root exudate of strawberry plants adsorbed and released from AC. Methyl esters of lactic acid (peak 1), benzoic acid (peak 2), succinic acid (peak 3), adipic acid (peak 4) and p-hydroxy benzoic acid (peak 5) are identified based on known standard retention times.

DWs of shoots and roots, and root length at all concentrations (Table 5). Therefore, it was thought that benzoic acid was the strongest inhibitor of vegetative and reproductive growth in strawberry. In conclusion, inhibition of the vegetative and reproductive growth in strawberry caused by non-renewed nutrient solution may occur through autotoxic root exudates, and the most potent inhibitor was benzoic acid. Reduction in the yields of strawberry grown closed hydroponic systems would, therefore, appear to be related to the allelochemicals exuded by the strawberry plant itself. Finally, for strawberry cultivation in a closed hydroponic system, AC should be added to the nutrient solution to relieve the autotoxicity caused by root exudates.

3.3 Autotoxicity in taro (*Colocasia esculenta* Schott.)

Taro plants do not grow well if cultivated consecutively for years on the same land (Takahashi, 1984). Rotation with other crops for at least three years (Miyoshi et al., 1971a), in combination with organic matter and soil disinfectants (Murota et al. 1984), has been suggested to improve the yield of taro. However, even in a fixed crop rotation system, there was a great difference in the growth and yields of taro plants. This depends upon the kinds of crops in rotation, and the order in which they were rotated. In combination with burdock, the yield of taro was equal to or more than that of taro in the first-year planting, and the extent of corm injury were slight (Murota et al., 1984). Harmful microbes (Atumi, 1956, 1957; Atumi & Nakamura, 1959; Nagae et al., 1971) and nematodes (Miyoshi et al., 1971b; Oashi, 1973; Matsumoto et al., 1973, 1974) in the soil are the main causes of damage in the successive culture of taro. Takahashi (1984) suggested that unknown factors were also involved whereas; Miyaji et al. (1979) found that taro residues in soils after harvest were inhibitory to its growth. Methanol extracts of taro residues alone or of soils with taro residues were found to strongly inhibit the elongation of

hypocotyls and radicle growth of turnip. The foregoing results reveal that growth inhibitors from taro were connected with replanting problems. Our laboratory has established used hydroponic culture system to assess autotoxicity in crop plants (Asao et al., 1999c; Pramanik et al., 2000). Thus, an attempt was made to identify the chemicals exuded by taro roots and to evaluate the allelopathic effects of these exudates on the growth and yield of taro through this established hydroponic culture system.

3.3.1 Cultivation of taro plants in hydroponics

Taro cv. Aichi-Wase was used for this experiment. Corms were planted in plastic tray (32 cm × 47 cm × 7 cm) containing vermiculite in the green house. At the third leaf stage, taro plantlets were transplanted into plastic containers following the cultivation method described above in section 2.2 (Fig. 4). At the end of this experiment, measurements were made on the longest leaf stalk, maximum leaf length and width, leaf number per plant, shoot dry weight and corm yield. Phytotoxic chemicals adsorbed in the ACs were extracted and identified by a Gas-chromatograph coupled with mass-spectrometer as mentioned earlier. Bioassay of the identified acids at concentrations of 0 (control) or 400 µM/l were prepared with a 75 % Enshi nutrient solution (EC 2.0 dS/m) in growth chamber condition. The taro plantlets were grown for 26 days and then the fresh and dry weights of shoots, number of leaves, longest root length and root dry weight were measured. Each treatment was replicated 15 times. We carried out further bioassays following the same procedure with benzoic and adipic acids at concentrations of 0 (control), 25, 50, 100 200 and 400 µM/l. In this case, the taro plantlets were grown for 20 days.

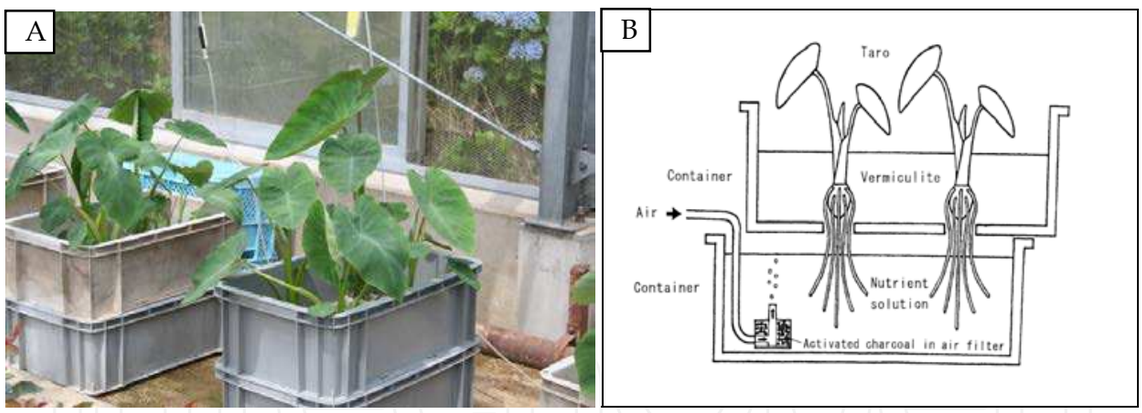


Fig. 4. Hydroponic system used for taro cultivation (A) taro plants in plastic containers, (B) sketch of taro hydroponics showing different components.

3.3.2 Effects of non-renewed nutrient solution on the growth and yield of taro

Results revealed that plants grown without AC had experienced significant shoot growth retardation compared to those grown with AC. The leaf numbers and shoot dry weights of the plants grown without AC decreased to about 90% and 67% of those grown with AC, respectively (Table 6). Addition of AC to the nutrient solution also improved yield significantly. The total yield per plant without AC decreased to about 34% compared to that on the addition of AC (Table 6). Larger corms were harvested from the nutrient solution with AC.

Charcoal supplement	No. of leaf/plant	DW of shoot/plant (g)	Total yield/ plant (g)	Yield/plant by corm size[z] (g)					
				2L	L	M	S	2S	3S
−	14.6	10.9	429	72	38	123	74	86	109
+	16.1	16.1	649	154	80	225	97	111	103
	*[y]	*	**[x]	**	**	**	NS	NS	NS

[z]2L (>60 g), L (35-59 g), M (20-34 g), S (15-19 g); 2S (10-14 g) and 3S (<10 g); [y]significant at the 5 % level (*); [x]significant at the 1 % level (**), and not significant (NS) by T-test.

Table 6. Influence of nutrient solutions in the absence and presence of activated charcoal on the vegetative growth, yield and yield components of taro plants grown by hydroponic culture.

