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Effect of Humic Substances Extracted from Compost to Plant Growth and Soil Microorganisms

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ABSTRACT

There are many products available for agricultural use falling into the broad category of biological soil conditioners, stimulants or additives. These product kinds enhance crop growth and yield through several mechanisms, such as microbial inoculation into soil, stimulation of soil microbial activity, increasing the activity of critical enzymes, production of phytohormones or supplementing micronutrients. Between these products, humic acids are nowadays being used as liquid organic amendment, since a close relationship exists between soil fertility and its organic matter content. In our work, the response of tomato plants to amendment with humic compounds (HCs) from several origins is reported in a pot trial. The different substrates used were sandy soil (SS) and another inert substrate (IS). The HCs used were potassium humates from compost based on horticultural waste (WCHs) and leonardite (LHs) were used. Two different cultivars of tomato (*Solanum lycopersicum* cv. 'Raf' type Marmande or 'Durinta') were tested in two pot substrates. Results were evaluated in terms of plant growth and biomass production. The effect of these HCs on soil microbial populations was also investigated. Results showed that the application of low rates of HCs had an overall stimulatory effect on heterotrophic aerobic bacteria, fungi and nitrogen fixing-bacteria growth. The WCHs addition had a positive influence on nitrogen fixing-bacteria and nitrifying bacteria population when 'Raf' or 'Durinta' were used, respectively. Both groups were favoured when IS was used. On the other hand, lower and thicker plants were observed when WCHs were applied to crops. Therefore, results showed the potential for improving the utilization of HCs extracted from compost based on plant waste (WCHs). The extraction of these substances (WCHs) by the process here described produced an extract which behaved as a stimulatory substance on soil-plant ecosystems and some microorganisms related to the plant roots.

Keywords: humic acids, soil fertility, soil microbial populations

INTRODUCTION

The effects of humic compounds (HCs), the major component of soil organic matter, on plant growth have been examined in recent works (Clapp *et al.* 2001; Varanini and Pinton 2001; Nardi *et al.* 2002). The beneficial effects of these substances may be either indirect, increasing fertilizer efficiency or reducing soil compaction, and direct, improving plant biomass production (Vaughan and Malcom 1985). In this sense, their direct effects on plants appear to be mainly exerted on cell membrane functions, promoting nutrient uptake, or plant development, by acting as hormone-like substances (Vaughan and Malcom 1985; Visser 1985a, 1985b; Nardi *et al.* 1996).

On the other hand, humic colloids also affect the growth of soil microbial populations. Many soil microorganisms from different taxonomic and functional groups respond favourably to the presence of HCs in *in vivo* or *in vitro* experiments (Visser 1985a, 1985b; Vallini *et al.* 1993; Valdrighi *et al.* 1995; Vallini *et al.* 1997). To explain the stimulatory effects of humic molecules on microbes, several authors have proposed the modification of cell membrane permeability to nutrients as the main mechanism involved (Schisler and Linderman 1989).

Composting consists of the aerobic biological decomposition of organic solid substrates, in which materials are converted to a stable end-product named compost (Goyal *et al.* 2005). This process is employed world-wide as a treatment for organic wastes such as sewage sludge and animal or agricultural residues (Gasser 1985; Huang *et al.* 2006; Lhadi *et al.* 2006).

During composting, organic matter is partially transformed into humus-like substances (de Bertoldi *et al.* 1983). Compost can therefore be used directly in agriculture as an organic amendment to enhance soil fertility. This process is of increasing importance in southeast Spain, where over 10^6 t of horticultural plant waste is produced annually (Cara and Rivera 1998).

The loss of organic matter in most agricultural soils, often to the level of 1% or less, implies the need to search for solutions such as the application of HCs to increase soil quality. The current present market for this product kind has therefore turned to compost as a possible economic source for the extraction of such substances (Valdrighi *et al.* 1995, 1996; Filip *et al.* 2000) instead of reliance on expensive fossil matrices such as leonardite.

The present study compares the effects of HCs extracted from compost based on horticultural waste and those from a commercial liquid fertilizer on plant growth and soil microbial populations using a pot experiment carried out with tomato plants (*Solanum lycopersicum* L.). The observation of the long-term response of soil microbial communities to the addition of HCs was one of the main objectives of the present work. The effect of these substances on plant growth and biomass were also investigated.

MATERIALS AND METHODS

Humic-like substances

A concentrated humic extract from leonardite (LHs) provided from an agricultural company (JISA, Jiloca Industrial, S.A) were used to represent humates of fossil origin. On the other hand, HCs from compost based on horticultural waste (WCHs) were prepared as follows. Dried and composted vegetable residues were digested with KOH 0.35% for 2 h at 121°C, in the ratio of 1:10 (w/v). The

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solute fraction was then separated from the undigested residue by sedimentation and decanting and concentrated 10 times by evaporation. KOH was used as an extractant instead of NaOH, since it is more suitable for the industrial production of humic amendments for agricultural applications.

The use of KOH to obtain humic extract has been above achieved by several authors (Valdrighi *et al.* 1995, 1996; Vallini *et al.* 1997; Charest *et al.* 2005).

Some chemical characteristics of humic extracts are shown in **Table 1**. In this sense, the humic content in LHs was higher than the observed in WCHs. However, on the basis of other research in which beneficial aspects were observed when these extracts were used under 1% (Pardo-Parra 2003), both products were applied in aqueous solution at 0.7%.

Plants and substrates used in pot trials

Two varieties of tomato were used to determine plant growth and production in cultivation experiments and they are certified cv. 'Raf', type Marmande and certified cv. 'Durinta' (Almeriplant Semilleros, Almería, Spain). Pot trials were carried out in the experimental greenhouse of the Plant Production Department, University of Almería (Spain). Both sandy soil (SS) and a semi-inert substrate (IS) on the basis of mainly "vermiculite" (PROJAR, S.A., El Ejido, Almería, Spain) were used separately to compare the effect of HCs on plant and microbial growth.

