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Involvement of soluble organic matter in increased plant growth in solarized soils

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Abstract Although soil solarization is used to control soil-borne pests, it also results in increased growth response (IGR) of plants, beyond the effect of pest control. IGR is attributed to various abiotic factors (e.g. increased mineral nutrient concentrations) and biotic factors. In this work, we studied the role played by dissolved organic matter (DOM) in soil extracts in the IGR. DOM concentrations were about twice as high in solarized soil than in untreated soil. In two out of three soils, solarization appeared to increase amino acid synthesis, indicating that it had a favorable effect on microbial activity. Elemental composition, carbohydrate levels, E_4:E_6 ratios and FTIR spectra did not differentiate between DOM extracted from solarized soils and DOM extracted from untreated soils. Growth of corn plants increased with increasing concentrations of DOM. Addition to the soil of DOM extracted from leonardite increased populations of fluorescent pseudomonads, known as beneficial bacteria, and reduced fungal populations. We conclude that the increase in DOM concentration following soil solarization is a potentially positive plant-growth-enhancement factor.

Key words Humic substances · Fulvic acid · Solarization · Growth response · Fluorescent pseudomonads

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Introduction

Various soil disinfestation methods, e.g. steaming or fumigation, are used to control pathogens and weeds. Soil solarization is a relatively new disinfestation method. It is based on the solar heating of moistened soil by mulching it with transparent polyethylene, thereby leading to thermal killing of pests (Katan and DeVay 1991). Pest control by soil disinfestation frequently leads to improved plant growth, even in the absence of major pests. This unexpected beneficial effect has been verified in many instances and is recognized as an important side effect of soil disinfestation. Increased growth response (IGR) is a term frequently used to describe this phenomenon (Chen et al. 1991).

IGR is common to most disinfestation techniques, including soil solarization, and is attributed to a number of biotic and abiotic factors, including stimulation of beneficial microorganisms such as fluorescent pseudomonad bacteria, and increased nutrient levels in the soil solution (Chen and Katan 1980; Stapleton et al. 1985; Schippers et al. 1987; Chen et al. 1991; Gamliel and Katan 1991; Gruenzweig et al. 1993). Root exudates strongly affect the populations of these microorganisms (Rovira 1991; Gamliel and Katan 1992). Chen and Katan (1980) found increased concentrations of dissolved organic matter (DOM) and minerals in saturated extracts of solarized soils, and tomato-seedling growth on these extracts was enhanced. The IGR exhibited by these plants could be explained by either enhanced levels of macronutrients (most likely NO_3^-), or by improved uptake of micronutrients resulting from their solubilization by humic substances (Chen and Aviad 1990; Chen et al. 1991). The fact that a higher concentration of DOM was found following solarization along with the observed IGR, leads to the assumption that either the quality or concentration of the organic matter is linked to IGR in solarized soil. However, the role of DOM, if any, in IGR has not been studied.

The purpose of this study was to compare the soluble organic substances in extracts of solarized and untreated soils and to examine their possible involvement in IGR.

Materials and methods

Soils

Three common agricultural soils from Israel, with different textures, were selected. An analysis of their particle size composition, texture, organic matter and carbonate content is shown in Table 1. Soil textures ranged from sand to clay loam, their organic matter content from 0.8 to 1.7% and their $CaCO_3$ level from 0.2 to 10.5% (Table 1).

Soil treatment and extraction

Solarization was achieved by mulching the moistened soils for 40 days with transparent polyethylene. Control soils were similarly treated, excluding mulching. Plots of these soils were exposed in the field, with or without solarization treatment, according to the procedure described by Chen and Katan (1980). Ten days after the termination of solarization, the soils were sampled and air-dried. The samples were collected from the upper 20 cm after removing the top 2 cm. Saturated soil extracts were then obtained from untreated (control) and solarized soils from each of the sites. The water contents of the saturated soils before extraction were 100, 30 and 53% for the Rehovot (R), Nir-Yitzhak (NY) and Ein-Dor (ED) soils, respectively. Four liters of extract were obtained from each soil. The organic matter concentration was determined by a modified wet-digestion procedure (the Walkley-Black method adapted to low concentrations of organic matter). The solutions were concentrated on a Rotovapor at temperatures of 34–38 °C. The concentrates, containing humic substances, were purified by the International Humic Substances Society (IHSS) procedure described by Calderoni and Schnitzer (1984). After purification, the materials were freeze-dried and stored. The following analyses were performed on these materials: elemental analyses using a C,H,N,S,O analyzer (Fisons, Milano, Italy), composition of amino acids using the method described by Sowden et al. (1977) and Chen et al. (1977), E_4:E_6 measurements (Stevenson 1994) on a PYE-UNICAM spectrophotometer (PU 8600 uv-vis), and Fourier transform infra-red (FTIR) spectra recorded on a Nicolet model nx-s spectrophotometer.