3.3.3 Phytotoxins in root exudates of taro and their phytotoxicity

Analysis of the extracted taro root exudates with GC-MS gave more than thirty peaks (Fig. 5). Based on the comparison of retention times and mass spectra with those of authentic samples, seven peaks were assigned as methyl esters of lactic acid, benzoic acid, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, vanillic acid, succinic acid, and adipic acid.

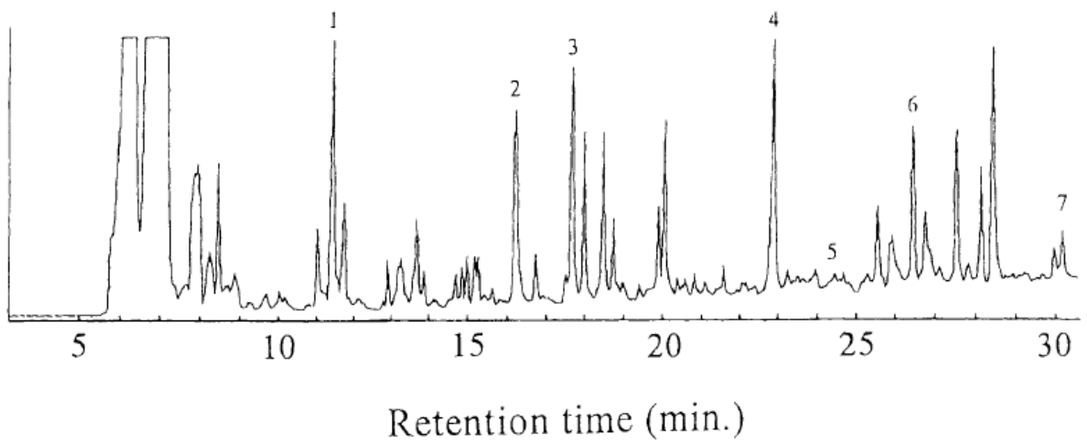


Fig. 5. Gas chromatograms of all components from root exudate of strawberry plants adsorbed and released from AC. Methyl esters of lactic acid (peak 1), benzoic acid (peak 2), succinic acid (peak 3), adipic acid (peak 4), *m*-hydroxybenzoic acid (peak 5), *p*-hydroxybenzoic acid (peak 6) and vanillic acid (peak 7) are identified based on known standard retention times.

The allelopathic potential of the identified compounds was evaluated using taro plantlets as the test material. Benzoic acid at 400 μM/l induced severe growth inhibition of shoots and roots, while adipic acid at the same concentration reduced only dry weight of roots (Table 7). Thus, we further evaluated growth inhibition potential of benzoic acid and adipic acid at concentrations ranged from 0 to 400 μM/l using taro plantlets. Both acids significantly inhibited the growth of plantlets (Table 8). Benzoic acid induced growth retardation even at 50 μM/l and growth decreased with increasing concentration of the acid. Benzoic acid at the highest concentration of 400 μM/l reduced fresh weight, shoot dry weight, root length and root dry weight in taro plants to 54 %, 53 %, 54 %, and 75 % of

Allelochemical[z]	FW of shoot/ plant (g)	DW of shoot/plant (g)	No. of leaf/plant	Root length (cm)	DW root/plant (g)
None(control)	26.58a[y]	2.05a	4.3	17.0a	0.52a
Lactic acid	35.40a	2.11a	4.1	18.8a	0.51a
Benzoic acid	20.79b	1.30b	3.6	14.5b	0.29b
m-hydroxybenzoic acid	27.23a	1.80a	4.0	16.5a	0.38a
p-hydroxybenzoic acid	32.87a	2.56a	4.2	16.7a	0.54a
Vanillic acid	27.37a	1.84a	3.5	17.7a	0.45a
Succinic acid	27.17a	1.84a	3.9	19.5a	0.36a
Adipic acid	23.54a	1.61a	4.3	16.7a	0.27b
			NS		

[z]400 µM/l; [y]different letters within a column indicate significance at the 5 % level and not significant (NS) by the Tukey test.

Table 7. Inhibition potentials of chemicals identified in taro root exudates (400 µM/l) on the growth of taro plantlets.

Allelochemical	Concentrations (µM/l)	FW of shoot/ plant (g)	DW of shoot/plant (g)	No. of leaf/plant	Root length (cm)	DW root/plant (g)
None(control)	0	3.76a[z]	0.235a	4.0	16.0a	24a
Benzoic acid	25	3.46a	0.200a	4.6	13.5a	24a
	50	3.14b	0.167b	4.2	14.2a	19a
	100	2.81b	0.159b	4.0	11.8a	20a
	200	2.16c	0.131c	3.0	10.5b	18b
	400	2.03c	0.125c	4.0	8.6c	18b
Significance						
Linear		*[y]	*	NS	*	*
Quadratic		*	*	NS	*	*
Adipic acid	25	3.91a	0.251a	4.2	16.6a	31a
	50	3.35a	0.020a	4.0	14.6a	23a
	100	3.43a	0.021a	3.7	12.3a	23a
	200	3.33a	0.195a	4.3	12.0a	27a
	400	3.18b	0.191a	4.3	10.8b	26a
Significance						
Linear		*	NS	NS	*	NS
Quadratic		*	NS	NS	*	NS

[z]Different letters within a column indicate significant at the 5 % level and not significant (NS) by the Tukey test; [y]significant at the 5 % level (*) and non-significant (NS) by regression analysis of the concentrations.

Table 8. Effects of benzoic acid and adipic acid at different concentrations on the growth of taro plantlets.

control values, respectively. Adipic acid only at 400 μM/l reduced fresh weight of shoots and root length. Lower concentrations of this acid did not affect shoot or root growth.

As the nutrient concentrations and growth environment in the hydroponic cultures of taro plants were apparently identical, the significant growth differences between the plants grown with and without AC could be attributed to the variation in the chemical composition of the nutrient solution. These chemicals would have exuded from taro roots. Tsuchiya and Ohno (1992) indicated that water extracts from soils used consecutively for taro cultivation over a period of years inhibited the growth of lettuce. Since the same phenomenon was observed even when the extracts were autoclaved, it was considered that the inhibition was caused by allelochemicals rather than by harmful soil microorganisms. There have been many reports that taro residues exhibited an allelopathic effect on plant growth (Miyaji et al., 1979; Tsuzuki et al., 1995; Pardales & Dingal, 1988). It was made clear here that the vegetative growth and corm yield of taro plants were decreased in the non-renewed culture solution and the loss was recovered by adding AC to the nutrient solution. This result suggests that the chemicals exuded from taro roots had induced the inhibition of growth and reduced yield. This inhibition was prevented by the adsorption of the exuded allelochemicals in AC.