Experimental design

Tomato plants were grown on seedbed during 30 days up to approximately 15 cm high. These were lined inside polyethylene bags (18 m^2) previously filled with SS or IS. The basic irrigation solution was prepared as described by Guzmán (2003) in **Table 2** being applied throughout the experiment on all tomato plants.

The final experimental design consisted of six blocks with 18 replicates (plants) per block. Plants were supplied weekly with LHs and WCHs in aqueous solutions at 0.7% but control plants were not amended with HCs. The weekly contribution of humates in aqueous solution was planned as described in **Table 3** based on water consumption by the plants.

Plants were located in a randomized block in the greenhouse and grown for 60 days at constant temperature of 24 ± 1°C and relative humidity of 75%.

Table 1 Some chemical characteristics of the humates tested.

Humics extract	C_o[1]	C_{ex}[2]	pH	CE[3]
WCHs	4.6%	20.8%	10.63	85
LHs	8.9%	30.3%	13.34	70.4

[1] Oxidizable carbon (g/100 g of concentrated extract)
[2] Alkaline-extractable carbon (g/100 g of dry matter)
[3] Electrical conductivity (mS/cm)

Table 2 Basic irrigation solution (Guzmán 2003).

Chemical composition[1]								
NO_3^-	$H_2PO_4^-$	SO^{2-}_4	Cl^-	NH_4^+	K^+	Ca^{2+}	Mg^{2+}	K+/ (Ca^{2+}+Mg^{2+})
15	2	6	2	1.5	7.5	12	4	0.47

[1] meq/L

Table 3 Contribution of humic extracts in aqueous solution throughout the sampling time.

Week	Water consuming (L/m^2)	L/plant IS	L/plant SS	Humic extract contribution (cc/m^2)	Humic extract contribution (cc/plant)
1	1.63	0.8	0.4	8.15	6
2	2.29	1.2	0.6	11.4	4
3	2.95	1.5	0.75	14.8	7
4	3.32	1.7	0.85	16.6	8
5	3.68	1.8	0.9	18.4	9
6	3.74	1.9	0.95	18.7	9
7	3.80	1.9	0.95	19.0	10
8	4.00	2.0	1.0	20.0	10
9	4.21	2.1	1.05	21.1	11
10	4.20	2.1	1.05	21.0	11

Estimation of microbial growth

After 7, 14, 28, 45 and 60 days, microbial counts were taken on 3 separate soil samples from each experimental block. Total number of aerobic bacteria (TB), actinomycetes (TA) and fungi (TF) were determined on nutrient agar, bengale rose and sodium caseinate agar plates, respectively. Cellulolytic population (CEL) was determined on aniline blue black agar (Karui and Kushner 1988), hemicellulolytic microorganisms (HEM) were estimated on xilane-agar medium (He *et al*. 1993) and ligninolytic microorganisms (LIG) were isolated using Poly-R-478 agar plates (Feritag and Morrell 1992). On the other hand, nitrogen-fixing bacteria (NF) were cultured in nitrogen-deficient medium (Burk 1930).

The other microbial groups were determined by the most probable number technique. Ammonifiers (AM) were evaluated in Winogradsky's saline solution plus oligoelements and L-asparagine as the only N and C source, as recommended by Pochon and Tardieux (1962); Nessler's reagent was used for assessing the presence of NH_3. The medium for nitrifying bacteria (NIT) contained Winogradsky's saline solution plus $CaCO_3$ and $(NH_4)_2SO_4$. Diphenylamine-sulfuric acid reagent was utilized to check positives tubes. The inoculated tubes and plates were incubated at 28°C for 15 and 2-7 days, respectively.

Estimation of plant growth and biomass

To assess tomato growth, six plants from each block were harvested approximately every 3-4 days for two months. The parameters measured were top height (H), leaf number (L) and fruit (F) number, stem diameter (D), inflorescence (I) number and height/diameter ratio (H/D). Additionally, fresh and dry weights of root, leaves, stem, inflorescences and fruits were also determined every 20 days, approximately. All reported values were mean of six measurements per treatment and sampling.

Statistical analysis

In relation to the effect of the humic extracts on plant growth and biomass, all values were expressed as the mean of six measurements for each parameter investigated (H, L, F, D, I, H/D ratio, RFB, SFB, LFB, IFB, FFB, RDB, SDB, LDB, IDB and FDB). Data were subjected to one multifactorial analysis of variance (ANOVA) in which mean values were compared for the different levels of humic treatment (LHs, WCHs and Control), sampling time and pot substrate type (IS and SS). On the other hand, relating to microbial growth experiment, counts of the different groups were compared for the different levels above cited. Statistical interactions between the different levels for each parameter were besides investigated (humic treatment X sampling time, humic treatment X substrate type and sampling time X substrate type). Only the most interesting interactions have been shown in **Figs. 2, 4, 6, 7** and **8**. Since 'Raf' and 'Durinta' showed noticeable differences, results were analysed irrespective of cultivar.

In order to determine which means were significantly different ($p < 0.05$), multiple comparison tests (Fisher's least significant difference) were used (Dowdy and Wearden 1991). All experiments were carried out twice.

RESULTS AND DISCUSSION

Effects of humic compounds on microbial populations from a plant-soil system

Microbial populations were affected when different HCs were added to all experimental blocks. The effect observed depended on the origin of the amendment applied (LHs or WCHs) as well as the tomato cultivar used ('Raf' or 'Durinta').