Plant growth experiment

In addition to determining chemical and spectroscopic properties of the DOM, a bioassay was performed using corn plants (*Zea mays* L. cv Jubilee). The plants were grown in an aerated nutrient solution composed of the following (Marschner et al. 1986):

$7(10^{-4}$ M $K_2SO_4)$; 10^{-4} M KCl; $2[10^{-3}$ M $Ca(NO_3)_2]$;
$5(10^{-4}$ M $MgSO_4)$; 10^{-4} M KH_2PO_4; 10^{-5} M H_3BO_3;
$5(10^{-7}$ M $MnSO_4)$; $5(10^{-7}$ M $ZnSO_4)$; $2(10^{-7}$ M $CuSO_4)$ and
10^{-8} M $(NH_4)_6Mo_7O_{24}$

Humic acid (HA) used in plant growth assays was extracted and purified from untreated and solarized R soils, according to the IHSS procedure, and used as a model compound for soil organic matter. Seedlings were prepared by germinating seeds for 7 days on paper dipped in a solution of 0.01 M $CaSO_4$ until they reached a length of about 7 cm. The seedlings were then transferred to the nutrient solution, placed in 0.5-l buckets, and 0, 25, 50 or 75 mg l^{-1} of HA added. The pH was maintained constant at 6.5 during the growth period using a 0.01 N MES buffer solution. The experiment was conducted in a growth chamber, under the following climatic conditions: day/night photoperiod, 16/8 h; day/night temperature, 28/25 ± 1.5 °C; light intensity, 26 W m^{-2} (fluorescent tubes, Osram L 40 W/20 S, cool white 3000 1 m). During the 3-week growth period, the solutions were discarded once (after 10 days) and replaced with new ones. At the end of the growth period, the plants were harvested and fresh and dry weights of roots and shoots were determined.

Statistical analysis of the results was performed using Duncan's multiple range test, at $P \leq 0.05$.

Microbial studies

A solution of humic substances extracted from leonardite was mixed with the R soil at various concentrations. Soil was untreated, or supplemented with tomato root exudate, at 4 mg g^{-1} soil. Fluorescent pseudomonad bacteria and fungi were counted after 4 days at 27 °C. Methods for extracting root exudates and assessing the number of fluorescent pseudomonads and fungi were as previously described (Gamliel and Katan 1991, 1992). Microbial counts were expressed as colony forming units (CFU) per gram of soil.

Results

The concentrations of DOM in the three different untreated and solarized soils (ED, NY and R) are shown in Fig. 1. DOM levels increased from about 35 mg l^{-1} in the untreated soils to 70–90 mg l^{-1} in the solarized soils.

The DOM was concentrated from samples of both solarized and untreated saturated paste soil extract, then purified to separate the DOM from the ash. The elemental analysis of the DOM is presented in Table 2, and it is essentially the same for solarized and untreated soils, suggesting that the elemental composition of this fraction was not affected by solarization.

Carbohydrates are of significance to soil-structure formation due to their involvement in soil-aggregate stabilization (Oades and Waters 1991). The hypothesis that the IGR effect is related to an increase in soil carbohydrates was therefore examined (Table 3). Differences between the carbohydrate contents of untreated vs. solarized soils ranged from 0.6 to 1.2%. In the solar-

Table 1 Soil type, particle size analysis, texture, organic matter and carbonate (*$CaCO_3$*) content of the three tested soils

Soil	Soil type	Particle size analysis (%)			Texture	Organic matter (%)	$CaCO_3$ (%)
		Sand	Silt	Clay			
Rehovot	Rhodoxeralf	96.2	0.0	3.8	Sand	0.8	0.2
Nir-Yitzhak	Psamment	87.5	2.5	10.0	Sand	0.5	10.5
Ein-Dor	Xerofluvent	25.0	32.5	42.5	Clay loam	1.7	2.7

