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Autotoxicity in beans and their allelochemicals

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ABSTRACT

The autotoxicity of *Pisum sativum*, *Phaseolus vulgaris*, and *Vicia faba* were investigated in hydroponics either with or without activated charcoal (AC) addition. Growth and yield of the three beans were significantly reduced when grown in the culture solution without AC addition. In *P. sativum* plants grown in non-renewed culture solution without AC, the number of pods, pod fresh mass, number of seeds, and seed fresh mass were reduced by about half compared with those with AC. The number of pods plant^{-1} and fresh mass of pods^{-1} plant in *P. vulgaris*, as well as pod number in *V. faba*, were decreased significantly to 49–67% without AC addition. The identified allelochemicals were benzoic, salicylic, and malonic acids in the root exudates of *P. vulgaris* and lactic, benzoic, *p*-hydroxybenzoic, vanillic, adipic, succinic, malic, glycolic, and *p*-hydroxyphenylacetic acids in *V. faba*. Bioassay of the identified allelochemicals revealed that benzoic, salicylic, and malonic acids significantly reduced the growth of *P. vulgaris* even at low concentrations. In *V. faba*, benzoic acid at 50 μM significantly reduced root length, and shoots fresh and dry mass by over 81% of those of the control, whereas adipic and *p*-hydroxyphenylacetic acids decreased root length to 87 and 88% of that of the control, respectively.

1. Introduction

Beans are grain legumes that belong to the family Leguminosae, which includes food and forage legumes. Bean plants are cultivated primarily for their seeds, which are harvested at maturity and are rich in protein and energy. They are used either for animal feed or for human consumption. The major grain legumes are *Pisum sativum*, *Vicia faba*, *Lens culinaris*, *Glycine max*, *Phaseolus vulgaris*, *Lupinus* spp., and *Cicer arietinum*. These grain legumes are generally intercropped with cereals to enhance crop yield, increase nitrogen use efficiency, and reduce weed infestation and the occurrence of plant disease (Willey, 1979; Jensen, 1996; Hauggaard-Nielsen et al., 2001, 2008). Among the grain legumes, some edible beans are used as vegetables and intensively cultivated in the same farmland year after year. The production of these common bean plants and other perennial legumes declines in replanting conditions owing to autotoxicity, a form of intraspecific allelopathy that occurs when a plant species releases chemical substances that inhibit or delay germination and growth of the same plant species (Putnam, 1985; Miller, 1996; Singh et al., 1999). Allelopathy has been investigated in some beans such as in *P. sativum* (Kato-Noguchi, 2003), *Mucun pruriens* (Fujii et al., 1991), *Glycine max* (Huber and Abney, 1986; Xiao et al., 2006; Yan and Yang, 2008), and *Cicer arietinum* (Yasmin et al., 1999). L-DOPA and cynamidine has been found to be potential allelochemicals identified in *Mucuna pruriens* and *Vicia villosa*, respectively (Fujii, 2003). It has been found that, in addition to common beans, several other species within the Leguminosae family contain secondary plant products that have allelopathic potential (Rice, 1984). In field experiments, it has been reported that residues and extracts of pea plants suppressed the growth and population size of several plant species (Purvis, 1990; Schenk and Werner, 1991; Tsuchiya and Ohno, 1992; Akemo et al., 2000). Phytotoxic substances in *P. sativum* root exudates have been reported by several researchers (Hatsuda et al., 1963; Yu and Matsui, 1999) and, recently, pisatin has been identified as an inhibitory chemical from its shoots (Kato-Noguchi, 2003). Aqueous leachates of dry shoot of *P. vulgaris* that contain phenolics showed allelopathic effects on several crop species (Nava-Rodríguez et al., 2005). Autotoxicity due to root exudates found to be involved in growth reduction in *Glycine max* monocropping, which decreased plant biomass and root triphenyl tetrazolium chloride-reducing activity as well as seedlings after exposure to root exudates, exhibited higher activities of superoxide dismutase and guaiacol peroxidase (Xiao et al., 2006).

