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Humic-Like Substances from Different Compost Extracts Could Significantly Promote Cucumber Growth*1

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ABSTRACT

The effects of direct extracts of compost (DEC), aerated fermentation extracts of compost (AFEC) and non-aerated fermentation extracts of compost (NAFEC) on cucumber growth and the action mechanisms were evaluated based on the structure and activity analysis of humic-like substances. AFEC increased cucumber growth most significantly, followed by DEC and NAFEC, which was insignificant compared to the control treatment. Humic-like substances from compost extracts played an important role in promoting cucumber growth. Application of humic-like substances stimulated auxin-like activity and increased chlorophyll content and nitrogen accumulation in plants. The positive auxin-like activity of humic-like substances could be attributed to the relative distribution of special carbon groups, such as those with a large amount of peptidic and carbohydratic groups or with a low content of phenolic groups. In conclusion, the best growth promotion by application of AFEC was mainly attributed to the humic-like substances in the AFEC.

Key Words: auxin-like activity, ^{13}C nuclear magnetic resonance, chemical structure, elemental composition analysis

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INTRODUCTION

Compost extracts, produced from mixing matured composts with water for a certain period, supply nutrients and beneficial organic compounds to plants (Scheuerell and Mahaffee, 2002, 2004). When applied as a drench, they have been shown to suppress certain plant diseases and to amend soil chemical and physical properties (Scheuerell and Mahaffee, 2002). Aerated and non-aerated methods are the two dominant approaches in the production of compost extracts. There is a debate about the necessity of aeration during the production of compost extracts (Brinton *et al.*, 1996; Ingham, 2000).

Several reports have investigated the effects of compost extracts on plant growth (Gamaley *et al.*, 2001; Zaller, 2006; Siddiqui *et al.*, 2008), but only a limited number of studies have compared the effects of aerated fermentation extracts of compost (AFEC) and non-aerated fermentation extracts of compost (NAFEC) on plant growth (Arancon *et al.*, 2007). Furthermore, the number of reports on the effects of applying direct extracts of compost (DEC), which do not require extraction time, is limited (Scheuerell and Mahaffee, 2002, 2004). Plant growth promotion by compost extracts can be attributed to the presence of many inorganic nutrients and beneficial organic compounds, such as humic acids, sugar and amino acids (Muscolo *et al.*, 2007a, b; Hargreaves *et al.*, 2009). Applications of humic-like substances have been shown to positively and stably promote plant growth (Arancon *et al.*, 2006; Puglisi *et al.*, 2009; Canellas *et al.*, 2010). However, the promotion mechanism is unknown. The growth promotion was putatively considered to the structure of humic-like substances (Canellas *et al.*, 2010), while some researchers have suggested that the growth promotion was also caused by auxin-like activity (Muscolo *et al.*, 2005, 2007a, b).

Only a limited number of investigations have studied the growth promotion, structure and activity of humic-like substances in compost extracts obtained through different methods (Scheuerell and Mahaffee, 2002; Arancon *et al.*, 2007). Therefore, this study was conducted to determine the effects of DEC, AFEC and NAFEC on cucumber growth and their action mechanisms based on the structure and activity analysis of

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humic-like substances.

MATERIALS AND METHODS

Production and analysis of compost extracts

Samples of a pig manure and rice straw compost in a 3:1 weight ratio were collected from a commercial composting facility in the city of Changshu, Jiangsu Province, China. The composting was carried out under aerobic conditions for 69 d, during which 44 d were in thermophilic phase and 25 d in cooling phase. Mature composts were used and the chemical properties were as follows: pH = 7.16; electrical conductivity (EC) = 5.24 mS cm^{-1}; total carbon (TC) = 249 g kg^{-1}; total nitrogen (TN) = 30.5 g kg^{-1}; total phosphorus (TP) = 25.1 g kg^{-1}; and total potassium (TK) = 14.7 g kg^{-1}.

The AFEC, NAFEC and DEC were extracted according to the methods described by Ingham (2000) and Scheuerell and Mahaffee (2002), with slight modifications. Extracts with a 1:8 weight-based ratio of compost and water were collected in 50-L plastic buckets at 20–25 °C. Before extraction, 40 L of tap water was placed in the plastic buckets and aerated for 2 h to reduce chlorine. Then, 5 kg of compost was added to the buckets. The mixture was continuously mixed for 15 min. The extracts from this method were defined as DEC. To produce AFEC, the mixture was mixed for 36 h and continuously aerated using aquarium pumps with outputs of 33 L min^{-1}. To produce NAFEC, the mixture was stirred vigorously for 5 min and then covered with a lid for 7 d. After extraction, all extracts were filtered with two layers of cheesecloth and stored at 4 °C until further use. The TN, TP, TK and total water-soluble organic carbon of 10 compost extract samples were determined using standard methods, and the results are presented in Table I.