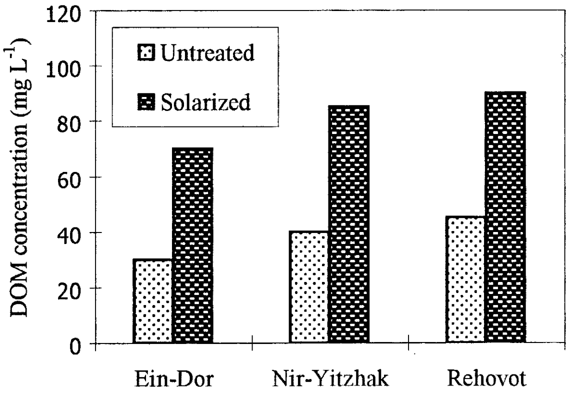

Fig. 1 Effect of soil solarization on dissolved organic matter (*DOM*) concentration in saturated soil extracts of three soils

Table 2 Elemental composition of the DOM (% of air-dried and ash-free basis)

Soil	Treatment	C	H	N	S	O
Rehovot	Untreated	49.1	5.6	2.9	1.1	41.3
	Solarized	50.7	5.6	1.5	1.3	40.9
Nir-Yitzhak	Untreated	49.3	5.5	2.6	2.5	40.1
	Solarized	51.4	5.3	2.3	1.4	38.3
Ein-Dor	Untreated	50.7	5.5	3.0	2.9	37.9
	Solarized	51.4	5.8	3.1	2.9	36.8

Table 3 Carbohydrate concentration in DOM extracted from solarized and untreated soils (% of dry weight)

Soil	Treatment	Total carbohydrates
Ein-Dor	Untreated	7.4 ± 0.4
	Solarized	8.0 ± .3
Nir-Yitzhak	Untreated	6.9 ± 0.3
	Solarized	5.7 ± 0.2
Rehovot	Untreated	5.4 ± 0.4
	Solarized	4.5 ± 0.3

ized ED soil, a slightly higher level of carbohydrates was detected, whereas in the NY and R soils, the carbohydrate concentrations were higher in the untreated soils. Thus, there was no evidence relating IGR to carbohydrate levels in solarized soils.

Soil humic substances contain a variety of amino acids, most of which appear to be of microbial origin. Earlier reports (Sowden et al. 1977) have shown that humic acids (HAs) contain a larger and smaller fraction of basic and acidic amino acids, respectively, than fulvic acids (FAs). Since FAs are produced first in the formation of humic substances (Stevenson 1994), changes in the relative composition of amino acids could indicate enhanced humification during solarization, and/or the formation of DOM factors relevant to the IGR phenomenon.

The results of the amino acid analyses, based on both the concentration of each amino acid in ash-free DOM and the percentage of total N in each amino acid, are presented in Tables 4 and 5, respectively, for DOM extracted from solarized and untreated soils. Total amino acid N concentration (Table 4) increased following solarization in the ED and NY soils, but decreased slightly in the R soil. The concentration of the S-containing amino acids decreased in all instances. The fact that only slight differences were observed between the amino acid compositions of solarized and untreated soils suggests that the process taking place in solarized soils involves mainly solubilization of low-molecular-weight humic substances, rather than enhancement of their formation.

FTIR spectra

DOM extracted from the three soils, both solarized and untreated, was freeze-dried, and the FTIR spectra recorded and compared to an IHSS standard FA (Armadale FA, Canada). The FTIR spectra (Fig. 2) are generally considered to be ‘fingerprints’, similar spectra indicating similarities in functionalities within the FA molecules. The main peaks observed in all spectra were at (cm^{-1}): 3300–3400 – H bond, OH stretching; 2900 – aliphatic CH, asymmetric stretch; 2850 – CH_2, asymmetric stretch; 2520–2580 – calcite CO_3^{2-}; 1720–1740 – CO stretch of carboxyl groups; 1650 – aromatic CC; 1450 – CH deformation of CH_2 or CH_3; 1250–1200 – aromatic C = C, CO stretch; 1000–1100 – CO stretch of polysaccharides.

The FTIR spectra presented in Fig. 2 clearly show that: (1) all spectra are similar to that of the Armadale FA, suggesting that the DOM fractions are indeed FAs; (2) great similarity exists between spectra obtained from DOM originating from solarized soils and those obtained from the untreated soils. This leads us to the conclusion that solarization had no significant effect on the composition of the DOM, in accordance with the observations described in Tables 2–5. It should be remembered, however, that the concentration of DOM was increased by solarization in the three tested soils (Fig. 1).