Successive culture of the same crop on the same land for years cause soil sickness or replanting injuries (Hirano, 1940; Bonner and

Abbreviations: AC, activated charcoal; EC, electrical conductivity; h, hour; GC–MS, gas chromatography–mass spectrometry; M, molar; DE, diethyl ether; EA, ethyl acetate; EI, electron impact; FM, fresh mass; DM, dry mass; HPLC, high performance liquid chromatography; rpm, revolutions per minute; μM, micro molar.

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Galson, 1944; Tsuchiya, 1990) resulting reduction in both crop yield and quality. This phenomenon is evidenced in agricultural cropping system especially in the production of horticultural crops (Young, 1984; Grodzinsky, 1992). It leads to the resurgence of disease pest, exhaustion of soil fertility, and developing chemical interference in the rhizosphere referring to allelopathy (Takahashi, 1984; Young, 1984; Hegde and Miller, 1990). Similar to successive culture, in closed hydroponics phytotoxic chemicals accumulated in the culture solution leading to the occurrence of autotoxicity and it was investigated in *Cucumis sativus* (Yu and Matsui, 1994, 1997), *Citrullus lanatus* (Kushima et al., 1998; Hao et al., 2007), *Colocasia esculenta* (Asao et al., 2003), *Fragaria ananassa* (Kitazawa et al., 2005), *Solanum lycopersicum* (Yu and Matsui, 1993), and *Lactuca sativa* (Lee et al., 2006). In this phenomenon, root exudates hamper the plant growth mainly by hampering water and mineral uptake. Previous studies have shown that allelochemicals released from plant roots play an important role in replant injuries of crops. Autotoxicity of root exudates is an important feature for understanding replanting problems in agroecosystem as it represents one of the largest direct inputs of allelochemicals into the rhizosphere environment with potent biological activity and great variation in chemical components (Inderjit and Weston, 2003). 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 (Pramanik et al., 2000; Inderjit and Weston, 2003). The removal of the inhibitory chemicals from soils or culture solution can permit continued crop cultivation in the same land for years. Hydroponic culture technique has the facility of trapping and isolating the chemicals released through plant roots. Elimination of these growth inhibitors from recycling culture solution is desirable from the viewpoint of conservation-oriented agriculture. Therefore, many researchers suggested addition of AC to the culture solution to improve growth and yield significantly by adsorbing organic compounds (mainly phenolics), for example in *Fragaria ananassa* (Kitazawa et al., 2005), *Colocasia esculenta* (Asao et al., 2003), *Cucumis sativus* (Yu and Matsui, 1994; Asao et al., 1998a, 1999, 2000), several leafy vegetables (Asao et al., 2004), and some ornamentals (Asao et al., 2007a).

The study of autotoxicity in commonly grown beans would provide useful knowledge of sustainable crop production. Thus, identification of the allelochemicals from bean root exudates, evaluation of their phytotoxicity, and their removal would facilitate the maintenance of profitable crop production. Previously, we found evidence of autotoxicity in *Lathyrus ordoratus*, a leguminous crop (Asao et al., 2007b); in this study, we investigated autotoxicity in three beans, namely, *P. sativum*, *P. vulgaris*, and *V. faba* as well as their allelochemicals, using hydroponic culture. The phytotoxicity of the identified allelochemicals was evaluated using seedling growth bioassay of the test plants.

2. Materials and methods

2.1. Plant materials

Bean plants viz. *P. sativum* cv. Kurume-yutaka, *P. vulgaris* cv. Taibyou-morokko, and *V. faba* cv. Nintoku-1-sun were used in this experiment.