TABLE I

Chemical characteristics of the compost extracts tested

Treatment[a)]	Total N	Total P	Total K	TWSOC[b)]
	mg L^{-1}			
DEC	411	420	1 098	94
AFEC	246	258	1 039	271
NAFEC	316	239	1 083	444

[a)]DEC = direct extracts of compost; AFEC = aerated fermentation extracts of compost; NAFEC = non-aerated fermentation extracts of compost.
[b)]Total water-soluble organic carbon.

Glasshouse experiment design

Pot experiments were conducted in the glasshouse on May 10 to June 10, 2009. Two cucumber (*Cucumis stativus* L.) seedlings of uniform size and vigor were transferred to plastic pots (18 cm in height × 15 cm in diameter) containing 3 kg of sand and irrigated with 150 mL of nutrient solution (half-Hoagland and Arnon). A completely randomized design was used in the pot experiments with four treatments: control (CK), DEC, AFEC and NAFEC. All treatments were replicated eight times, and each treatment consisted of 16 plants. Plants were irrigated with equal amounts of tap water when irrigation was required. One hundred and fifty milliliters of half-Hoagland and Arnon nutrient solution was added to each pot every two weeks, and 150 mL of extract was added every week until the end of the experiment. In the control treatment, extract was replaced with the half-Hoagland and Arnon solution. After 30 d, cucumber plants were harvested, and the fresh and dry weights of shoots and roots were calculated.

Extraction of humic-like substances and plant growth experiments

Humic-like substances were extracted using the IHSS (International Humic Substances Society) methodology, as described by Muscolo *et al.* (1999). The total organic carbon (TOC) concentrations of three humic-like substance solutions were calculated. The TOC concentrations were 5.07 mg L^{-1} for the DEC, 47.2 mg L^{-1} for the AFEC and 89.6 mg L^{-1} for the NAFEC. Three cucumber seedlings of uniform size and vigor were transferred to plastic pots (18 cm in height × 15 cm in diameter) containing 300 g of sand and irrigated with 100 mL of nutrient solution (half-Hoagland and Arnon). A completely randomized design was used in the pot experiments with four treatments: control (CK), humic-like substances extracted from DEC (H-DEC), humic-like substances extracted from AFEC (H-AFEC) and humic-like substances extracted from NAFEC (H-NAFEC). All treatments were replicated for 5 times, and each treatment consisted of 15 plants. The plants were irrigated with equal amounts of tap water when irrigation was required. One week after planting, 100 mL of the half-Hoagland and Arnon nutrient solution was added to each pot, and it was repeated at one-week intervals for all treatments. Eighty milliliters of humic-like substances were added every 4 d after planting until 10 applications had been administered. In the control treatments, 80 mL of the half-Hoagland and Arnon solution was used in place of extracts.

Forty five days afterwards, the chlorophyll content (SPAD readings) in the 4th leaf from the shoot apex was measured with a Minolta SPAD-502 meter, according to the method described by Wu *et al.* (2007). Then, the cucumber plants were harvested, and the fresh and dry weights of shoots and roots were calculated. Dry plant samples were digested in 98% H_2SO_4 and 30% H_2O_2, and the total N content was measured by a continuous flow autoanalyzer (Autoanalyzer 3, Bran + Luebbe GmbH, Germany) (Bao, 2000). Nitrogen accumulation was calculated by multiplying the dry weights of shoots and roots with the N content in the dry matter.

Auxin-like activity of the normalized humic-like substances

The auxin-like activity of the humic-like substances was assessed by investigating their effects on the growth of lettuce roots because lettuce seeds are more sensitive to humic-like substances than cucumber seeds, as described by Muscolo *et al.* (1999, 2007b). Whatman filter paper was placed inside a 9-cm diameter, sterilized, disposable Petri dish, and 30 seeds were placed on the filter paper. The Petri dishes were wetted with 5 mL of 1 mmol L^{-1} $CaSO_4$ (control) or 5 mL of indole-3-acetic acid (IAA) with concentrations of 10, 5, 1, 0.1, 0.01 and 0.001 mg L^{-1}, respectively, and other Petri dishes of treatments were wetted with 5 mL of normalized humic-like substances from H-DEC, H-AFEC or H-NAFEC with concentrations of 10, 5, 1, 0.4, 0.1 and 0.01 mg L^{-1} TOC, respectively. Petri dishes were stored at 25 ± 2 °C in darkness for 3 d. The promotion and root lengths were recorded. Each treatment consisted of 5 replicates, and the experiments were replicated twice.