Table 4 shows the significant influence of the different factors on all microbial groups analysed as well as the influence of the interactions between them. In general, several differences were observed when humic extracts were added to different cultivars. In this sense, the humic treatment significantly influenced on TB, TF, CEL, LIG and NF populations when 'Raf' was used while this effect was observed

Table 4 Effect of Sampling time, Substrate kind and Humic treatment on microbial populations from a plant-soil system. Significant differences are observed at 95% confidence level ($p < 0.05$).

'Raf'									
Factors	**TB[1]**	**TA[2]**	**TF[3]**	**CEL[4]**	**HEM[5]**	**LIG[6]**	**NF[7]**	**NIT[8]**	**AM[9]**
	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Sampling time	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000
Substrate kind	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0158	0.0010
Humic treatment	0.0087	ns[10]	0.0009	0.0000	ns	0.0000	0.0001	ns	ns
Interactions	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Sampling time X Substrate kind	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000
Sampling time X Humic treatment	ns	ns	0.0357	0.0000	0.0005	0.0000	0.0029	ns	0.0000
Humic treatment X Substrate kind	ns	ns	ns	0.0000	0.0000	0.0000	ns	0.0010	0.0029
'Durinta'									
Factors	**TB[1]**	**TA[2]**	**TF[3]**	**CEL[4]**	**HEM[5]**	**LIG[6]**	**NF[7]**	**NIT[8]**	**AM[9]**
	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Sampling time	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Substrate kind	0.0001	0.0000	0.0000	0.0294	0.0000	0.0000	0.0000	0.0048	0.0001
Humic treatment	0.0005	ns	0.0060	ns	0.0000	ns	0.0002	0.0000	ns
Interactions	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Sampling time X Substrate kind	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Sampling time X Humic treatment	0.0393	ns	0.0036	0.0000	0.0197	0.0159	0.0025	0.0000	ns
Humic treatment X Substrate kind	ns	ns	ns	0.0000	ns	0.0000	ns	0.0005	ns

[1] Total Bacteria
[2] Total *Actinomycetes*
[3] Total Fungi
[4] Cellulolytic Microorganisms
[5] Hemicellulolytic Microorganisms
[6] Ligninolytic Microorganisms
[7] Nitrogen-Fixing Bacteria
[8] Ammonifiers Microorganisms
[9] Nitrifying Bacteria
[10] non significant

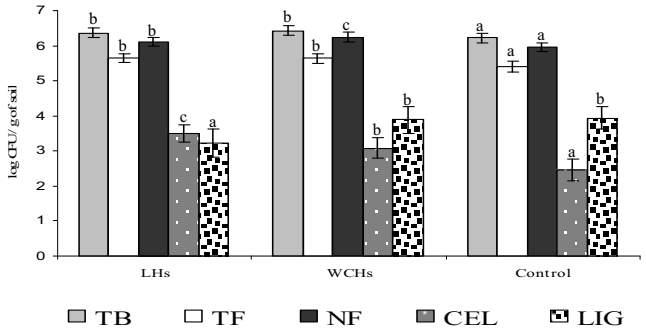

Fig. 1 Microbiological analyses of *Solanum lycopersicum* cv. 'Raf'-soil system treated with two humic compounds, LHs or WCHs. For each microbial group, columns with the same letter(s) are not significantly different ($P < 0.05$). CEL: cellulolytic microorganisms; LIG: lignocellulolytic microorganisms; NF: nitrogen-fixing bacteria; TB: total aerobic bacteria; TF: total fungi.

on TB, TF, HEM, NF and NIT when 'Durinta' was used (**Table 4**). On the other hand, in both cultivars, the interactions between the different factors significantly influenced TA, TF, CEL, LIG and NF populations.

Figs. 1 and **3** show the results previously treated as a whole, irrespective of the sampling time. The most interesting interactions between the different factors are shown in **Figs. 2** and **4**.

In the 'Raf'-soil system, when HCs were added to the soil, populations of TA, HEM, AM and NIT did not differ from those observed in the control treatment (data not shown). On the other hand, counts of TB, TF, NF and CEL populations in LHs and WCHs treatments were, in general, higher than those obtained in control soils (**Fig. 1**). The population of nitrogen-fixing bacteria (NF) was particularly higher in the WCHs treatment than in the LHs, and values from control fell in between them (**Fig. 1**). This effect was more evident from 28 days and when IS was used (**Fig. 2**).

In the 'Durinta'-soil system, microbial counts of TA, CEL, LIG and AM were not affected by the addition of HCs to the soil. On the other hand, as observed in the case of 'Raf', counts of TB, TF and NF populations were higher

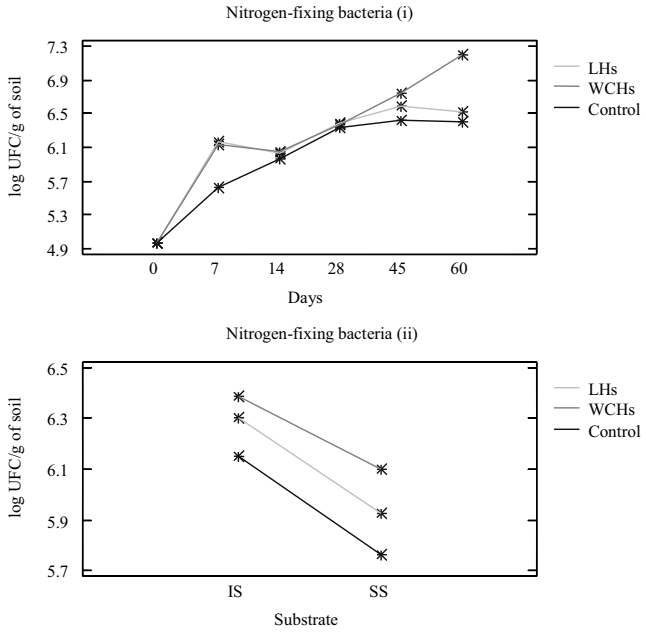

Fig. 2 Effect of the interactions between humic compounds and time (i) or substrate (ii) on nitrogen-fixing bacteria (NF) population: *Solanum lycopersicum* cv. 'Raf'-soil system. The ANOVA test decomposes the variability of NF into contributions due to the interactions between various factors. Since P-values are less than 0.05, these interactions have a statistically significant effect on NF at the 95.0% confidence level.

when LHs or WCHs were added (**Fig. 3**). Contrary to the results obtained from the 'Raf'-soil system, microbial counts of HEM and NIT were also higher in amended substrates (**Fig. 3**). NIT showed higher counts than those obtained in the case of substrates added with LHs. These differences were higher during the second month and when IS was used rather than SS (**Fig. 4**).