2.2. Plant cultivation either with or without AC

Seeds of the beans under study were germinated on vermiculite on a plastic tray with tap water. The seedlings were transplanted to plastic containers (50 cm × 60 cm × 21 cm) in the greenhouse of Shimane University (Fig. 1). Twelve plants were planted in each container and three containers were used for each treatment (plants either with or without AC) following a randomized block design. The container was filled with 50 l of 75% Enshi nutrient solution with electrical conductivity (EC) of 2.0 dS m^{-1} (Hori, 1966). Full-strength nutrient solution contains the following amounts of salts 1000 l^{-1} of tap water: 950 g of $Ca(NO_3)_2{\cdot}4H_2O$; 810 g of KNO_3; 500 g of $MgSO_4{\cdot}7H_2O$; 155 g of $NH_4H_2PO_4$; 3 g of H_3BO_3; 2 g of $ZnSO_4{\cdot}7H_2O$; 0.05 g of $CuSO_4{\cdot}5H_2O$; 0.02 g of $NaMoO_4$; and 25 g of NaFe-EDTA. The nutrient solution in the containers were continuously aerated (3.8 l min^{-1}) 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 °C for later extraction. $FeSO_4{\cdot}7H_2O$ (0.75 g) was added to each 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. 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). The pH of the nutrient solutions ranged from 5.7 to 7.1 irrespective of either with or without AC addition. At the end of the experiment, plant length, fresh and dry mass of shoots, dry mass of roots, root length, numbers of pods and seeds, and fresh mass of pods and seeds were recorded.

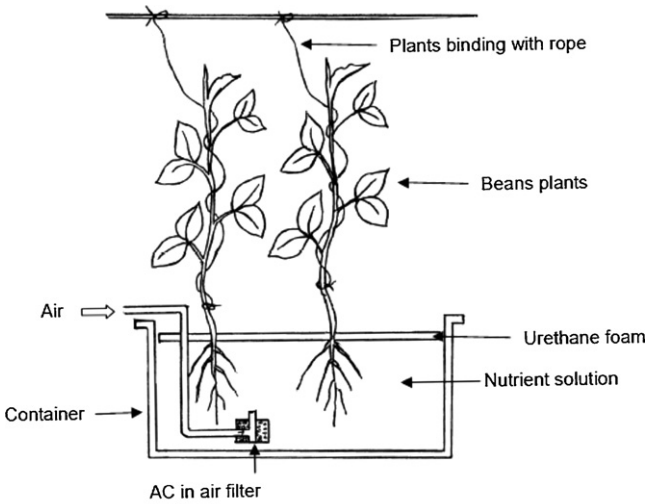

Fig. 1. Hydroponic system used for bean plant cultivation.

2.3. GC–MS analysis of root exudates adsorbed in AC

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 °C) 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 °C. Organic compounds in the concentrate were then extracted according to Yu and Matsui

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Table 1
Growth and yield of three beans either with or without AC addition in hydroponics.

Beans	AC[a]	Plant length (cm)	FM[b] of shoot (g)	DM of shoot (g)	Root length (cm)	DM of root (g)	No. of pod plant^{-1}	FM of pods plant^{-1} (g)	No. of seeds plant^{-1}	FM of seeds plant^{-1} (g)
Pisum sativum	–	176.2	512.1	141.0	–[d]	41.2	36.2	288.9	199.5	118.5
	+	223.0	838.8	211.2	–	70.3	69.9	557.0	374.5	220.8
		*[c]	*	*		*	**	**	**	**
Phaseolus vulgaris	–	39.4	56.1	9.7	72.4	3.89	9.8	74.3	–	–
	+	48.0	78.4	14.0	81.7	5.85	14.3	116.3	–	–
		**	**	**	**	**	**	**		
Vicia faba	–	74.1	73.4	13.4	57.3	6.0	3.9	–	–	–
	+	88.1	96.2	17.5	60.6	6.2	7.9	–	–	–
		**	*	*	NS	NS	*			

[a] AC added (+), non-AC (–).
[b] Fresh mass (FM), dry mass (DM).
[c] Significant at the 1% (**) and 5% levels (*), and not significant (NS) by *t*-test.
[d] No data.

(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 °C. 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 °C. 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 min and then raised at 5 °C min^{-1} to a final temperature of 260 °C for 10 min. 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