The substances adsorbed on the AC were extracted, analyzed and some of them identified as phenolic and aliphatic acids although many compounds in the root exudates are yet to be detected. The allelopathic potential of each identified compound was evaluated and found that almost all the compounds inhibited the growth of taro plantlets (Table 7 & 8). Benzoic induced significant growth inhibition in taro plantlets even at concentration of 50 μM/l. Inhibitory effects of phenolic acids (Pramanik et al., 2001) and aliphatic acids (Yu & Matsui, 1997) to plant growth have been well recognized. In a bioassay, Blum (1996) found that 30 % reduction of absolute leaf expansion brought about at 0.23 μM of phenolic acid per gram soil, while it required only 0.05 μM in the presence of 0.06, 0.17, and 0.04 μM of *p*-coumaric, *p*- hydroxybenzoic, and vannilic acids per gram of soil, respectively. Thus, mixture of allelochemicals can below their inhibitory levels. This indicates that taro plants exude a number of compounds (Fig. 5) into its surroundings and those inhibit the growth taro plants by synergistic or additive actions. In conclusion, taro roots exude a number of allelochemicals including aromatic acids such as benzoic acid and aliphatic acids such as adipic acid which inhibit the growth of taro plants by additive or synergistic actions. Benzoic acid induced strongest inhibition. Thus, the decline in yield on the successive culture of taro would appear to be related to the allelochemicals exuded from the taro plant itself.

3.4 Autotoxicity in some ornamental plants

Plants synthesize, store, and exude various kinds of organic compounds in their surroundings as exudates, volatiles, or residues of decomposition (Hale & 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 renew of nutrient solution (Asao et al., 1998a, 2001a). 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, 2004b; Shafer & Garrison, 1986; Yang, 1982; Yu & Matsui, 1993). 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 et al., 1993) and cucumber (Asao et al., 1998a; Pramanik et al., 2000) has been recovered by addition of 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. Therefore, we attempted to investigate autotoxicity, if any, in selected ornamentals along with a possible remedial measure to overcome the growth inhibition from autotoxicity.

3.4.1 Cultivation of ornamental plants in hydroponics

Thirty-seven different ornamentals belonging to 16 different families were chosen for this experiment (Table 9). Plant cultivation was carried out according to Pramanik et al. (2000) as described above. 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. 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.

3.4.2 Bioassay of the identified autotoxic chemicals in nutrient solution

Gas chromatography-mass spectroscopy analysis of root exudates adsorbed in activated charcoal identified the responsible autotoxic chemicals. Bioassay of aqueous solutions of the identified compounds was carried out according to Asao et al. (1998b) at concentrations of 0 (control), 50, 100, 200, and 400 μM with 50% Enshi nutrient solution (EC 1.3 dS/m) having ten replications. The plants were grown for 2 weeks and then the fresh and dry weights of shoots were measured.

3.4.3 Bioassay in soils amended with activated charcoal

Bioassay in soils amended with activated charcoal has also been carried out. Soils were collected from a field successively cultivated with prairie gentian [*Eustoma grandiflorum* (Raf.) Shinn.] 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/10a. 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. Irrigation (500 ml water) was applied to each container at 2-week intervals and 500 ml Enshi nutrient solution (50%) with

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 annuns* 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 I'
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 9. Planting materials used for investigating autotoxicity from their root exudates in hydroponics.

EC of 1.3 dS/m 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.

3.4.4 Growth performances of the ornamental plants grown in hydroponics

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 10). 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 approx. 85% and 74%, respectively, followed by prairie gentian with growth reduced to 55%. Root length of lily was reduced to approx. 58%, whereas that in prairie-gentian was reduced to approx. 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 et al., 1993). Asao et al. (2001a) 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 non-renewed nutrient solution. The exact reasons for this growth stimulation in the latter plants were not discovered. 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 & 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.

3.4.5 Growth and yield of prairie gentian plants when grown its replant soil

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 (Table 11). 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 12 and 13). 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 followed by a gradual decline at the highest dose of AC (480 kg/10a). This high dose of AC might have affected other chemical properties in soil. Results

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

zGrowth performance (%) = Growth in absence of AC/Growth in presence of AC × 100; ysignificant at 5% level (*), 1% level (**) and not significant (NS) by t-test (n=36); xno data.

Table 10. Growth performances of some ornamental plants grown in hydroponics in the presence or absence of activated charcoal (AC) in the nutrient solution (%)z.

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

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

Table 11. 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.

Allelochemicals	Pot marigold	Toritelia	Lily	Rocket larkspur	Sweet pea	Stock	Farewell -to- spring	Bishop's week	Snap-dragon	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 12. The compounds identified in the exudates of some ornamentals adsorbed on activated charcoal (AC) added in the nutrient solution.

Allelochemicals	Conc. (μM)	Pot marigold		Lily		Rocket larkspur		Sweet pea		Stock		Prairie gentian	
		F W shoot	D W root	F W shoot	D W root	F W shoot	D W root	F W shoot	D W root	F W shoot	D W root	F W shoot	D W root
None(control)	0	530b[z]	4.7b	1640a	63a	140a	4.4a	1210a	18a	130a	1.1a	560a	23a
Lactic acid	50	420c	3.1c	–	–	130a	3.5a	–	–	150a	1.1a	–	–
	100	420c	3.3c	–	–	140a	3.7a	–	–	120a	0.9a	–	–
	200	430c	3.4c	–	–	160a	4.5a	–	–	120a	1.1a	–	–
	400	420c	3.4c	–	–	150a	4.6a	–	–	110a	0.8a	–	–
Malonic acid	50	–	–	–	–	–	–	1340a	23a	110a	1.1a	530a	24a
	100	–	–	–	–	–	–	1320a	32a	140a	1.2a	510a	24a
	200	–	–	–	–	–	–	1330a	21a	140a	1.1a	510a	25a
	400	–	–	–	–	–	–	1070b	17b	110a	1.5a	490b	29a
Maleic acid	50	–	–	–	–	–	–	–	–	–	–	490b	17b
	100	–	–	–	–	–	–	–	–	–	–	460b	17b
	200	–	–	–	–	–	–	–	–	–	–	420b	18b
	400	–	–	–	–	–	–	–	–	–	–	390c	18b
n-Caproic acid	50	–	–	1410a	43b	–	–	–	–	–	–	530a	22a
	100	–	–	1380a	41b	–	–	–	–	–	–	580a	25a
	200	–	–	940b	34b	–	–	–	–	–	–	550a	23a
	400	–	–	850b	35b	–	–	–	–	–	–	530a	25a
Succinic acid	50	510b	4.7b	–	–	120a	4.3a	–	–	130a	1.1a	–	–
	100	490b	4.3b	–	–	160a	4.7a	–	–	120a	1.7a	–	–
	200	510b	3.7b	–	–	140a	3.9a	–	–	120a	1.2a	–	–
	400	490b	4.1b	–	–	140a	3.9a	–	–	110a	1.3a	–	–
Benzoic acid	50	470b	4.2b	810b	34b	–	–	1150b	18a	–	–	460b	19b
	100	750a	6.2a	810b	34b	–	–	1110b	18a	–	–	470b	18b
	200	530b	4.3b	800b	34b	–	–	1090b	21a	–	–	480b	17b
	400	440c	3.1c	890b	42b	–	–	1110b	21a	–	–	470b	16b
Malic acid	50	–	–	–	–	–	–	–	–	–	–	510a	22a
	100	–	–	–	–	–	–	–	–	–	–	480b	22a
	200	–	–	–	–	–	–	–	–	–	–	380c	22a
	400	–	–	–	–	–	–	–	–	–	–	390c	22a
m-Hydroxybenzoic acid	50	–	–	–	–	–	–	–	–	–	–	520a	19b
	100	–	–	–	–	–	–	–	–	–	–	510a	18b
	200	–	–	–	–	–	–	–	–	–	–	510a	18b
	400	–	–	–	–	–	–	–	–	–	–	420b	16b
p-Hydroxybenzoic acid	50	–	–	990b	28b	–	–	1330a	21a	–	–	510a	22a
	100	–	–	1170b	35b	–	–	1310a	34a	–	–	550a	23a