Soil degradation caused by intensive agriculture, the use of inappropriate technologies or the application of great quantities of pesticides and fertilizers represents a major environmental problem. Indeed, current horticultural methods

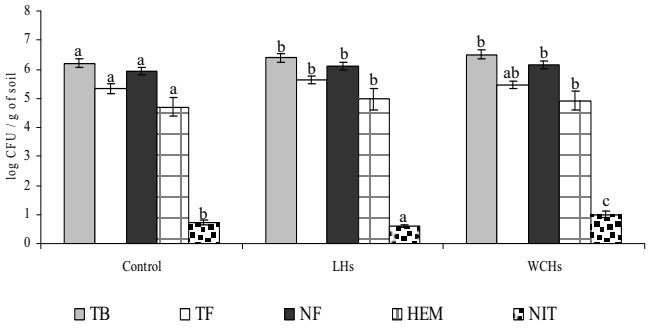

Fig. 3 Microbiological analyses of *Solanum lycopersicum* cv. 'Durinta'-soil system treated with two humic compounds, LHs or WCHs. For each microbial group, columns with the same letter(s) are not significantly different ($P < 0.05$). HEM: hemicellulolytic microorganisms; NF: nitrogen-fixing bacteria; NIT: nitrifying microorganisms; TB: total aerobic bacteria; TF: total fungi.

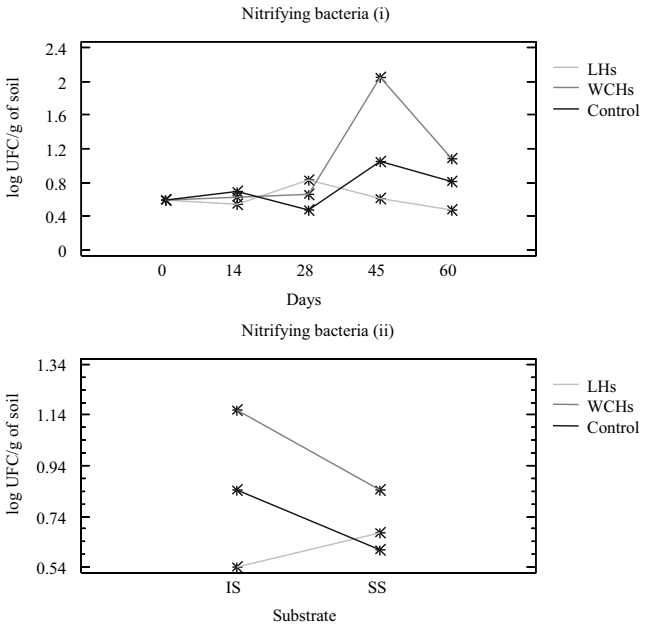

Fig. 4 Effect of the interactions between humic compounds and time (i) or substrate (ii) on nitrifying bacteria (NIT) population: *Solanum lycopersicum* cv. 'Durinta'-soil system. The ANOVA test decomposes the variability of NIT into contributions due to the interactions between various factors. Since P-values are less than 0.05, these interactions have a statistically significant effect on NIT at the 95.0% confidence level.

lead to deterioration of the physical, chemical and biological properties of soil (Albiach *et al.* 2000).

The use of organic amendments in deteriorated soils improves soil structure and is a source of carbon, nitrogen and other nutrients, thus favouring the microbial diversity and activity. The preservation of soil microbial groups is essential, as they play an important role in carbon and nitrogen cycling, the decomposition of organic matter and maintenance of soil fertility (Potter and Meyer 1990).

The results obtained in this work have shown that the application of HCs has a significant impact on several soil microbial groups. This response could be attributed to the nutritive value of humates from WCHs and LHs. Also, Valdrighi *et al.* (1996) suggest that potassium added to WCHs has no stimulatory effects on microbial populations. Several authors have confirmed that molecular characteristics of HCs may result in higher biological activity due to enzymatic activation of nutrient uptake or modification of bacterial cell permeability to nutrients (Valdrighi *et al.* 1995; Tejada *et al.* 2006). Counts of total aerobic bacteria, fungi and nitrogen-fixing bacteria were higher in soils treated with HCs than in control soils (**Figs. 1-3**). This effect was observed in both plant cultivars tested ('Raf' and 'Durinta'). However, treatment with WCHs promoted the highest counts of nitrogen-fixing (NF) bacteria with 'Raf' (**Fig. 1**), while the highest counts of nitrifying bacteria were obtained with 'Durinta' (**Fig. 3**). It is therefore possible that different plant-soil systems react differently to the presence of HCs (Vaughan and Malcom 1985).

Soil microorganisms involved in nitrogen cycling have previously been studied as regards their response to the application of HCs. Populations of autotrophic ammonia and nitrite oxidizers increased in soil or axenic cultures amended with humates from composted vegetable waste, especially at high rates (Valdrighi *et al.* 1995, 1997). On the other hand, Acea *et al.* (2003) confirmed the importance of nitrogen-fixing bacteria to promote microbial crust forma-

Table 5 Effect of Sampling time, Substrate kind and Humic treatment on parameters related to plant growth. Significant differences are observed at 95% confidence level ($p < 0.05$).