The bioassay was carried out according to the method of Asao et al. (1998b). In nature, plant roots exudates allelochemical at a low concentration in the rhizosphere and it depends greatly on the environmental factors. In case of *Cucumis sativus*, the exudation rates varied markedly with the kind of acids, temperatures, and photoperiods, ranging from 0.2 to 4.17 mg day^{-1} plant^{-1} (Pramanik et al., 2000). We prepared a series of aqueous solutions of the identified allelochemicals at concentration of 0 (control), 50, 100, 200 and 400 μM with a 75% Enshi nutrient solution (EC 2.0 dS m^{-1}). To determine the most inhibitory concentration, high concentrations (400 μM) of the allelochemicals were bioassayed for their phytotoxicity. The inhibitions of the test solution were assayed by their effect on bean seedlings. Each treatment was replicated 10 times. Test solutions were added to 420 ml flasks wrapped with black polyethylene to avoid direct light on the roots of test plants. The selected plants were transplanted to each flask with urethane foam as support. We planted the bean plants in such a way that roots were inserted into the nutrient solution inside the flask keeping the shoot outside. Urethane foam blocks were used for holding the plants tight and upright at the neck of the flask. The planted flask was placed in a growth chamber at 25 °C with a light intensity of 74–81 μmol s^{-1} m^{-2} and 16 h photoperiod. To minimize the effect of aeration and the microbial degradation of organic compounds (Sundin and Waechter-Kristensen, 1994) on the bioassay, we renewed the test solutions in the planted flask at every 3 or 4 days. The plants were grown for two weeks and then the number of leaves, maximum leaf length and width, plant length, root length, the fresh and dry mass of shoots, and dry mass of roots were measured.

2.5. Statistical analysis

The growth and yield data obtained from bioassay and hydroponics of bean plants were compiled and analyzed for statistical differences among the treatments and means were separated by the analysis of variation with Tukey–Kramer test and *t*-test (Statcel 2 Software, OMS Publication, Tokorozawa, Saitama, Japan) at $P < 0.05$.

3. Results and discussion

3.1. Growth and yield of Pisum sativum, Phaseolus vulgaris, and Vicia faba in hydroponics

The growth and yield of the above three beans were significantly affected by the AC addition in the culture solution (Table 1). In *P. sativum*, the plant length, shoot fresh mass, shoot dry mass, and root dry mass declined significantly in the plants grown without AC compared with those grown with AC. The reductions were about 79%, 61%, 67%, and 59% of the values without AC addition, respectively. Similarly, the addition of AC to the nutrient solution greatly improved growth in *P. sativum* (Yu and Matsui, 1999). In *P. vulgaris*, growth was severely retarded in terms of the plant length, shoot fresh mass, shoot dry mass, and root length and dry mass (reductions of about 82, 72, 69, 89, and 66%, respectively), when cultivated without AC. A similar pattern of reduction in growth parameters of *V. faba* also appeared. However, there was no significant difference in root length and root dry mass between plants cultivated with and without AC addition. Growth inhibition of the beans without AC addition might be due to the endogenous chemicals from root exudates in the culture solution. In a previous study, the aqueous extracts from leguminous crop residues found to be phytotoxic to crop plants, the aqueous extracts of alfalfa shoots, reduced the germination of alfalfa to 35% and radish to 80% at higher concentrations (Nakahisha et al., 1993). In addition, alfalfa plant extracts significantly affected root growth and morphological differentiation of alfalfa and barnyard grass with increasing concentration, resulting in the reduction of their biomass in the presence of either autotoxic or allelopathic compounds (Chon et al., 2002). In other studies, growth inhibition was recovered by the supplementation of AC to nutrient solution or soil, which removed the phytotoxic chemicals

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from root exudates of *Cucumis sativus* and *Lomandra longifolia* (Asao et al., 1999, 2007b).