Allelochemicals	Conc. (µM)	Pot marigold		Lily		Rocket larkspur		Sweet pea		Stock		Prairie gentian	
		F W shoot	D W root	F W shoot	D W root	F W shoot	D W root	F W shoot	D W root	F W shoot	D W root	F W shoot	D W root
	200	–	–	1010b	31b	–	–	980b	15b	–	–	610a	26a
	400	–	–	1010b	35b	–	–	870b	16b	–	–	470b	25a
Adipic acid	50	–	–	1370a	36b	–	–	–	–	–	–	–	–
	100	–	–	1210a	28b	–	–	–	–	–	–	–	–
	200	–	–	910b	29b	–	–	–	–	–	–	–	–
	400	–	–	970b	22c	–	–	–	–	–	–	–	–
o-Hydroxyphenylacetic acid	50	–	–	–	–	110a	3.0b	–	–	–	–	–	–
	100	–	–	–	–	110a	2.8b	–	–	–	–	–	–
	200	–	–	–	–	110a	2.4b	–	–	–	–	–	–
	400	–	–	–	–	60b	2.2b	–	–	–	–	–	–
Vanillin	50	–	–	1340a	38b	–	–	–	–	–	–	–	–
	100	–	–	1310a	34b	–	–	–	–	–	–	–	–
	200	–	–	1030b	27b	–	–	–	–	–	–	–	–
	400	–	–	1010b	26b	–	–	–	–	–	–	–	–
Vanillic acid	50	–	–	–	–	140a	4.6a	1230a	19a	–	–	–	–
	100	–	–	–	–	140a	4.6a	1190a	19a	–	–	–	–
	200	–	–	–	–	120a	2.8b	1010b	23a	–	–	–	–
	400	–	–	–	–	110a	2.4b	1110b	21a	–	–	–	–

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

Table 13. Effects of the identified compounds at different concentrations on the fresh and dry weights (mg) of shoot and root of some ornamental plants.

revealed that the test plant length was increased by 96% over control as a result of the addition of AC (60 kg/10a). Shoot dry weight and root length were increased by 90% and 94%, respectively, over control for the same concentration (60 kg/10a). Flower setting was also increased at 60 kg AC/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. 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.

3.4.6 Phytotoxicity of the identified allelochemicals

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 12). 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 and root dry weight in pot marigold to 79% and 66% of control, respectively, even at low concentration (50 μM) (Table 13). Benzoic and *p*-hydroxybenzoic acid in lily, even at 50 μM, significantly reduced fresh weight to 49% and 60% of over control, *n*-caproic, benzoic, phydroxybenzoic, and adipic acid and vanillin decreased root dry weight to 68%, 54%, 44%, 57%, and 60% of control, respectively. *o*-Hydroxyphenylacetic acid at 50 μM reduced root dry weight in rocket larkspur to 68% of control (Table 13). 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 mg/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 13). So, it appears that the identified compounds would be toxic enough to affect growth of the ornamental plants by additive or synergistic effects.

We found a number of ornamental plants with autotoxic potential due to their root exudation. Growth and yield of the ornamentals under investigation were found to be improved in culture solution with AC supplementation where it used to trap the exudates. Therefore, cultivation through hydroponics enables us to isolate the responsible allelochemicals. Most of the autotoxic ornamental plants released mainly aliphatic acids or phenolic compounds to the nutrient solution. Phytotoxicity was evaluated in terms of fresh weight of shoot and dry weight of root and some compounds like lactic, benzoic acid and *p*-hydroxybenzoic acid showed phytotoxicity even at lower concentration (50 μM). The aforesaid results would be useful for sustainable production of ornament plants. We have tried AC to remove the allelochemicals from culture solution; however, more practical measures should be investigated to manage the rhizosphere free of inhibitory exudates.

4. Conclusion

Intensive and continuous culture of vegetable crops on the same land for several years causes replanting injuries like outbreak of disease and insect pest, exhaustion of soil fertility, development of chemical interference (allelopathy) leading to growth and yield reduction. Similarly in closed hydroponic system for the commercial cultivation of vegetables, autotoxicity is also evidenced. Hydroponic culture solution accumulates root exudates which in terns hamper water and mineral uptake due to root injuries. This managed culture technique has the facility of trapping and isolating the chemicals released through plant

roots. Therefore, autotoxicity phenomenon can clearly be investigated through hydroponics. Our lab aims to investigate autotoxicity in a number of vegetables and ornamental crops, to identify potential allelochemicals, their phytotoxicity through bioassay and suggest the control measures. We cultured the plants in hydroponics using plastic containers in growth chamber or in the greenhouse. Mineral nutrients were supplied as Enshi nutrient solution and nutrient concentration were adjusted throughout the culture period. Activated charcoals were supplemented in the air filters for trapping allelochemicals. Root exudates were extracted and analyzed through GC-MS. Phytotoxicity of the identified chemicals was assayed at several concentrations and potential growth inhibitors were identified for each crop. We have showed species differences in the susceptibility to autotoxicity among leaf vegetables in hydroponics. Therefore, knowledge regarding autotoxicity in vegetable crops, autotoxic chemicals, and their phytotoxicity with control measures are useful for sustainable crop production.