'Raf'						
Factors	**H[1]**	**L[2]**	**F[3]**	**D[4]**	**I[5]**	**H/D[6]**
	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Sampling time	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Substrate kind	0.0000	0.0000	0.0001	0.0000	0.0000	0.0003
Humic treatment	0.0000	0.0147	ns	0.0000	0.0088	0.0000
Interactions	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Sampling time X Substrate kind	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Sampling time X Humic treatment	0.0000	ns	ns	0.0000	ns	ns
Humic treatment X Substrate kind	0.0000	0.0006	ns	0.0000	0.0304	0.0245
'Durinta'						
Factors	**H[1]**	**L[2]**	**F[3]**	**D[4]**	**I[5]**	**H/D[6]**
	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Sampling time	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Substrate kind	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Humic treatment	0.0000	0.0000	ns	ns	0.0000	0.0000
Interactions	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Sampling time X Substrate kind	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Sampling time X Humic treatment	ns	ns	ns	ns	0.0062	ns
Humic treatment X Substrate kind	ns	0.0000	0.0052	0.0000	0.0004	0.0013

[1] Top Height
[2] Leaf Number
[3] Fruit Number
[4] Stem Diameter
[5] Inflorescence Number
[6] Height/Diameter ratio
[7] non significant

tion, enhancing C and N cycling microorganisms and increasing organic matter and nutrient content in deteriorated soils.

Effects of humic compounds on plant growth and biomass

Table 5 shows the significant influence of the different factors on all parameters related to plant growth (H, L, F, D, I and H/D) as well as the statistical influence of the interactions between them. In this sense, excepting "Fruit Number" and "Stem Diameter" factors, significant influences were in general observed when the humic treatment was independently analysed. Several differences between both cultivars were detected when statistically significant interactions were analysed (**Table 5**). On the other hand, **Fig. 5** shows the results previously treated as a whole, irrespective of the sampling time. The most interesting interactions between the different factors were besides shown in **Figs. 6-8**.

Therefore, plant growth was affected when tomato plants were amended with HCs depending on the plant cultivar and substrate used. Contrary to expectations, for several parameters treatments showed no effect in comparison with control plants (**Fig. 5**).

In 'Raf', leaf (L) and inflorescence (I) number and top height (H) increased when plants were amended with WCHs rather than plants LHs. However, significant differences were not observed respect to control plants (**Fig. 5**). On the contrary, measure of stem diameter (D) was highest in plant treated with WCHs, especially in IS (**Fig. 6**), and as a result these plants also had the lowest H/D values (**Fig. 5**). This last observation occurred in both sandy soil (SS) and inert substrate (IS) with added WCHs (**Fig. 7**).

The results obtained were different to 'Durinta', except for H/D. These values were lower when WCHs were applied in both substrates, but especially so in IS (**Fig. 8**). Leaf (L) and inflorescence (I) number and top height (H) were lower when plants were amended with WCHs, but stem diameter (D) was not affected by either of the HCs in this cultivar (**Fig. 5**).

Finally, **Table 6** shows the significant influence of the different factors on all parameters related to fresh and dry vegetable biomass (RFB, SFB, LFB, IFB, FFB, RDB, SDB, LDB, IDB and FDB) as well as the statistical influence of the interactions between them. In this case, results obtained both in 'Raf' and 'Durinta' were in general significantly influenced by the sampling time and substrate kind. Opposite to expected, vegetable biomass parameters were not affected by the humic treatment (**Table 6**).

Therefore, these results show a weak effect of HCs on vegetable biomass parameters. This effect was surely due to the low concentrations applied. However, biomass values of plants amended with WCHs were very similar to those of samples treated with LHs. Indeed, for 'Raf', only stem fresh biomass (SFB) was higher for WCHs treatment than LHs treatment (**Fig. 9**). The opposite effect was observed for 'Durinta'. We must emphasize that **Fig. 9** show the results previously treated as a whole, irrespective of the sampling time.

Although neither LHs nor WCHs added to the soil at 0.7% showed a marked beneficial influence on tomato growth (**Fig. 5**), an interesting effect was observed with respect to H/D. The thickest plants were observed when WCHs were applied (**Fig. 5**), possibly making less breakable plants. In 'Durinta', H/D was significantly lower for WCHs treatment than LHs (**Fig. 5**).

The other parameters relating to plant growth or biomass production were only slightly affected by the application of HCs. This may be explained by the low concentration of HCs added to the crop. Indeed, several authors have confirmed the absence of stimulatory effects when humates were added to soil at rates below 1000-2000 mg.kg^{-1} (Valdrighi *et al.* 1995, 1996).

The physiological effects of HCs on some aspects of plant growth have been extensively examined (Nardi *et al.*