Characterization of humic-like substances

Samples of the humic-like substances were freeze-dried and ground. Elemental analysis was conducted using dry combustion on a CHNOS elemental analyzer (Vario EL III, Germany) to analyze carbon (C), hydrogen (H), nitrogen (N) and sulfur (S) contents. Oxygen (O) content was calculated by the formula: $O\% = 100\% - (C + H + N + S)\%$. Each sample was analyzed for 3 times. Two mg of the freeze-dried sample were also mixed with 200 mg of KBr (Fourier-transform infrared grade, Aldrich Chemical Co.) and compressed under vacuum for 1 min. Carbon group content was estimated by the Fourier transform infrared (FTIR) absorbance spectra of humic-like substances on a Nicolet FTIR spectrometer (PerkinElmer 1 600) from 400 to 4 000 cm^{-1}.

Solid-state cross-polarization magic-angle-spinning ^{13}C nuclear magnetic resonance (CP-MAS ^{13}C NMR) spectra were acquired with a Chemagnetics AVANCE III 400 MHz spectrometer, equipped with a 7 mm HX-MAS probe. A 20 to 50 mg portion of humic-like substance powder was packed into a 7 mm zirconium rotor. The acquisition parameters were as follows: rotor spin rate = 14 kHz; spectral frequency = 100 MHz; contact time = 2 ms; pulse delay = 0.5 s; scan times = 66 001; and line broadening = 100 Hz.

Data analysis

All data were subjected to analysis of variance (ANOVA) and tested for significance with the Tukey's HSD tests in SPSS 13.0 for Windows (SPSS, Chicago, IL). Statistical significance was defined for $P < 0.05$. All spectra were constructed using the Sigmaplot 10.0 software.

RESULTS AND DISCUSSION

Effect of compost extracts on cucumber growth

Application of compost extracts significantly affected cucumber growth (Table II). Shoot biomass was increased by all compost extracts compared to CK. The maximum shoot fresh weight was obtained with applications of AFEC and it was 50.2%, 59.2% and 82.1% higher than the weights with the applications of DEC, NAFEC and CK, respectively. The same trend was found for the dry weight of cucumber. However, no significant difference in the dry weights of cucumber plants was observed between NAFEC and CK. AFEC increased the root fresh weight by 14.7% and 36.1% compared to DEC and NAFEC, respectively. The dry root weight from applications of NAFEC was 37.2%,

TABLE II

Biomass of cucumber plants growing in different compost extracts

Treatment[a)]	Shoot biomass		Root biomass	
	Fresh weight	Dry weight	Fresh weight	Dry weight
	g pot^{-1}			
CK	39.1±1.26[b)]c[c)]	3.67±0.29b	26.9±3.97b	1.29±0.26a
DEC	47.4±1.27b	4.45±0.75b	29.7±4.20ab	1.25±0.29a
AFEC	71.2±2.86a	6.01±0.66a	34.0±4.50a	1.30±0.26a
NAFEC	44.7±2.38b	3.84±0.49b	25.0±4.28b	0.81±0.24b

[a)] CK = control treatment; DEC = direct extracts of compost; AFEC = aerated fermentation extracts of compost; NAFEC = non-aerated fermentation extracts of compost.
[b)] Mean±standard deviation ($n = 8$).
[c)] Values followed by the same letter(s) within a row are not significantly different at $P < 0.05$ according to the Tukey's HSD test.

35.2% and 37.7% lower than those for applications of CK, DEC and AFEC, respectively.

Previous studies have demonstrated that compost extracts produced by different methods significantly promoted plant growth (Keeling *et al.*, 2003; Zaller, 2006; Siddiqui *et al.*, 2008). Keeling *et al.* (2003) reported that compost extracts from mature green waste composts that had been diluted for 12 times increased shoot weight compared to unused compost extracts (fertilizer only) because of the presence of humic acids. Arancon *et al.* (2007) found that the height and leaf area of tomato and cucumber plants were significantly greater after treatments with aerated vermi-compost teas than those treated with non-aerated vermi-compost teas. We observed a similar phenomenon that the amount of cucumber biomass was significantly greater in the AFEC treatments than in the NAFEC and CK treatments (Table II). However, the amount of cucumber biomass in the DEC treatment was much greater than that in the NAFEC treatment.

Growth promotion by humic-like substances

The H-AFEC treatment yielded the highest chlorophyll contents, which were 1.8%, 3.7% and 5.9% higher than the contents in the H-DEC, CK and H-NAFEC treatments, respectively (Fig. 1). The H-NAFEC treatment gave the lowest chlorophyll content value for all treatments including the control. Yang *et al.* (2004) also reported that soil humic substances in concentrations of 0.125, 0.25, and 0.5 mg mL^{-1} stimulated