5. References

Asao, T.; Hasegawa, K.; Sueda, Y.; Tomita, K.; Taniguchi, K.; Hosoki, T.; Pramanik, M.H.R. & Matsui, Y. (2003). Autotoxicity of root exudates from taro. *Scientia Horticulturae*, Vol. 97, No. 3-4, pp. 389–396, ISSN 0304-4238

Asao, T.; Kitazawa, H.; Ban, T. & Pramanik, M.H.R. (2004b). Search of autotoxic substances in some leaf vegetables. *Journal of the Japanese Society for Horticultural Science*, Vol. 73, No. 3, pp. 247–249, ISSN 1347-2658 (in Japanese with English summary)

Asao, T.; Kitazawa, H.; Ban, T. & Pramanik, M.H.R. (2008). Electrodegradation of root exudates to mitigate autotoxicity in hydroponically grown strawberry (*Fragaria* × *ananassa* Duch.) plants. *HortScience*, Vol. 43, No. 7, pp. 2034-2038, ISSN 0018-5345

Asao, T.; Kitazawa, H.; Tomita, K.; Suyama, K.; Yamamoto, H.; Hosoki, T. & Pramanik, M.H.R. (2004a). Mitigation of cucumber autotoxicity in hydroponic culture using microbial strain. *Scientia Horticulturae*, Vol. 99, No. 3-4, pp. 389-396, ISSN 0304-4238

Asao, T.; Kitazawa, H.; Ushio, K.; Sueda, Y.; Ban, T. & Pramanik, M.H.R. (2007). Autotoxicity in some ornamentals with the means to overcome it. *HortScience*, Vol. 42, No. 6, pp. 1346-1350, ISSN 0018-5345

Asao, T.; Ohba, Y.; Tomita, K.; Ohta, K. & Hosoki, T. (1999d). Effects of activated charcoal and dissolved oxygen levels in the hydroponic solution on the growth and yield of cucumber plants. *Journal of the Japanese Society for Horticultural Science*, Vol. 68, No. 6, pp. 1194-1196, ISSN 1347-2658 (in Japanese with English summary)

Asao, T.; Ohtani, N.; Shimizu, N.; Umeyama, M.; Ohta, K. & Hosoki, T. (1998b). Possible selection of cucumber cultivars suitable for a closed hydroponics system by the bioassay with cucumber seedlings. *Journal of Society of High Technology in Agriculture*, Vol. 10, No. 2, pp. 92-95, ISSN 0918-6638 (in Japanese with English summary)

Asao, T.; Pramanik, M.H.R.; Tomita, K.; Ohba, Y.; Ohta, K.; Hosoki, T. & Matsui, Y. (1999a). Identification and growth effects of compounds adsorbed on activated charcoal from hydroponic nutrient solutions of cucumber. *Allelopathy Journal*, Vol. 6, No. 2, pp. 243-250, ISSN 0971-4693

Asao, T.; Pramanik, M.H.R.; Tomita, K.; Ohba, Y.; Ohta, K.; Hosoki, T. & Matsui, Y. (1999c). Influences of phenolics isolated from the nutrient solution nourishing growing

cucumber (*Cucumis sativus* L.) plants on fruit yield. *Journal of the Japanese Society for Horticultural Science*, Vol. 68, No. 4, pp. 847-853, ISSN 1347-2658 (in Japanese with English summary)

Asao, T.; Shimizu, N.; Ohta, K. & Hosoki, T. (1999b). Effect of rootstocks on the extension of harvest period of cucumber (*Cucumis sativus* L.) grown in non-renewal hydroponics. *Journal of the Japanese Society for Horticultural Science*, Vol. 68, No. 3, pp. 598-602, ISSN 1347-2658 (in Japanese with English summary)

Asao, T.; Taniguchi, H.; Suyama, K.; Yamamoto, H.; Itoh, K.; Tomita, K.; Taniguchi, K. & Hosoki, T. (2001b). Reversibility of the action of 2,4-dichlorobenzoic acid on cucumber seedlings by strains of soil microorganisms. *Journal of the Japanese Society for Horticultural Science*, Vol. 70, No. 3, pp. 393-395, ISSN 1347-2658 (in Japanese with English summary)

Asao, T.; Taniguchi, K.; Tomita, K. & Hosoki, T. (2001a). Species differences in the susceptibility to autotoxicity among leaf vegetables grown in hydroponics. *Journal of the Japanese Society for Horticultural Science*, Vol. 70, No. 4, pp. 519– 521, ISSN 1347-2658 (in Japanese with English summary)

Asao, T.; Tomita, K.; Taniguchi, K.; Hosoki, T.; Pramanik, M.H.R. & Matsui, Y. (2000). Effects of activated charcoal supplementation in the nutrient solution on the harvested fruit number of cucumbers grafted on the bloomless rootstock in hydroponics. *Journal of Society of High Technology in Agriculture*, Vol. 12, No. 1, pp. 61-63, ISSN 0918-6638 (in Japanese with English summary)

Asao, T.; Umeyama, M.; Ohta, K.; Hosoki, T.; Ito, N. & Ueda, H. (1998a). Decrease of yield of cucumber by non-renewal of the nutrient hydroponic solution and its reversal by supplementation of activated charcoal. *Journal of the Japanese Society for Horticultural Science*, Vol. 67, No. 1, pp. 99-105, ISSN 1347-2658 (in Japanese with English summary)

Atumi, K. & Nakamura, M. (1959). Studies on the soil sickness of taro plants (*Colocasia esculenta* Schott.). III. On the effect of soil sterilization on the recovering of sick soil. *Research Bulletin of Faculty of Agriculture*, Gifu University, Japan, Vol. 11, pp. 32-42 (in Japanese with English abstract)

Atumi, K. (1956). Studies on the soil sickness of taro plants (*Colocasia esculenta* Schott.). *Research Bulletin of Faculty of Agriculture*, Gifu University, Japan, Vol. 7, pp. 34-40 (in Japanese with English abstract)

Atumi, K. (1957). Studies on the soil sickness of taro plants (*Colocasia esculenta* Schott.). II. On the effect of soil sterilization on the recovering of sick soil, *Research Bulletin of Faculty of Agriculture*, Gifu University, Japan, Vol. 8, pp. 39-42 (in Japanese with English abstract)

Blum, H. (1996). Allelopathic interactions involving phenolic acids. *Journal of Nematology*, Vol. 28, No. 3, pp. 259-267, ISSN 0022-300X