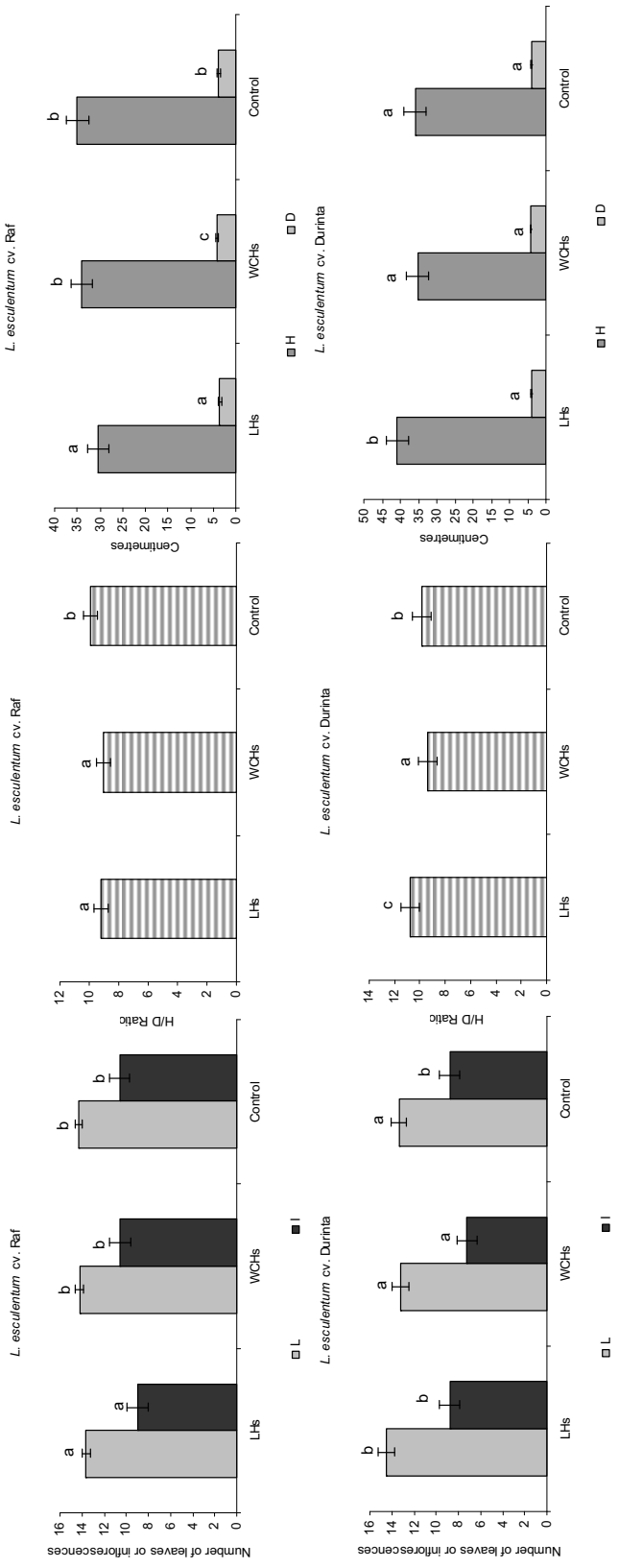

Fig. 5 Effects of two humic compounds, LHs and WCHs, on the growth of tomato plants. For each growth parameter, columns with the same letter(s) are not significantly different ($P < 0.05$). H: top height; L: leaf number; F: fruit number; D: stem diameter; I: inflorescence; H/D: height/diameter ratio.

2002). These effects mainly depend on the source, concentration and molecular weight of the HCs applied. However, many of the important functions of these substances remain obscure as their nature is not clear. In this sense, a low molecular size fraction is the major candidate for determining the positive effects of HCs on plant growth, since this frac-

Table 6 Effect of Sampling time, Substrate kind and Humic treatment on parameters related to fresh and dry vegetable biomass. Significant differences are observed at 95% confidence level ($p < 0.05$).

'Raf'										
Factors	**RFB**[1]	**SFB**[2]	**LFB**[3]	**IFB**[4]	**FFB**[5]	**RDB**[6]	**SDB**[7]	**LDB**[8]	**IDB**[9]	**FDB**[10]
	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Sampling time	0.0000	0.0000	0.0000	0.0000	0.0020	0.0000	0.0000	0.0000	0.0126	0.0004
Substrate kind	0.0000	0.0000	0.0000	0.0000	0.0375	0.0000	0.0000	0.0000	0.0179	0.0220
Humic treatment	ns[11]	0.0438	ns	ns	ns	ns	ns	ns	ns	ns
Interactions	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Sampling time X Substrate kind	0.0000	0.0000	0.0000	0.0003	0.0024	0.0000	0.0000	0.0000	ns	0.0005
Sampling time X Humic treatment	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Humic treatment X Substrate kind	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
'Durinta'										
Factors	**RFB**[1]	**SFB**[2]	**LFB**[3]	**IFB**[4]	**FFB**[5]	**RDB**[6]	**SDB**[7]	**LDB**[8]	**IDB**[9]	**FDB**[10]
	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Sampling time	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Substrate kind	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000
Humic treatment	ns	ns	0.0359	ns	ns	ns	ns	ns	ns	ns
Interactions	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Sampling time X Substrate kind	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Sampling time X Humic treatment	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Humic treatment X Substrate kind	ns	0.0097	0.0021	ns	ns	ns	ns	ns	ns	ns

[1] Root Fresh Biomass
[2] Stem Fresh Biomass
[3] Leaf Fresh Biomass
[4] Inflorescence Fresh Biomass
[5] Fruit Fresh Biomass
[6] Root Dry Biomass
[7] Stem Dry Biomass
[8] Leaf Dry Biomass
[9] Inflorescence Dry Biomass
[10] Fruit Dry Biomass
[11] non significant

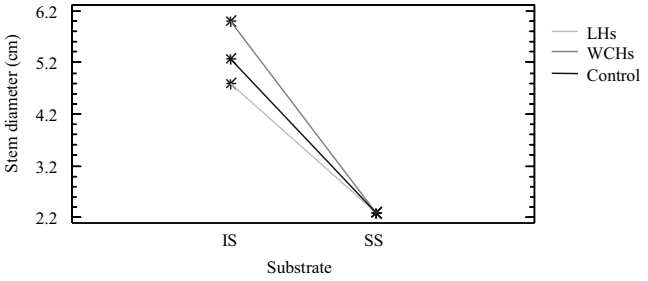

Fig. 6 Effect of the interactions between humic compounds and substrate on stem diameter: *Solanum lycopersicum* cv. 'Raf'-soil system. The ANOVA test decomposes the variability of stem Diameter into contributions due to the interactions between various factors. Since P-values are less than 0.05, these interactions have a statistically significant effect on stem diameter at the 95.0% confidence level.