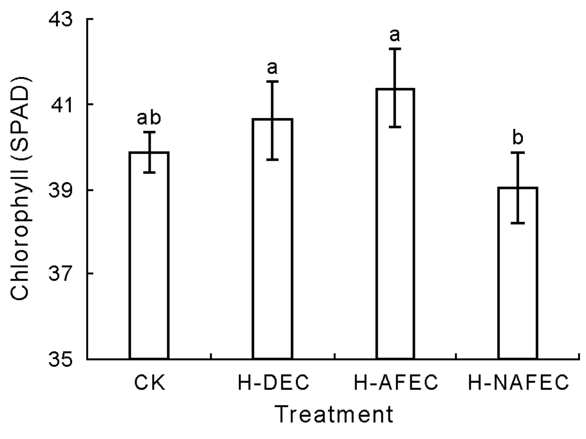

Fig. 1 Chlorophyll content (SPAD) of cucumber plants supplied with different humic-like substances. Error bars represent the standard deviations of the means (n = 5). Values marked by the same letter(s) are not significantly different at $P < 0.05$ by Tukey's test. CK = control treatment; H-DEC = humic-like substances extracted from direct extracts of compost; H-AFEC = humic-like substances extracted from aerated fermentation extracts of compost; H-NAFEC = humic-like substances extracted from non-aerated fermentation extracts of compost.

the activities of chlorophyll *a* and *b*. The concentration of H-NAFEC could have been too high to stimulate chlorophyll synthesis. Conversely, it could have inhibited chlorophyll synthesis. Varying interaction mechanisms between chlorophyll and humic-like substances could putatively attributed to the concentration difference but this should be further studied.

The amounts of cucumber biomass produced with different humic-like substances are presented in Table III. The lowest shoot fresh weight of cucumber plants was found in the H-NAFEC treatment, 13.0%, 8.1% and 9.4% less than those in the CK, H-AFEC and H-DEC treatments, respectively. The effect of humic-like substances on root fresh and dry weights was not significant.

TABLE III

Effect of different humic-like substance treatments on cucumber biomass

Treatment[a)]	Shoot biomass		Root biomass	
	Fresh weight	Dry weight	Fresh weight	Dry weight
	g pot^{-1}			
CK	18.6±0.93[b)]a[c)]	5.51±0.17a	6.24±0.71a	3.25±0.05a
H-DEC	17.8±0.55a	5.42±0.05a	6.61±1.22a	3.32±0.29a
H-AFEC	17.6±0.40a	5.21±0.06b	6.77±0.76a	3.26±0.08a
H-NAFEC	16.2±0.90b	5.13±0.11b	6.37±1.11a	3.24±0.08a

[a)] CK = control treatment; H-DEC = humic-like substances extracted from direct extracts of compost; H-AFEC = humic-like substances extracted from aerated fermentation extracts of compost; H-NAFEC = humic-like substances extracted from non-aerated fermentation extracts of compost.
[b)] Mean±standard deviation (n = 5).
[c)] Values followed by the same letter within a row are not significantly different at $P < 0.05$ according to the Tukey's HSD test.

Numerous reports have demonstrated that humic acids extracted from various materials and composts promoted plant growth (Atiyeh *et al.*, 2002; Arancon *et al.*, 2006; Canellas *et al.*, 2010). However, compared to the nutrient solution (half-Hoagland and Arnon), humic-like substances did not promote the growth of cucumber plants. The H-NAFEC treatment significantly reduced cucumber biomass (Table III), which could be caused by high quantities of humic-like substances. In our experiment, the TOC of humic-like substances were 13.5, 126 and 239 mg kg^{-1} dry sand for the H-DEC, H-AFEC and H-NAFEC treatments, respectively. Similar results were reported by Arancon *et al.* (2006). These results suggested that humic-like substances could stimulate plant growth only in low or adequate concentrations.

All humic-like substances increased the nitrogen

(N) accumulation of cucumber plants (Table IV). The H-AFEC treatment increased the N accumulation in shoots by 19.2%, 9.2% and 21.8% compared to the CK, H-DCE and H-NAFEC treatments, respectively. However, no significant difference was observed between the CK and H-NAFEC treatments. The CK treatment yielded the lowest root N accumulation, which was 4.8%, 29.2% and 14.3% lower than those obtained in the H-DEC, H-AFEC and H-NAFEC treatments, respectively.

TABLE IV

Effect of different humic-like substance treatments on cucumber N accumulation

Treatment[a)]	Shoot N	Root N	Total N
	mg pot^{-1}		
CK	62.8±4.01[b)]b[c)]	42.3±5.51b	105±7.82b
H-DEC	68.5±4.95ab	44.4±3.92b	113±4.68b
H-AFEC	74.8±1.23a	59.7±4.23a	135±3.67a
H-NAFEC	61.4±5.50b	49.4±4.87b	111±8.89b

[a)]CK = control treatment; H-DEC = humic-like substances extracted from direct extracts of compost; H-AFEC = humic-like substances extracted from aerated fermentation extracts of compost; H-NAFEC = humic-like substances extracted from non-aerated fermentation extracts of compost.
[b)]Mean±standard deviation ($n = 5$).
[c)]Values followed by the same letter(s) within a row are not significantly different at $P < 0.05$ according to the Tukey's HSD test.