The yields of the bean plants under investigation were significantly affected by the non-renewed nutrient solution either with or without AC addition (Table 1). In control (without AC) plants, the number of pods, their fresh mass, the number of seeds, and fresh mass of seeds in *P. sativum* were reduced by half (to about 52%, 52%, 53%, and 54% of the values with AC, respectively). However, the number of pods and pod fresh mass in *P. vulgaris*, and the number of pods in *V. faba* were decreased significantly to about 67%, 64%, and 49%, respectively in without AC supplementation compared to with AC. The results showed that the addition of AC in nutrient solution improved the yield of the beans by adsorbing organic compounds or allelochemicals released by roots. Activated carbon was found to adsorb allelopathic plant exudates in the soil with only small effects on soil nutrients (Callaway and Aschehoug, 2000). Decrease of yield in the non-renewed nutrient solution and its reversal by supplementation of AC were found in *Cucumis sativa* (Asao et al., 1998a), *Colocasia esculenta* (Asao et al., 2003), and *Fragaria ananassa* (Kitazawa et al., 2005) hydroponics. By its very large surface-to-volume ratio, AC has long been known as an adsorbent of organic compounds in soils (Zackrisson et al., 1996), and has been used in greenhouse and field experiments (Prati and Bossdorf, 2004; Callaway et al., 2005; Kulmatiski and Beard, 2006). In laboratory conditions, addition of AC to the medium for plant tissue cultures was found to improve growth by adsorbing toxic metabolites (Wang and Huang, 1976). In field experiments, activated carbon has been shown to adsorb phenols released by *Empetrum hermaphroditum* vegetation and to eliminate the inhibitory effects of *E. hermaphroditum* on tree seedling establishment and growth (Zackrisson and Nilsson, 1992; DeLuca et al., 2002; Thoss et al., 2004).

3.2. Allelochemicals in the root exudates of Phaseolus vulgaris and Vicia faba

Root exudates from *P. vulgaris* and *V. faba* were analyzed and a number of compounds were detected (Table 2). Benzoic, salicylic, and malonic acids were identified in the root exudates of *P. vulgaris* whereas lactic, benzoic, *p*-hydroxybenzoic, vanillic, adipic, succinic, malic, glycolic, and *p*-hydroxyphenylacetic acids were detected in the root exudates of *V. faba*. In *V. faba*, it has been found that phenolic compounds were rapidly released from the emerging root and the amount of phenolics in exudates peaked the first day after seed germination (Bekkara et al., 1998); in another study, salicylic acid was identified as an allelochemical from its root exudates (Schulz and Friebe, 1999). Benzoic, cinnamic, *p*-hydroxybenzoic, 3,4-dihydrobenzoic, vanillic, *p*-coumaric, and sinapic acids were identified from the phytotoxic acidic fraction of the root exudates of *P. sativum* (Yu and Matsui, 1999). Several investigations have been conducted to identify the allelochemicals responsible for autotoxicity in *Glycine max*. Exogenously supplied *p*-coumaric acid (≥0.25 μM) induced premature cessation of root growth, and increased peroxidase activity and lignin content in it (Zanardo et al., 2009). Allelochemicals like benzoic, vanillic, cinnamic, and ferulic acids showed inhibition in P uptake of *Glycine max* (Baziramakenga et al., 1997); likewise, benzoic acid and *trans*-cinnamic acid reduced root and shoot dry biomass, lowered the amounts of P, K, Mg, Mn, Cl^-, and SO_4^{2-} and reduced leaf chlorophyll content (Baziramakenga et al., 2005). The germinating seeds were responsible for a large portion of the total aliphatic and aromatic acid exudation of seedling plant grown aseptically for 14 days and lactic acid was the predominant aliphatic acid detected in *P. sativum* and *Hordeum vulgare* root exudates, whereas malic acid was the predominant acid found in *P. sativum*, *Gossypium* spp. and *Hordeum vulgare* seed exudates (Kovacs, 1971).

Table 2
The allelochemicals identified in the exudates of *Phaseolus vulgaris* and *Vicia faba* adsorbed on AC added in the nutrient solution.

Allelochemicals	*Phaseolus vulgaris*	*Vicia faba*
Lactic acid	–[a]	+
Benzoic acid	+	+
p-Hydroxybenzoic acid	–	+
Vanillic acid	–	+
Adipic acid	–	+
Succinic acid	–	+
Malic acid	–	+
Glycolic acid	–	+
Salicylic acid	+	+
Malonic acid	+	–
p-Hydroxyphenylacetic acid	–	+

[a] Detected (+) and not detected (–).

3.3. Bioassay with the identified allelochemicals

Seedling growth bioassays were carried out to evaluate the allelopathic potential of the identified chemicals at several concentrations. The phytotoxic effects of the allelochemicals were assayed for growth parameters of *P. vulgaris* and *V. faba* at several concentrations (Tables 3 and 4). In *P. vulgaris* grown in benzoic acid solution, the number of leaves, maximum leaf width, shoot fresh mass, and shoot dry mass were significantly reduced to 67, 83, 78, and 84% compared with those of the control, respectively, even at a low concentration (50 μM). Salicylic and malonic acids decreased

Table 3
Effects of the identified phenolic acids at different concentrations on the growth of *Phaseolus vulgaris*.