Bonner, J. & Galson, A.W. (1944). Toxic substances from the culture media of guayule which may inhibit growth. *Botanical Gazette*, Vol. 106, pp. 185-198, ISSN 0006-8071

Chon, S.U.; Choi, S.K.; Jung, S.; Jang, H.G.; Pyo, B.S. & Kim, S.M. (2002). Effects of alfalfa leaf extracts and phenolic allelochemicals on early seedling growth and root morphology of alfalfa and barnyard grass. *Crop Protection*, Vol. 21, No. 10, pp. 1077-1082, ISSN 0261-2194

Chung, I.M.; Seigler, D.; Miller, D.A. & Kyung, S.H. (2011). Autotoxic compounds from fresh alfalfa leaf extracts: Identification and biological activity. *Journal of chemical ecology*, Vol. 26, No. 1, pp. 315-327, ISSN 0098-0331

Davis, E.F. (1928). The toxic principle of *Junlans nigra* as identified with synthetic jaglone and its toxic effect on tomato and alfalfa plants. *American Journal of Botany*, Vol. 15, No. 10, pp. 620–629, ISSN 00029122

Grodzinsky, A.M. (1992). Allelopathic effects of cruciferous plants in crop rotation. In: *Allelopathy: Basic and Applied Aspects*, S.J.H. Rizvi & V. Rizvi, (Ed.), 77-86, Chapman & Hall, ISBN 0 412 394006, London

Hale, M.G. & Orcutt, D.M. (1987). Allelochemical stress, In: *The physiology of plants under stress*, M.G. Hale & D.M. Orcutt, (Ed.), 117-127, John Wiley & Sons, ISBN 0471031526, 9780471031529, New York

Hao, Z.P.; Wang, Q.; Christie, P. & Li, X.L. (2007). Allelopathic potential of watermelon tissues and root exudates. *Scientia Horticulturae*, Vol. 112, No. 3, pp. 315-320, ISSN 0304-4238

Hartung, A.C.; Nair, M.G. & Putnum, A.R. (1990). Isolation and characterization of phytotoxic compounds from asparagus *(Asparagus officinalis* L.) roots. *Journal of Chemical Ecology,* Vol. 16, No. 5, pp. 1707-1718, ISSN 0098-0331

Hegde, R. & Miller, D.A. (1990). Allelopathy and autotoxicity in alfalfa: characterization and effects of preceding crops and residue incorporation. *Crop Science*, Vol. 30, No. 6, pp. 1255-1259, ISSN 0011-183X

Hirano, T. (1940). Studies on soil sickness of tomatoes. *Japanese Journal of Soil Science and Plant Nutrition*, Vol. 14, pp. 521-530, ISSN 1747-0765 (in Japanese).

Hori, H. (1966). *Gravel culture of vegetables and ornamental crops*. pp. 60-80, Youkendo Co. Ltd., OCLC No. 673083780, Tokyo, Japan (in Japanese)

Inderjit & Weston, L.A. (2003). Root exudates: an overview. In: *Root ecology*, de Kroon, H. & E.J.W. Visser, (Ed.), 235-255, , Ecological Studies 168, Springer, ISBN 3-540-00185-9, Verlag Berlin, Heidelberg, New York

Inderjit, (1996). Plant phenolics in allelopathy. *Botanical Review*, Vol. 62, No. 2, pp. 186-202, ISSN 0006-8101

Kaul, K. (2000). Autotoxicity in *Tagets erecta* L. on its own germination and seedling growth. *Allelopathy Journal*, Vol. 7, pp. 109-113, ISSN 0971-4693

Kitazawa, H.; Asao, T.; Ban, T.; Hashimoto, Y. & Hosoki, T. (2007). 2,4-D and NAA supplementation mitigates autotoxicity of strawberry in hydroponics. *Journal of Applied Horticulture*, Vol.9, No. 1, pp. 26-30, ISSN 0972-1045

Kitazawa, H.; Asao, T.; Ban, T.; Pramanik, M.H.R. & Hosoki, T. (2005). Autotoxicity of root exudates from strawberry. *The Journal of Horticultural Science & Biotechnology*, Vol. 80, No. 6, pp. 677-680, ISSN 1462-0316

Koda, T.; Ogiwara, S. & Hiroyasu, T. (1977). Effects of addition of activated charcoal into the nutrient solution on the growth of mitsuba (*Cryptotaenia japonica* Hassk.) in hydroponics. *Journal of the Japanese Society for Horticultural Science*, Vol. 46 (Supplement 1), pp. 270-271, ISSN 1347-2658 (in Japanese with English summary)

Koda, T.; Ogiwara, S.; Udagawa, Y. & Hiroyasu, T. (1980). Effects of addition of activated charcoal into the nutrient solution on the growth of mitsuba (*Cryptotaenia japonica* Hassk.) in hydroponics. 2. Effects of organic acids on the growth of mitsuba and its

removal. *Journal of the Japanese Society for Horticultural Science*, Vol. 49 (Supplement 2), pp. 224-225, ISSN 1347-2658 (in Japanese with English summary)

Komada, H. (1988). The occurrence, ecology of soil-borne diseases and their control. Takii Seed Co. Ltd., Japan (in Japanese)

Koshikawa, K. & Yasuda, M. (2003). Studies on the bench culture with closed hydroponic system in strawberry (Part 1). *Journal of the Japanese Society for Horticultural Science*, Vol. 72 (Supplement 2), pp. 394, ISSN 1347-2658 (in Japanese with English summary)

Kushima, M.; Kakuta, H.; Kosemura, S.; Yamamura, S.; Yamada, S.; Yokotani-Tomita, K. & Hasegawa, K. (1998). An allelopathic substance exuded from germinating watermelon seeds. *Plant Growth Regulation,* Vol. 25, No. 1, pp. 1-4, ISSN 0167-6903

Lee, J.G.; Lee, B.Y. & Lee, H.J. (2006). Accumulation of phytotoxic organic acids in reused nutrient solution during hydroponic cultivation of lettuce (*Lactuca sativa* L.). *Scientia Horticulturae*, Vol. 110, No. 2, pp. 119-128, ISSN 0304-4238

Markus, A.; Klages, U.; Krauss, S. & Lingens, F. (1984). Oxidation and dehalogenation of 4-chlorophenylacetate by a two-component enzyme system from *Pseudomonas* sp. strain CBS3. *Journal of Bacteriology*, Vol. 160, No. 2, pp. 618-621, ISSN 0021-9193

Matsumoto, M.; Yoshioka, K.; Sumida, S.; Kondo, T.; Takahashi, S.; Nanjo, H. & Manabe, Y. (1973). Harmful effects of consecutive planting of taros on their growth in Ehime prefecture, Japan. *Proceedings of Association for Plant Protection of Shikoku, Japan,* Vol. 8, pp. 57-63, (in Japanese)