The beneficial effects of humic substances on plant growth could be direct and indirect. Previous research has shown that humic substances promoted plant N uptake, when applied in appropriate concentrations (Muscolo *et al.*, 1999; Nardi *et al.*, 2000; Quaggiotti *et al.*, 2004). In this research, humic-like substances also promoted plant N uptake, thus increasing the chlorophyll content (SPAD). However, N accumulation in the H-NAFEC treatment was less than that in the H-AFEC treatment. This phenomenon could be explained as follows: firstly, the concentrations of humic-like substances in H-AFEC might be suitable for plant growth (Arancon *et al.*, 2006), and secondly, the H-AFEC treatment showed much more humic-like substance activity than the H-NAFEC and H-DEC treatments, which could be caused by different elemental compositions and structures (Nardi *et al.*, 2000; Muscolo *et al.*, 2007a, b; Muscolo and Sidari, 2009; Canellas *et al.*, 2010).

Auxin-like activity of the humic fraction

The auxin-like activities of the three humic-like substances are shown in Table V. Compared to the lettuce root growth promotion of IAA, all humic-like substances promoted lettuce root growth and showed auxin-like activity, but the promotion effect was inconsistent. When the concentration of IAA reached to 1 mg L^{-1}, lettuce had the longest root length, but when the concentration of IAA decreased to 0.01 mg L^{-1}, growth promotion almost disappeared. However, diluted humic-like substances promoted the lettuce root growth (Table V). When TOC was normalized at 10 mg L^{-1}, the root length in the H-NAFEC treatment was lowest and 14.0% and 4.4% less than the lengths in the H-DEC and H-AFEC treatments, respectively. However, when TOC was normalized to 5 mg L^{-1}, the H-DEC treatment showed the highest lettuce root growth promotion, which was 12.4% and 11.8% higher than the H-AFEC and H-NAFEC treatments, respectively. The mostly promoted growth of lettuce root was obtained in the H-NAFEC treatments diluted to 1 mg L^{-1} of TOC, and root growth was 20.1% and 20.1% higher than those in the H-DEC and H-AFEC treatments, respectively. As the concentration of TOC was decreased, the lettuce root lengths in the H-DEC and H-AFEC treatments were equivalent and greater than the length in the H-NAFEC treatment. When

TABLE V

Lettuce seedling root growth promotion by indole-3-acetic acid and humic-like substances

Treatment[a)]	Root length	Promotion
	cm	%
Control	1.80±0.30[b)]	
IAA(10 mg L^{-1})	2.05±0.23	13.7
IAA (5 mg L^{-1})	2.11±0.24	17.3
IAA (1 mg L^{-1})	2.30±0.27	28.0
IAA (0.1 mg L^{-1})	2.17±0.30	20.3
IAA (0.01 mg L^{-1})	2.04±0.26	13.2
IAA (0.001 mg L^{-1})	1.82±0.29	1.2
H-DEC (10 mg L^{-1})	2.00±0.03	11.1
H-DEC (5 mg L^{-1})	2.08±0.29	15.4
H-DEC (1 mg L^{-1})	2.04±0.16	13.4
H-DEC (0.1 mg L^{-1})	2.18±0.21	21.3
H-DEC (0.01 mg L^{-1})	2.23±0.20	23.9
H-AFEC (10 mg L^{-1})	1.80±0.18	0.3
H-AFEC (5 mg L^{-1})	1.85±0.11	2.9
H-AFEC (1 mg L^{-1})	2.04±0.28	13.3
H-AFEC (0.1 mg L^{-1})	2.17±0.09	20.6
H-AFEC (0.01 mg L^{-1})	2.16±0.19	19.9
H-NAFEC (10 mg L^{-1})	1.72±0.16	−4.3
H-NAFEC (5 mg L^{-1})	1.86±0.23	3.6
H-NAFEC (1 mg L^{-1})	2.45±0.04	35.9
H-NAFEC (0.1 mg L^{-1})	2.07±0.16	15.0
H-NAFEC (0.01 mg L^{-1})	1.91±0.15	6.4

[a)]IAA = indole-3-acetic acid; H-DEC = humic-like substances extracted from direct extracts of compost; H-AFEC = humic-like substances extracted from aerated fermentation extracts of compost; H-NAFEC = humic-like substances extracted from non-aerated fermentation extracts of compost.
[b)]Mean±standard deviation ($n = 5$).

TOC was 0.01 mg L^{-1}, H-DEC treatments increased the lettuce root length by 3.2% and 16.8% compared to the H-AFEC and H-NAFEC treatments, respectively.