Allelochemicals	Conc. (μM)	No. of leaves plant^{-1}	Max. leaf length (mm)	Max. leaf width (mm)	Plant length (mm)	Root length (mm)	FM of shoot (g)	DM of shoot (g)	DM of root (g)
None (control)	0	4.2a[a]	110.3a	133.2a	180.8a	141.8a	5.19a	0.55a	0.11a
Benzoic acid	50	2.8b	107.2a	111.0b	173.7a	147.0a	4.05b	0.46b	0.09a
	100	2.8b	98.3b	107.8b	148.0b	146.6a	4.07b	0.43b	0.09a
	200	2.7b	90.2b	90.8b	145.5b	149.2a	3.71b	0.41b	0.08a
	400	2.2c	82.3b	82.5b	132.0c	141.8a	3.44c	0.35c	0.09a
Salicylic acid	50	3.2b	112.5a	126.7a	182.7a	144.2a	4.23b	0.49b	0.11a
	100	2.5c	107.8a	107.8b	148.3b	120.2b	3.34c	0.44b	0.10a
	200	2.3c	89.0b	97.7b	152.8b	125.2b	3.37c	0.31c	0.09a
	400	2.5c	60.5c	65.5c	122.2c	114.2c	3.13c	0.26c	0.06c
Malonic acid	50	3.0b	123.5a	130.2a	178.8a	127.3b	4.45b	0.50b	0.11a
	100	3.0b	122.5a	128.5a	152.2b	125.3b	4.35b	0.46b	0.11a
	200	3.0b	125.2a	125.7a	157.5b	127.2b	4.21b	0.47b	0.11a
	400	3.0b	93.2b	90.3b	145.0b	121.0b	3.87b	0.42b	0.09a

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

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Table 4
Effects of the identified phenolic acids at different concentrations on the growth of *Vicia faba*.