Effects of humic substances extracted from solarized or untreated soils on plant growth

Corn plants were grown in nutrient solutions containing HA at increasing concentrations (0–75 mg l^{-1}). The HA originated from the R soil, either solarized or untreated. These humic substances were used as model compounds to test the effects of organic matter from solarized and untreated soils on plant growth. We could not use DOM due to the large volumes of soil extracts that would be required to conduct plant growth assays. Following a growth period of 3 weeks, the seedlings were tested for both shoot and root fresh weight. The

Table 4 Amino acid concentration ($\mu g\ g^{-1}$) in the DOM extracted from untreated and solarized soils

Amino acid	Ein-Dor		Nir-Yitzhak		Rehovot	
	Untreated	Solarized	Untreated	Solarized	Untreated	Solarized
Acidic						
Aspartic	461	641	403	604	612	656
Glutamic	348	501	408	362	430	413
Basic						
Lysine	250	373	261	561	422	443
Histidine	84	131	143	190	112	76
Arginine	190	232	193	204	231	205
Neutral						
Threonine	289	351	246	240	294	269
Serine	279	347	275	486	337	287
Proline	305	408	273	247	303	340
Glycine	983	1276	1216	1213	1095	1027
Alanine	515	677	356	429	534	452
Valine	234	346	203	201	283	293
Isoleucine	134	178	127	107	142	154
Leucine	185	243	174	174	203	220
Tyrosine	36	44	60	45	53	35
Phenylalanine	69	103	78	68	78	84
S-containing						
Cystine	23	9	54	0	23	12
Methionine	43	50	48	0	57	0
Total amino acid N	4428	5910	4518	5131	5209	4966
Total acidic	809	1142	811	966	1042	1069
Total basic	52	736	597	955	765	724
Total neutral	3029	3973	3008	3210	3322	3161
Total S-containing	66	59	102	0	80	12

Table 5 Distribution of N in the various amino acids (% of total amino acid N in soluble organic matter)

Amino acid	Ein-Dor		Nir-Yitzhak		Rehovot	
	Untreated	Solarized	Untreated	Solarized	Untreated	Solarized
Acidic						
Aspartic	10	11	9	12	12	13
Glutamic	8	8	9	7	8	8
Basic						
Lysine	6	6	6	11	8	9
Histidine	2	2	3	4	2	2
Arginine	4	4	4	4	4	4
Neutral						
Threonine	7	6	5	5	6	5
Serine	6	6	6	9	6	6
Proline	7	7	6	5	6	7
Glycine	22	22	27	24	21	21
Alanine	12	11	8	8	10	9
Valine	5	6	4	4	5	6
Isoleucine	3	3	3	2	3	3
Leucine	4	4	4	3	4	4
Tyrosine	1	1	1	1	1	1
Phenylalanine	2	2	2	1	1	2
S-containing						
Cystine	1	0	1	0	0	0
Methionine	1	1	1	0	1	0
Total amino acid N	100	100	100	100	100	100
Total acidic	18	19	18	19	20	22
Total basic	12	12	13	19	15	15
Total neutral	68	67	67	63	64	64
Total S-containing	1	1	2	0	2	0

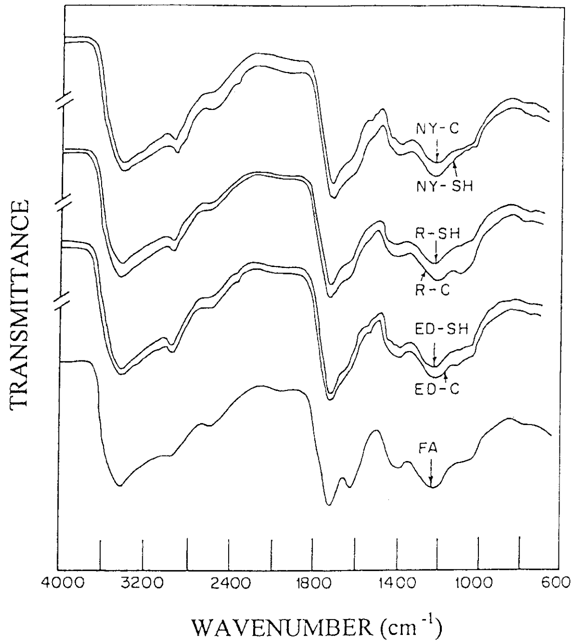

Fig. 2 Infrared spectra of DOM extracted from three soils, which had been either solarized (*SH*) or untreated (*C*), compared to fulvic acid (*FA*) extracted from Armadale soil (Canada). *NY* Nir-Yitzhak; *R* Rehovot; *ED* Ein-Dor