Previous researches have reported that humic substances had auxin-like activity and affected nitrate metabolism and promoted plant growth (Muscolo *et al.*, 2005; Eyheraguibel *et al.*, 2008). Muscolo and Sidari (2009) found that humic substances added at low concentrations (1 mg L^{-1}) promoted callus growth. However, in our experiment, all humic-like substances promoted lettuce root growth when the TOC concentration was 1 mg L^{-1}. Conversely, regardless of increases in the TOC concentration from 0.01 to 5 mg L^{-1}, the humic-like substances from all extracts showed auxin-like activity. This could be a result of H-DEC, H-AFEC and H-NAFEC having the same humic substance source. However, when the three humic-like substances were normalized to the same TOC concentration, H-DEC produced the maximum lettuce root length, and H-AFEC produced more growth than H-NAFEC. H-AFEC promoted more growth than H-DEC and H-NAFEC when the TOC concentrations were 5.07, 47.2 and 89.6 mg L^{-1}, respectively. Thus, the quantity of humic-like substances made a main contribution to the growth promotion of cucumber plants, and the quantity of substances in H-AFEC could be appropriate for cucumber growth. The quantity of humic-like substances in H-NAFEC was too high to promote cucumber growth. The varying auxin-like activities of these humic-like substances could also play an important role in growth promotion in the pot experiment.

However, even if the TOC concentration was consistently normalized, the promotion of lettuce root growth by H-DEC, H-AFEC and H-NAFEC was inconsistent. This was attributed to variable auxin-like activity. Muscolo *et al.* (1998, 1999, 2007a) and Nardi *et al.* (1994, 2000) reported that auxin-like activity was related to the chemical structure of humic substances.

Chemical and conformational properties of humic-like substances

The elemental compositions of humic-like substances are reported in Table VI. In all samples, C and N were the major constituents (> 85%). The contents of C and N increased, and the contents of O decreased in the following order: H-DEC > H-AFEC > H-NAFEC. However, the contents of H were similar in all humic-like substances. The maximum S contents were found in H-AFEC and were 25.9% and 18.4% greater than those in H-DEC and H-NAFEC, respectively. The maximum C/N, O/C and O/H ratios were found in H-DEC, while the maximum C/H ratios were found in H-AFEC and H-NAFEC.

TABLE VI

Elemental compositions of different humic-like substances[a)]

Elemental	H-DEC	H-AFEC	H-NAFEC
C (%)	43.83±0.05[b)]c[c)]	50.88±0.04b	54.54±0.08a
N (%)	1.92±0.07b	2.80±0.01a	2.85±0.01a
H (%)	2.19±0.04a	2.07±0.09a	2.29±0.25a
S (%)	7.97±0.24b	10.03±0.16a	8.47±0.07b
O (%)	44.09±0.23a	34.22±0.03b	31.85±0.08c
C/N	22.84±0.87a	18.20±0.06b	19.14±0.07b
C/H	20.06±0.30a	24.66±1.08a	24.01±2.64a
O/C	1.01±0.01a	0.67±0.01b	0.58±0.01c
O/H	20.19±0.22a	16.59±0.73ab	14.03±1.55b

[a)]H-DEC = humic-like substances extracted from direct extracts of compost; H-AFEC = humic-like substances extracted from aerated fermentation extracts of compost; H-NAFEC = humic-like substances extracted from non-aerated fermentation extracts of compost.

[b)]Mean±standard deviation ($n = 3$).

[c)]Values followed by the same letter(s) within a row were not significantly different at $P < 0.05$ according to the Tukey's HSD test.

According to He *et al.* (2008) and Pedra *et al.* (2008), the C, H, O and N concentrations in humic acids were related to the source of the humic substances. In our experiment, the humic-like substances had the same sources, but aerated and non-aerated extraction procedures increased the C and N content and decreased the O content. Aerated extraction increased the content of S. The atomic ratios of C/N, C/H, O/C and O/H are often used to monitor structural changes of humic substances and to elucidate the structural formulae for humic substances from different sources (Steelink, 1985; Adani *et al.*, 2006). The similar C/N and C/H ratios for these humic-like substances suggested similar stability and condensation degrees and an extended humification degree (Lu *et al.*, 2000). The O/C ratio was considered to be an indicator of the carbohydrate and carboxylic acid contents in humic substances (He *et al.*, 2008). In this study, the decrease in the O/C ratio in H-AFEC and H-NAFEC suggested an increase in the degree of aromatic condensation and a decrease in O-alkyl and carboxylic acids (Muscolo and Sidari, 2009). H-NAFEC showed the highest degree of aromatic condensation.

The FTIR spectra of the three humic-like substances are shown in Fig. 2. The main absorption bands in Fig. 2 were as follows: a broad band at 3 200 cm^{-1} was attributed to O-H stretching of carboxylic and alcoholic groups (Muscolo and Sidari, 2009); the peak between 1 620 and 1 660 cm^{-1} represented the C=C vibration of aromatic components and the C=O vibration of bonded conjugated ketones, quinones, carboxylic acids and esters; the another band at 1 380 to 1 420 cm^{-1} might encompass O-H deformation, C=C stretch band of vinyl ethers, aliphatic C-H deformation and anti-symmetric COO^- stretching; the wavelength between 1 000 to 1 260 cm^{-1} was attributed to unconjugated C-N linkage in primary, secondary and tertiary aliphatic amines and C-O stretch vibrations in alcohols and phenols; and the stretch of 675–900 cm^{-1} was attributed to aromatic compounds. All the results indicated that, in general, all humic-like substances exhibited similar FTIR spectra, suggesting that they had similar functional groups.