Allelochemicals	Conc. (μM)	No. of leaves per plant	Max. leaf length (mm)	Max. leaf width (mm)	Plant length (mm)	Root length (mm)	FM of shoot (g)	DM of shoot (g)	DM of root (g)
None (control)	0	5.9a[a]	53.0a	102.8a	256.4a	175.8a	6.36a	0.63a	0.32a
Lactic acid	50	6.0a	55.4a	110.7a	249.3a	176.9a	6.18a	0.66a	0.38a
	100	5.9a	56.9a	113.0a	256.0a	176.6a	6.49a	0.64a	0.34a
	200	5.7a	54.6a	105.9a	257.4a	186.6a	6.52a	0.65a	0.34a
	400	5.5a	54.8a	103.7a	267.0a	182.4a	6.95a	0.65a	0.32a
Benzoic acid	50	5.9a	52.6a	105.4a	225.6a	156.1b	5.29b	0.51b	0.34a
	100	6.4a	57.5a	102.1a	252.1a	154.4b	5.41b	0.48b	0.24b
	200	5.9a	56.6a	105.0a	260.6a	151.8b	5.48b	0.45b	0.23b
	400	5.1a	37.9b	86.0b	209.9b	152.3b	4.69b	0.44b	0.18b
p-Hydroxybenzoic acid	50	5.6a	53.8a	103.6a	255.9a	177.5a	6.15a	0.69a	0.35a
	100	5.9a	56.4a	99.5a	262.4a	181.8a	6.22a	0.62a	0.32a
	200	5.8a	50.4a	101.0a	245.8a	168.5a	5.86a	0.69a	0.40a
	400	5.8a	51.3a	98.8a	248.1a	169.4a	5.71a	0.68a	0.37a
Vanillic acid	50	5.9a	49.6a	99.6a	258.8a	184.3a	6.28a	0.68a	0.37a
	100	5.9a	54.3a	108.8a	251.6a	176.9a	6.14a	0.66a	0.37a
	200	6.3a	51.6a	95.3a	256.3a	139.4b	5.95a	0.68a	0.32a
	400	6.3a	50.3a	106.1a	245.4a	145.1b	6.04a	0.70a	0.40a
Adipic acid	50	6.1a	54.3a	99.8a	274.8a	152.6b	6.95a	0.70a	0.36a
	100	6.1a	52.3a	93.5a	261.5a	152.1b	5.89a	0.69a	0.35a
	200	6.1a	51.9a	93.3a	244.4a	145.6b	5.75a	0.63a	0.28a
	400	5.8a	41.9b	100.1a	240.5a	143.3b	5.42b	0.49b	0.24b
Succinic acid	50	5.8a	55.9a	99.4a	276.8a	175.6a	6.45a	0.59a	0.30a
	100	5.9a	56.1a	100.4a	261.0a	176.8a	5.99a	0.59a	0.37a
	200	6.0a	56.9a	98.5a	277.4a	174.9a	6.01a	0.58a	0.30a
	400	6.1a	56.9a	103.6a	269.8a	170.1a	6.07a	0.59a	0.29a
Malic acid	50	6.0a	57.0a	109.1a	273.0a	185.5a	6.77a	0.73a	0.42a
	100	6.1a	54.5a	102.6a	259.8a	176.9a	6.31a	0.65a	0.37a
	200	5.6a	52.1a	105.5a	243.5a	175.1a	5.89a	0.64a	0.39a
	400	5.8a	49.4a	103.9a	253.3a	141.5b	6.02a	0.72a	0.33a
Glycolic acid	50	6.0a	51.4a	119.6a	266.1a	191.4a	6.06a	0.63a	0.23b
	100	6.0a	56.6a	106.8a	273.5a	184.3a	6.45a	0.60a	0.24b
	200	6.1a	55.5a	102.8a	272.0a	182.4a	6.58a	0.62a	0.23b
	400	5.8a	53.8a	100.1a	271.9a	191.3a	6.08a	0.59a	0.21b
p-Hydroxyphenylacetic acid	50	5.9a	50.6a	105.0a	276.9a	154.1b	6.11a	0.66a	0.31a
	100	6.0a	55.3a	100.9a	264.0a	155.3b	6.12a	0.58a	0.28a
	200	6.1a	56.1a	105.6a	254.8a	141.8b	6.20a	0.56a	0.28a
	400	6.0a	55.6a	99.0a	247.9a	146.0b	6.25a	0.63a	0.28a

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

the number of leaves, shoot fresh mass, and shoot dry mass in snap bean compared with those of the control. *V. faba* shoot length, fresh mass, and transpiration rates were affected by salicylic acid at concentrations higher than 3.5 μM after long-term treatments and it was found that guard cells in epidermal peels exhibited a high sensitivity at concentrations as low as 0.001 μM, resulting in stomatal closing. HPLC analysis of methanolic extracts from roots and leaves revealed the presence of free salicylic acid and a metabolite, the amount of which increased with time in plants previously incubated with a medium containing salicylic acid (Barbara et al., 1992).

In *V. faba*, benzoic acid at 50 μM significantly reduced root length, and shoots fresh and dry mass to 89, 83, and 81% those of the control, respectively. Adipic and *p*-hydroxyphenylacetic acids decreased root length to 87 and 88% of that of the control, respectively. When three-day-old soybean seedlings were cultivated in nutrient solution containing ferulic or vanillic acid (0.1–1 μM) for 48 h, both compounds (at 0.5 and 1 μM) decreased root length, fresh mass, and dry mass and increased phenylalanine ammonia-lyase contents (Herrig et al., 2002).

4. Conclusion

The above results clearly indicate that the root exudates from bean plants create autotoxicity in non-renewed culture solution (without AC), which leads to retardation in growth and the poor yield. This growth and yield retardation was significantly improved by the addition of AC in the non-renewed culture solution. The potent allelochemicals were detected as benzoic acid, salicylic acid, and malonic acid in *P. vulgaris*; in *V. faba*, they were benzoic acid, adipic acid, glycolic acid, and *p*-hydroxyphenylacetic acid.

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