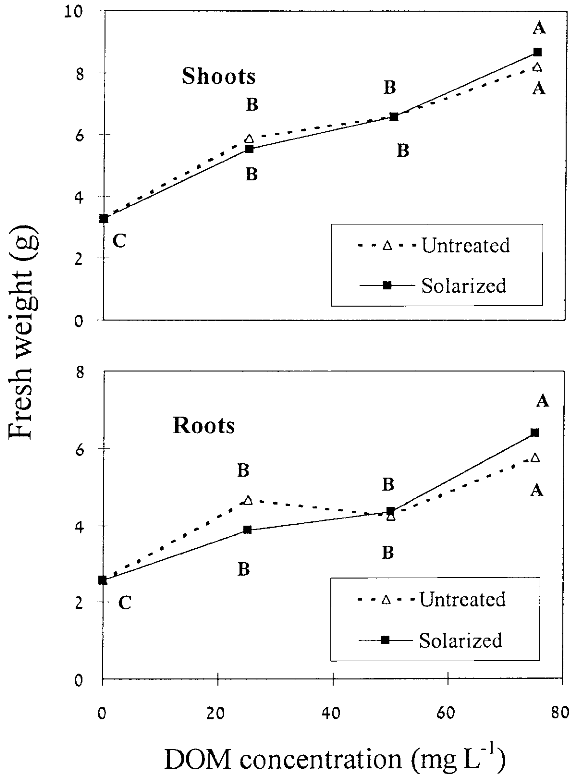

Fig. 3 Effect of the concentration of dissolved organic matter (*DOM*) extracted from Rehovot soil, which had been either untreated (*open triangles*) or solarized (*solid squares*), on the fresh weight of shoots (*upper*) and roots (*lower*) of corn plants in nutrient solution. A two-way factorial design with interaction was employed. Pairs of means were compared using Duncan's test at the 0.05 level. Values with the same letter do not differ significantly

results of this experiment are shown in Fig. 3. The fresh weights of both roots and shoots of corn plants grown in solutions containing identical concentrations of HA originating from either solarized or untreated soils were similar. However, in both cases increasing concentrations of HA significantly improved both shoot and root growth.

Effect on microbial populations

The population of fluorescent pseudomonad bacteria in the tested soil was below detection level (Fig. 4). The addition of root exudates, or a leonardite extract, or both, increased their populations to a great extent. Leonardite extract increased the bacterial population to a higher extent than root exudates alone. The highest number of these bacteria was obtained at 100 μg/g of leonardite with root exudates. Root exudates increased the number of fungi by 117%, but in contrast to bacteria, leonardite reduced the number of fungi, especially at 100 μg leonardite extract (dry weight) per g soil.

Discussion

The data presented in this study (three soils; Fig. 1) and in an earlier publication (nine soils) by Chen and Katan (1980) show that solarization resulted in increased concentrations of DOM. However, carbohydrate analyses and FTIR showed that the DOM extracted from untreated soils was very similar to that extracted from solarized soils. Elemental analyses of the DOM in samples extracted from solarized and untreated soils were also very similar. FTIR measurements revealed that the spectra of the DOM extracted from solarized and untreated soils resembled that of a reference FA extracted from Armadale soil in Canada, so that FA appeared to be the major component of the DOM extracted from the three soils before and after solarization. Also, humic substances that were extracted from either untreated or solarized soil had similar effects on plant growth (Fig. 3).