Matsumoto, M.; Yoshioka, K.; Sumida, S.; Kondo, T.; Tanbara, K. & Kurihara, H. (1974). Seasonal prevalence of *Pratylenchus coffeae* (Zimmerman.) in taro fields. *Proceedings of Association for Plant Protection of Shikoku, Japan*, Vol. 9, pp. 41-47, (in Japanese)

Miller, D.A. (1983). Allelopathic effects of alfalfa. *Journal of Chemical Ecology*, Vol. 9, No. 8, pp. 1059-1072, ISSN 0098-0331

Miyaji, T. & Shirazawa, N. (1979). Researches on the injuries of continuous cropping and those countermeasures in taro plant. *Bulletin of Kagoshima Agricultural Research Centre*, Vol. 7, pp. 5-15 (in Japanese)

Miyoshi, S.; Yamada, T. & Yoshika, S. (1971a). Studies on soil-borne diseases of taro-plant by continuous cropping. (1) Appearances of diseases by continuous cropping and its control effect of cultivating by alternation of land usage between dry and flooded conditions. *Bulletin of Fukuoka Agricultural Research Centre*, Vol. 9, pp. 45-48 (in Japanese)

Mizutani, F.; Hirota, R. & Kadoya, K. (1988). Growth inhibiting substances from peach roots and their possible involvement in peach replant problems. *Acta Horticulturae*, Book No. 233, pp. 37-43, ISSN 0567-7572, ISBN 978-90-66053-13-7

Murota, M.; Sakamoto, Y.; Tomiyama, K.; Ozaki, M.; Manabe, M.; Iwahashi, T. & Fukukawa, T. (1984). Actual conditions of taro planting in the main producing districts of Miyazaki prefecture and some methods to prevent injury by continuous cropping. *Bulletin of Miyazaki Agricultural Experimental Station*, pp. 39-53 (in Japanese with English abstract)

Nagae, S.; Samesima, T. & Watanabe, B. (1971). Studies on the root rot of taro (I). The causal agents of taro root rot. *Kyusyu Agricultural Research*, pp. 33-90 (in Japanese)

Nakahisa, K.; Tsuzuki, E. & Mitsumizo, T. (1993). Study on the allelopathy of alfalfa (*Medicago sativa* L.). I. Observation of allelopathy and survey for substances

inducing growth inhibition. *Japanese Journal of Crop Science*, Vol. 62, No. 2, pp. 294-299, ISSN 0011-1848

Nakahisa, K.; Tsuzuki, E.; Terao, H. & Kosemura, S. (1994). Study on the allelopathy of alfalfa (*Medicago sativa* L.). II. Isolation and identification of allelopathic substances in alfalfa. *Japanese Journal of Crop Science*, Vol. 63, No. 2, pp. 278-284, ISSN 0011-1848

Nanbu, K. (1990). Fate of pesticides in soil. *Plant Protection*, Vol. 11, pp. 477-482 (in Japanese)

Oashi, K. (1973). On the damages of taro caused by *Pratylenchus coffeae* (Zimmerman.) and their chemical control. *Kyusyu Agricultural Research*, Vol. 35, pp. 103-104 (in Japanese)

Oka, S. (2002). Development of the labor-saving cultivation techniques by raising the labor-saving cultivars of vegetables (Part 1). *Bulletin of the National Agricultural Research Center for Western Region*, pp. 26-27 (in Japanese)

Overland, L. (1966). The role of allelopathic substances in the "smother crops" barley. *American Journal of Botany*, Vol. 53, No. 5, pp. 423-432, ISSN 00029122

Pardales, J.R.Jr. & Dingal, A.G. (1988). An allelopathic factor in taro residues. *Tropical Agriculture*, Vol. 65, No. 1, pp. 21-24, ISSN 0041-3216

Petrova, A.G. (1977). Effect of phytoncides from soybean, gram, chickpea and bean on uptake of phosphorus by maize. In: *Interaction of Plants and Microorganisms in Phytocenoses,* A.M. Grodzinsky, (Ed.), 91-97, Naukova Dumka, Kiev (in Russian, English summary).

Pramanik, M.H.R.; Asao, T.; Yamamoto, T. & Matsui, Y. (2001). Sensitive bioassay to evaluate toxicity of aromatic acids to cucumber seedlings. *Allelopathy Journal*, Vol. 8, No. 2, pp. 161-170, ISSN

Pramanik, M.H.R.; Nagai, M.; Asao, T. & Matsui, Y. (2000). Effects of temperature and photoperiod on phytotoxic root exudates of cucumber (*Cucumis sativa*) in hydroponic culture. *Journal of Chemical Ecology*, Vol. 26, No. 8, pp. 1953-1967, ISSN 0098-0331

Putnam, A.R. (1985a). Allelopathic research in agriculture: Past highlights and potential. In: *The Chemistry of allelopathy: Biochemical interactions among plants*, A.C. Thompson, (Ed.), pp. 1-8, American Chemical Society, ISBN13: 9780841208865, Washington, D.C.

Rice, E.L. (1984). Allelopathy (Second Edition). Academic Press, ISBN 0125870558, 9780125870559, Orlando, FL

Rizvi, S.J.H. & Rizvi, V. (1992). Allelopathy: Basic and Applied Aspects. pp. 480, Chapman & Hall, ISBN 0 412 39400 6, London

Ruijs, M.N.A. (1994). Economic evaluation of closed production systems in glasshouse horticulture. *Acta Horticulturae*, Book No. 340, pp. 87-94, ISSN 0567-7572, ISBN 978-90-66054-25-7

Sato, N. (2004) Effect of the substances accumulated in the nutrients solution by the rock wool circulated hydro culture to the rose seedlings growth. *Journal of the Japanese Society for Horticultural Science*, Vol. 73 (Supplement 2), pp. 497, ISSN 1347-2658 (in Japanese)

Shafer, W.E. & Garrison, S.A. (1986). Allelopathic effects of soil incorporated asparagus roots on lettuce, tomato, and asparagus seedling emergence. *HortScience*, Vol. 21, No. 1, pp. 82-84, ISSN 0018-5345

Singh, H.P.; Batish, D.R. & Kohli, R.K. (1999). Autotoxicity: concept, organisms and ecological significance. *Critical Review on Plant Science*, Vol. 18, No. 6, pp. 757-772, ISSN 0735-2689

Sunada, K.; Ding, X.G.; Utami, M.S.; Kawashima, Y.; Miyama, Y. & Hashimoto, K. (2008). Detoxification of phytotoxic compounds by TiO_2 photocatalysis in a recycling hydroponic cultivation system of asparagus. *Journal of Agricultural and Food Chemistry,* Vol. 56, No. 12, pp. 4819-4824, ISSN 0021-8561