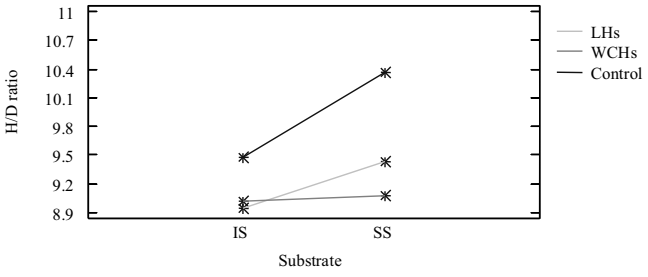

Fig. 7 Effect of the interactions between humic compounds and substrate on H/D ratio: *Solanum lycopersicum* cv. 'Raf'-soil system. The ANOVA test decomposes the variability of H/D ratio into contributions due to the interactions between various factors. Since P-values are less than 0.05, these interactions have a statistically significant effect on H/D ratio at the 95.0% confidence level.

tion easily reaches the inside of plant cells. Humates extracted from mature compost usually have a smaller molecular size than those extracted from fossil or soil samples (Guzmán 2003). Our results have shown that soil microorganisms easily use these amendments as a nutrient source causing the quick loss of these substances from the soil. Since they were added at a low rate, the subsequent availability of these substances for plants was minimal.

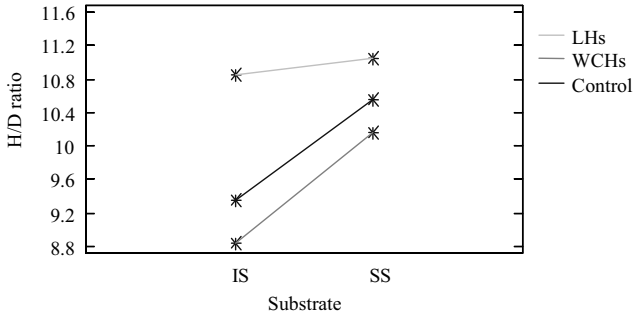

Fig. 8 Effect of the interactions between humic compounds and substrate on H/D ratio: *Solanum lycopersicum* cv. 'Durinta'-soil system. The ANOVA test decomposes the variability of H/D ratio into contributions due to the interactions between various factors. Since P-values are less than 0.05, these interactions have a statistically significant effect on H/D ratio at the 95.0% confidence level.

CONCLUSIONS

The application of low rates of HCs has an overall stimulatory effect on heterotrophic aerobic bacteria, fungi and nitrogen fixing-bacteria growth. The WCHs addition has a positive influence on nitrogen fixing-bacteria and nitrifying bacteria population when 'Raf' or 'Durinta' were used, respectively. Both groups were favoured when IS was used and generally during the second month of the assay. On the whole, lower and thicker plants were observed when WCHs were applied to crops.

Therefore, results derived from this preliminary work showed the potential for improving the utilization of HCs extracted from compost based on plant waste (WCHs). The extraction of these substances (WCHs) by the process here described produced an extract which behaved as a stimulatory substance on soil-plant ecosystems and some microorganisms related to the plant roots.

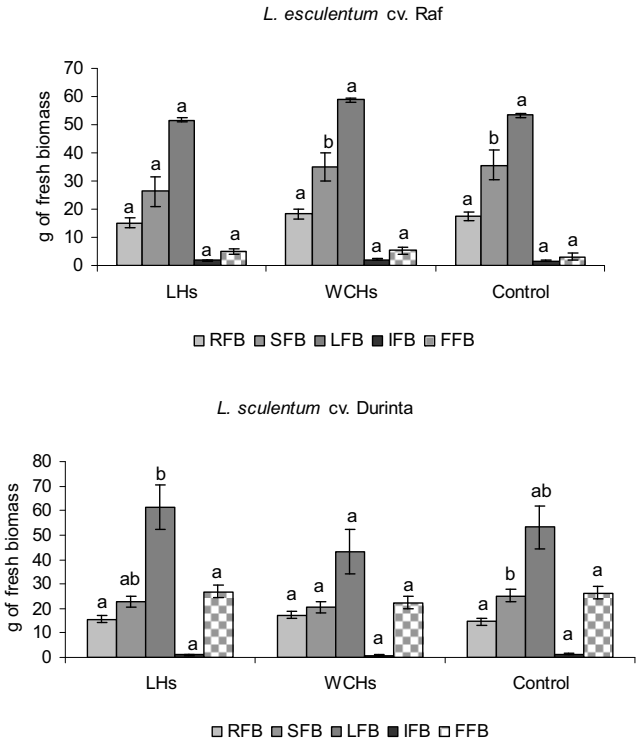

Fig. 9 Effects of two humic compounds, LHs and WCHs, on fresh vegetable biomass. For each growth parameter columns with the same letter(s) are not significantly different ($P < 0.05$). FFB: fruit fresh biomass; IFB: inflorescence fresh biomass; LFB: leaf fresh biomass; RFB: root fresh biomass; SFB: stem fresh biomass.