Our hypothesis is that the increased concentration of DOM results from a mild hydrolysis or depolymerization of the organic matter in the soil, at the elevated temperatures induced by solarization. The wet (nearly field-capacity) and high-temperature conditions (40–50 °C for several hours each day, for 40 days) prevailing in the solarized soils (Katan and DeVay 1991) apparently enhanced mild hydrolysis and conversion of small fractions of the DOM into soluble forms. Similar observations have been made during the composting of

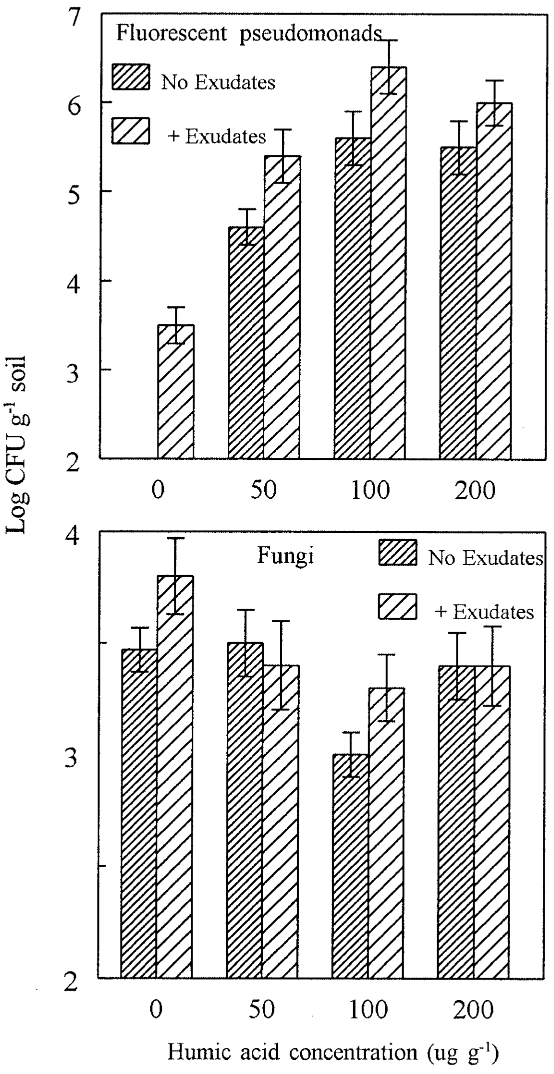

Fig. 4 Effect of humic acid (*HA*) extracted from leonardite and of tomato root exudates (at 4 mg g^{-1} soil) on populations of fluorescent pseudomonads (*upper*) and fungi (*lower*) in Rehovot soil. *CFU* Colony forming units. *Bars* represent ± SE

organic matter, which takes place over a similar temperature range (Inbar et al. 1989). Other investigators (Yarden et al. 1989) have shown that an increase in hydrolytic activity occurs at elevated reaction temperatures in the tested soils. The fact that significant increases in amino acid concentrations after solarization in two of the three soils suggests that solarization created favorable conditions for the microbial synthesis of amino acids, which were subsequently detected after hydrolysis.

Many investigators have shown that humic substances can enhance plant growth when present in the nutrient or soil solution at low concentrations. Comprehensive reviews summarizing these observations have been published by Vaughan and Malcolm (1985), Chen and Aviad (1990) and Chen et al. (1994). These investigators reported optimum plant-growth enhancement when humic substances in concentrations of 25 to 150 mg l^{-1} were added to the nutrient solution. The mechanism suggested by a number of researchers is the enhanced solubility of (mostly microelements) in solution and therefore also an enhanced nutrient uptake and plant growth.

In this study, we also show that humic substances increase the population of fluorescent pseudomonads. The beneficial effects of siderophores produced by this group of microorganisms have been demonstrated by Jurkevitch et al. (1986), who studied interactions of various strains of *Pseudomonas fluorescens* with Fe-deficient plants. Gamliel and Katan (1991) have shown that fluorescent bacteria stimulate the growth of tomato plants. Moreover, fluorescent pseudomonads play an important role in the biological control of pathogens in many soils (Thomashow and Weller 1990; Keel 1992). Thus, humic substances (HAs and FAs), in the presence of pathogens, may also have beneficial effects on plant health, beyond effects in IGR. Some fungi have adverse effects on plant growth (Schippers et al. 1987; Gamliel and Katan 1991). Since leonardite applications reduced fungal populations (Fig. 4), this microbial population shift may also contribute to IGR.

In summary, we suggest that the observed increase in DOM concentration in the soil solutions following solarization, from about 35 mg l^{-1} to values ranging from 70–90 mg l^{-1} is a potentially positive plant-growth enhancement factor. IGR is obviously a complex phenomenon attributed to various chemical, physical and biological factors. Humic substances are an additional important factor to be considered in future studies.

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