Sundin, P. & Waechter-Kristensen, B. (1994). Degradation of phenolic acids by bacteria from liquid hydroponic culture of tomato. In: *Plant Production on the Threshold of a New Century,* P.C. Struik; W.J. Vredenverg; J.A. Renkama, & J.E. Parlevliet, (Ed.), 473-475, Kluwer Academic Publishers, Dordrecht, ISBN 0 7923 2903 1, The Netherlands

Takahashi, K., (1984). The replant failures of vegetables. *Research Reports of National Research Institute of Vegetable and Tea Science*, Japan, Vol. 18, pp. 87-99 (in Japanese)

Takeuchi, T. (2000). The nourishment uptake of strawberry cultivar 'Akihime' in rockwool hydroponics with a nutrient solution circulating system. *Bulletin of the Shizuoka Agricultural Experiment Station*, Vol. 45, pp. 13-23 (in Japanese with English summary)

Tang, C.S. & Young, C.C. (1982). Collection and identification of allelopathic compounds from the undisturbed root system of bitalta limpograss (*Helmarthria altissima*). *Plant Physiology*, Vol. 69, No. 1, pp. 155-160, ISSN 0032-0889

Tsuchiya, K. & Ohno, Y. (1992). Analysis of allelopathy in vegetable cultivation. I. Possibility of occurrence of allelopathy in vegetable cultivation. *Bulletin of the National Research Institute of Vegetable, Ornamental Plants and Tea Science*, Vol. 5, pp. 37-44 (in Japanese with English abstract)

Tsuchiya, K. (1990). Problems on allelopathy in vegetable cropping. *Agriculture and Horticulture*, Vol. 65, No. 1, pp. 9-16, ISSN 0369-5247 (in Japanese)

Tsuzuki, E.; Shimazaki, A.; Naivalulevu, L.U. & Tomiyama, K. (1995). Injury by continuous cropping to taro and its related factors. *Japanese Journal of Crop Science*, Vol. 64, No. 2, pp. 195-200, ISSN 0011-1848

Tukey, H.B., Jr. (1969). Implications of allelopathy in agricultural plant science. *Botanical Review*, Vol. 35, No. 1, pp. 1-16, ISSN 0006-8101

van den Tweel, W.J.J.; Kok, J.B. & de Bont, J.A. (1987). Reductive dechlorination of 2,4-dichlorobenzoate to 4-chlorobenzoate and hydrolytic dehalogenation of 4-chloro, 4-bromo-, and 4-iodobenzoate by *Alcaligenes denitrificans* NTB-1. *Applied Environmental Microbiology*, Vol. 53, No. 4, pp. 810-815, ISSN 0099-2240

Van Os, E.A. (1995). Engineering and environmental aspects of soilless growing systems. *Acta Horticulturae*, Book No. 396, pp. 25-32, ISSN 0567-7572, ISBN 978-90-66053-13-7

Yang, H.J. (1982). Autotoxicity of *Asparagus officinalis* L. *Journal of the American Society for Horticultural Science*, Vol. 107, No. 5, pp. 860-862, ISSN 0003-1062

Young, C.C. & Chou, T.C. (1985). Autointoxication in residues of *Asparagus officinalis* L. *Plant and Soil*, Vol. 85, No. 3, pp. 385-393, ISSN 0032-079X

Young, C.C. (1984). Autotoxication in root exudates of *Asparagus officinalis* L. *Plant and Soil*, Vol. 82, No. 2, pp. 247-253, ISSN 0032-079X

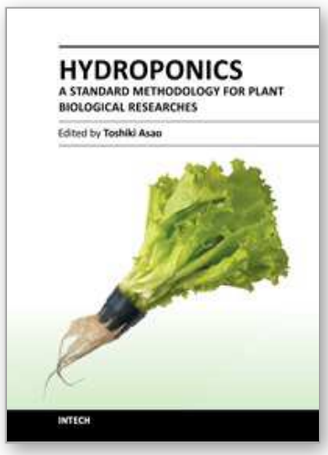
Yu, J.Q & Matsui, Y. (1994). Phytotoxic substances in root exudates of *Cucumis sativus* L. *Journal of Chemical Ecology*, Vol. 20, No. 1, pp. 21-31, ISSN 0098-0331

Yu, J.Q. & Matsui, Y. (1993a). Effect of addition of activated charcoal to the nutrient solution on the growth of tomato grown in the hydroponic culture, Soil Science and Plant Nutrition, Vol. 39, No. 1, pp. 13-22, ISSN 1747-0765

Yu, J.Q. & Matsui, Y. (1993b). Extraction and identification of the phytotoxic substances accumulated in the nutrient solution for the hydroponic culture of tomato, *Soil Science and Plant Nutrition*, Vol. 39, No. 4, pp. 691-700, ISSN 1747-0765

Yu, J.Q. & Matsui, Y. (1997). Effects of root exudates of cucumber (*Cucumis sativus*) allelochemicals on ion uptake by cucumber seedlings. *Journal of Chemical Ecology*, Vo. 23, No. 3, pp. 817-827, ISSN 0098-0331

Yu, J.Q.; Lee, K.S. & Matsui, Y. (1993). Effect of addition of activated charcoal to the nutrient solution on the growth of tomato grown in the hydroponic culture. *Soil Science and Plant Nutrition*, Vol. 39, No. 1, pp. 13-22, ISSN 1747-0765



Hydroponics - A Standard Methodology for Plant Biological Researches

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Hydroponics-A standard methodology for plant biological researches provides useful information on the requirements and techniques needs to be considered in order to grow crops successfully in hydroponics. The main focuses of this book are preparation of hydroponic nutrient solution, use of this technique for studying biological aspects and environmental controls, and production of vegetables and ornamentals hydroponically. The first chapter of this book takes a general description of nutrient solution used for hydroponics followed by an outline of in vitro hydroponic culture system for vegetables. Detailed descriptions on use of hydroponics in the context of scientific research into plants responses and tolerance to abiotic stresses and on the problems associated with the reuse of culture solution and means to overcome it are included. Some chapters provides information on the role of hydroponic technique in studying plant-microbe-environment interaction and in various aspects of plant biological research, and also understanding of root uptake of nutrients and thereof role of hydroponics in environmental clean-up of toxic and polluting agents. The last two chapters outlined the hydroponic production of cactus and fruit tree seedlings. Leading research works from around the world are brought together in this book to produce a valuable source of reference for teachers, researcher, and advanced students of biological science and crop production.

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