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REFERENCES

Acea MJ, Prieto-Fernández A, Diz-Cid N (2003) Cyanobacterial inoculation of heated soils: effect on microorganisms of C and N cycles and on chemical composition in soil surface. *Soil Biology and Biochemistry* **35**, 513-524

Albiach R, Canet R, Pomares F, Ingelmo F (2000) Organic matter components and aggregate stability after the application of different amendments to a horticultural soil. *Bioresource Technology* **76 (2)**, 125-129

Burk D (1930) The influence of nitrogen gas upon the catalysis of nitrogen fixation by *Azotobacter*. *Journal of Physical Chemistry* **34**, 1174-1194

Bertoldi M de, Vallini G, Pera A (1983) The biology of composting. *Waste Management Resources* **1**, 157-176

Cara J (1998) Residuos en la agricultura intensiva. El caso de Almería. Encuentro medio ambiental Almeriense: En busca de soluciones. Gestión de residuos. Almería, pp 128-132

Charest MH, Beauchamp CJ, Antoun H (2005) Effects of the humic substances of de-inking paper sludge on the antagonism between two compost bacteria and *Pythium ultimun*. *FEMS Microbiology and Ecology* **52**, 219-227

Clapp CE, Chen Y, Hayes MHB, Cheng HH (2001) Plant growth promoting activity of humic substances. In: Swift RS, Sparks KM (Eds) *Understanding and Managing Organic Matter in Soils, Sediments, and Waters*, International Humic Science Society, Madison, pp 243-255

Dowdy S, Wearden S (1991) *Statistics for Research* (2nd Edn), John Wiley and Sons, New York, pp 291-295, 302-305

Feritag M, Morrell JJ (1992) Decolorization of the polymeric dye Poly R-478 by wood-inhabiting fungi. *Canadian Journal of Microbiology* **38**, 811-822

Filip Z, Pecher W, Berthelin J (2000) Microbial utilization and transformation of humic acid-like substances extracted from a mixture of municipal refuse and sewage sludge disposed of in a landfill. *Environmental Pollution* **109**, 83-89

Gasser JRK (1985) *Composting of Agriculture and Other Wastes*, Elsevier, London, 320 pp

Goyal S, Dhull SK, Kapoor KK (2005) Chemical and biological changes during composting of different organic wastes and assessment of compost maturity. *Bioresource Technology* **96**, 1584-1591

Guzmán M (2003) Nutrición y Fertirriego. Suelo y Materia Orgánica. Servicio de Publicaciones de la Universidad de Almería. RNM 0151 PAI-UAL

He L, Bickerstaff GF, Paterson A, Buswell JA (1993) Purification and partial characterization of two xylanases that differ in hydrolysis of soluble and insoluble xylan fractions. *Enzyme Microbial Technology* **15**, 13-18

Huang GF, Wu QT, Wong JWC, Nagar BB (2006) Transformation of organic matter during co-composting of pig manure with sawdust. *Bioresource Technology* **97**, 1834-1842

Karui T, Kushner DJ (1988) Detection of cellulolytic activity of bacteria and fungi growing on agar surfaces. *Biotechnological Techniques* **2**, 149-152

Lhadi EK, Tazi H, Aylaj M, Genevini PL, Adani F (2006) Organic matter evolution during co-composting of the organic fraction of municipal waste and poultry manure. *Bioresource Technology* **97**, 2117-2123

Nardi S, Concheri G, Dell'Agnola G (1996) Biological activity of humic substances. In: Piccolo A (Ed) *Humic Substances in Terrestrial Ecosystems*, Elsevier, Amsterdam, pp 361-406

Nardi S, Pizzeghello D, Muscolo A, Vianello A (2002) Physiological effects of humic substances on higher plants. *Soil Biology and Biochemistry* **34**, 1527-1536

Pardo Parra JA (2003) Determinación de fitotoxicidad o efectos estimuladores inespecificos en un extracto húmico mediante bioensayos. Monographic Project. Higher Technical College. University of Almería, Spain, 155 pp

Pochon J, Tardieux P (1962) *Techniques d' Analyse en Microbiologie du Sol*, La Tourelle, France, 111 pp

Potter C, Meyer R (1990) The role of soil biodiversity in sustainable dryland farming systems. *Advance in Soil Science* **13**, 241-251

Schisler DA, Linderman RG (1989) Influence of humic-rich organic amendments to coniferous nursery soils on Douglas-fir growth, damping-off and associated soil microorganisms. *Soil Biology and Biochemistry* **21**, 403-408

Tejada M, García C, González JL, Hernández MT (2006) Use of organic amendment as a strategy for saline soil remediation: Influence on the physical, chemical and biological properties of soil. *Soil Biology and Biochemistry* **38**, 1413-1421

Valdrighi MM, Pera A, Scatena S, Agnolucci M, Vallini G (1995) Effects of humic acids extracted from mined lignite or composted vegetable residues on plant growth and soil microbial populations. *Compost Science Utilization* **3 (1)**, 30-38

Valdrighi MM, Pera A, Agnolucci M, Frassinetti S, Lunardi D, Vallini G (1996) Effects of composted-derived humic acids on vegetable biomass production and microbial growth within a plant (*Cichorium intybus*)-soil system: A comparative study. *Agriculture Ecosystem and Environment* **58**, 133-144

Vallini G, Pera A, Avio L, Valdrighi M, Giovannetti M (1993) Influence of humic acids on laurel growth, associated rhizospheric microorganisms, and mycorrhizal fungi. *Biology and Fertility of Soils* **16**, 1-4

Vallini G, Pera A, Agnolucci M, Valdrighi MM (1997) Humic acids stimulate growth and activity of in vitro tested axenic cultures of soil autotrophic nitrifying bacteria. *Biology and Fertility of Soils* **24**, 243-248

Varanini Z, Pinton R (2001) Direct versus indirect effects of soil humic substances on plant growth and nutrition. In: Pinton R, Varanini Z, Nannipieri P (Eds) *The Rizosphere*, Marcel Dekker, Basel, pp 141-158

Vaughan D, Malcom RE (1985) Influence of humic substances on growth and physiological processes. In: Vaughan D, Malcom RE (Eds) *Soil Organic Matter and Biological Activity*, Martinus Nijhoff/Junk W, Dordrecht, pp 37-76

Visser SA (1985a) Effects of humic acids on numbers and activities of microorganisms within physiological groups. *Organic Geochemistry* **8**, 81-85

Visser SA (1985b) Physiological action of humic substances of microbial cells. *Soil Biology and Biochemistry* **17**, 457-462