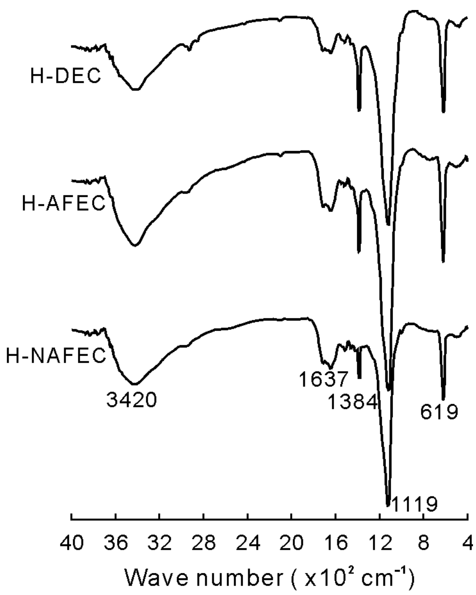

Fig. 2 Fourier transform infrared (FTIR) spectra of humic-like substances. H-DEC = humic-like substances extracted from di rect extracts of compost; H-AFEC = humic-like substances extracted from aerated fermentation extracts of compost; H -NAFEC = humic-like substances extracted from non-aerated fermentation extracts of compost.

Carballo *et al.* (2008) reported no changes in the functional group variety between aerated and non-aerated compost extracts and little difference in intensity. Similar findings were observed in our experiments (data not shown). However, in this experiment, humic-like substances from all compost extracts contained similar functional groups, including aromatic groups, carbohydrates, phenols and amides (Fig. 2).

The CPMAS ^{13}C NMR spectra of humic-like substances and the C-containing functional group contents are presented in Fig. 3 and Table VII, respectively. The spectral area integration (0–48, 48–105, 105–145, 145–165, and 165–190 ppm) was conducted with the Win NMR software (Fukushima *et al.*, 2009). The CPMAS ^{13}C NMR spectra of H-AFEC and H-NAFEC exhibited major peaks at 30 ppm (alkyl C), 56 and 74 ppm (alkyl-O carbons), 130 ppm (aromatic carbons), 153 ppm (phenol carbons), 174 ppm (carboxylic carbon), and 196 ppm (carbonyl carbon) (He *et al.*, 2008). The persistence of a signal at 20 ppm in the dipolar dephasing spectra (Fig. 3) was related to a methyl group (Spaccini and Piccolo, 2008). However, the signal intensity at 74 ppm in the H-DEC spectra was much less than that in the H-AFEC and H-NAFEC spectra, but an additional peak at 103 ppm, indicating peptide and carbohydrate carbons, was observed in the H-DEC spectra. Signal intensity decreased in the H-AFEC and H-NAFEC spectra (Fig. 3). Compared to H-DEC and H-NAFEC, the relative distribution of aliphatic carbon in H-AFEC was greatest and 8.4% and 14.5% greater than the values in H-DEC and H-NAFEC, respectively (Table VII). The relative distribution of peptides and carbohydrate carbons in H-DEC was 32.4% and 39.3% greater than those in H-AFEC and H-NAFEC, respectively. The relative distributions of aromatic and phenol carbons in H-NAFEC were greatest and 1.6% and 15.3% greater than the distributions in H-AFEC and 29.2% and 46.3% greater than the distribution in H-DEC, respectively. The lowest relative distribution of carboxyl carbon was found in H-AFEC and was 9.4% and 24.8% less than the values in H-DEC and H-NAFEC, respectively.

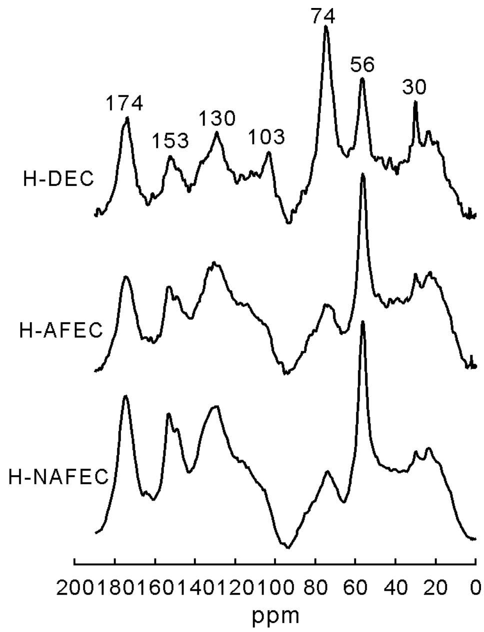

Fig. 3 CPMAS ^{13}C NMR spectra of humic-like substances. H-DEC = humic-like substances extracted from direct extracts of compost; H-AFEC = humic-like substances extracted from aerated fermentation extracts of compost; H-NAFEC = humic-like substances extracted from non-aerated fermentation extracts of compost.

TABLE VII

Relative distribution of signal area over chemical shift regions (ppm) in the CPMAS ^{13}C NMR spectra of humic-like substances from different compost extracts[a)]

Compound group	H-DEC	H-AFEC	H-NAFEC
		%	
Aliphatic C (0–48 ppm)	24.9	27.0	23.1
Peptide and carbohydrate C (48–105 ppm)	35.8	27.0	25.7
Aromatic C (105–145 ppm)	20.3	25.8	26.2
Phenol C (145–165 ppm)	8.2	10.3	11.9
Carboxyl C (165–190 ppm)	10.9	9.8	13.1

[a)] H-DEC = humic-like substances extracted from direct extracts of compost; H-AFEC = humic-like substances extracted from aerated fermentation extracts of compost; H-NAFEC = humic-like substances extracted from non-aerated fermentation extracts of compost.

Typically, O-alkyl and alkyl groups were biodegraded during composting and produced aromatic and carboxylic groups (Tang *et al.*, 2006; Spaccini and Piccolo, 2008). In this research, compared to H-DEC, aerated extraction and non-aerated extraction decreased the relative distributions of peptides and carbohydrates and increased the relative distributions of aromatic and carboxyl carbons. According to the elemental composition analysis, the O/C ratio of H-NAFEC was lowest. H-NAFEC had low contents of O-alkyl groups and carboxylic acids and the highest relative distribution of carboxylic carbons. The difference could be attributed to the decrease of O-alkyl carbon as opposed to the increase of carboxylic carbon. According to Table VII, the degree of aromatic condensation of H-NAFEC was higher than those of H-DEC and H-AFEC, and the degree of H-AFEC was higher than that of H-DEC. Similar results were found in the elemental compositions analysis.

Lower amounts of carbohydrates and polysaccharides were found in the NMR spectra of humic-like substances isolated at increased stages of compost maturity (Spaccini and Piccolo, 2008). H-NAFEC was the most mature compost and had the greatest aromatic degree. Meanwhile, the relative distributions of phenols and carboxyl carbons in H-NAFEC were higher than those in H-DEC and H-AFEC, and the distributions in H-AFEC were higher than those in H-DEC. The relative distributions of peptides and carbohydrate carbons in H-DEC were much greater than those in H-AFEC and H-NAFEC. According to Andelković *et al.* (2006), O-bearing carboxyl and phenol hydroxyl groups were considered to be responsible for interactions between humic substances and plants. Canellas *et al.* (2010) and Muscolo *et al.* (2007a) also reported that variable humic substance activity was related to diverse chemical compositions. In this study, biological and auxin-like activity differed among H-DEC, H-AFEC and H-NAFEC. H-DEC was rich in carbohydrate carbons and increased lettuce root growth. Conversely, H-NAFEC was rich in phenol carbons and negatively affected lettuce root growth. This result suggested that higher amounts of phenolic compounds in the humic-like substances of H-NAFEC decreased the biological activity of lettuce and cucumber plants. Similar results were reported by Muscolo and Sidari (2009). The inhibitory effects of phenolic acids on seed germination (Muscolo *et al.*, 2005; Muscolo and Sidari, 2006) and plant growth (Chon *et al.*, 2004) were well documented. Hättenschwiler and Vitousek (2000) and Whitehead *et al.* (1981) found that phenolic compounds are potential inhibitors of N uptake and influence supplies of available N. Similar results were found in this study. Thus, the effects of H-DEC, H-AFEC and H-NAFEC on biological activity were attributed to varying amounts of peptides, carbohydrates and phenolic carbons.

CONCLUSIONS

Humic-like substances played an important role in growth promotion by compost extracts. AFEC promoted growth much more than DEC and NAFEC. H-NAFEC had the most humic-like substances, followed by H-AFEC and H-DEC. All humic-like substances produced auxin-like activity. H-DEC was rich in peptidic and carbohydratic groups and showed the highest auxin-like activity. H-NAFEC was rich in phenolic groups and showed the lowest auxin-like activity. Thus, positive auxin-like activity could be attributed to the relative distribution of special carbon groups, such as the peptidic and carbohydratic groups, and the low content of phenolic groups. However, compared to H-DEC and H-NAFEC, H-AFEC promoted cucumber growth most significantly, and the promotion effect of H-AFEC were attributed to the quantity and auxin-like activity.

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