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The influence of humic acids derived from earthworm-processed organic wastes on plant growth

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Abstract

Some effects of humic acids, formed during the breakdown of organic wastes by earthworms (vermicomposting), on plant growth were evaluated. In the first experiment, humic acids were extracted from pig manure vermicompost using the classic alkali/acid fractionation procedure and mixed with a soilless container medium (Metro-Mix 360), to provide a range of 0, 50, 100, 150, 200, 250, 500, 1000, 2000, and 4000 mg of humate per kg of dry weight of container medium, and tomato seedlings were grown in the mixtures. In the second experiment, humates extracted from pig manure and food wastes vermicomposts were mixed with vermiculite to provide a range of 0, 50, 125, 250, 500, 1000, and 4000 mg of humate per kg of dry weight of the container medium, and cucumber seedlings were grown in the mixtures. Both tomato and cucumber seedlings were watered daily with a solution containing all nutrients required to ensure that any differences in growth responses were not nutrient-mediated. The incorporation of both types of vermicompost-derived humic acids, into either type of soilless plant growth media, increased the growth of tomato and cucumber plants significantly, in terms of plant heights, leaf areas, shoot and root dry weights. Plant growth increased with increasing concentrations of humic acids incorporated into the medium up to a certain proportion, but this differed according to the plant species, the source of the vermicompost, and the nature of the container medium. Plant growth tended to be increased by treatments of the plants with 50–500 mg/kg humic acids, but often decreased significantly when the concentrations of humic acids derived in the container medium exceeded 500–1000 mg/kg. These growth responses were most probably due to hormone-like activity of humic acids from the vermicomposts or could have been due to plant growth hormones adsorbed onto the humates.

Keywords: Vermicompost; Compost; Earthworm; Humic acid; Plant growth; Hormone activity

1. Introduction

It is well established that earthworms have beneficial physical, biological and chemical effects on soils and many researchers have demonstrated that these effects can increase plant growth and crop yields in both natural and managed ecosystems (Edwards and Bohlen, 1996; Edwards, 1998). These beneficial effects have been attributed to improvements in soil properties and structure (Kahsnitz, 1992), to greater availability of mineral nutrients to plants (Gilot, 1997), and to increased microbial populations and biologically active metabolites such as plant growth regulators (Tomati and Galli, 1995; Doube et al., 1997). In recent years, the applied use of earthworms in the breakdown of a wide range of organic residues, including sewage sludge, animal wastes, crop residues, and industrial refuse, to produce vermicomposts has increased tremendously (Mitchell et al., 1980; Reinecke and Venter, 1987; Edwards and Neuhauser, 1988; Chan and Griffiths, 1988; Hartenstein and Bisesi, 1989; Haimi, 1990; van Gestel et al., 1992; Dominguez and Edwards, 1997; Edwards, 1998; Kale, 1998). The earthworms fragment the organic waste substrates, stimulate microbial activity greatly and increase rates of mineralization, rapidly converting the wastes into humus-like substances with a finer structure than composts but possessing a greater and more diverse microbial activity, commonly referred to as vermicomposts (Elvira et al., 1996, 1998; Atiyeh et al., 2000b). The effects of vermicomposts on the growth of a variety of crops including cereals and legumes, vegetables, ornamental and flowering plants have been assessed in the greenhouse and to a lesser degree in field crops (Chan and Griffiths, 1988; Edwards and Burrows, 1988; Wilson and Carlile, 1989; Mba, 1996; Thankamani et al., 1996;

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Buckerfield and Webster, 1998; Vadiraj et al., 1998; Buckerfield et al., 1999; Nethra et al., 1999; Atiyeh et al., 1999, 2000c,d). These investigations have demonstrated consistently that vermicomposted organic wastes have beneficial effects on plant growth independent of nutrient transformations and availability. Whether they are used as soil additives or as components of horticultural soilless container media, vermicomposts have consistently improved seed germination, enhanced seedling growth and development, and increased plant productivity much more than would be possible from the mere conversion of mineral nutrients into more plant-available forms. The greatest plant growth responses and yields have occurred usually when vermicomposts constituted a relatively small proportion (10–40%) of the total volume of the plant growth medium in which they are incorporated. Usually, greater proportions of vermicomposts substituted in growth media have not increased plant growth as much as smaller proportions (Subler et al., 1998; Atiyeh et al., 1999, 2000a,c,d, 2001, 2002). There are very few data in the literature on possible mechanisms by which vermicomposts produce these growth enhancement effects. However, it has been shown that the incidence of plant diseases can be limited by vermicomposts (Szczech et al., 1993; Nakamura, 1996), the activity of vesicular arbuscular mycorrhizae is enhanced (Cavender et al., 1999), and plant parasitic nematode population are suppressed.

Additionally, there are a number of references in the literature that show that plant growth regulators, such as indole-acetic acids (auxins), gibberellins and cytokinins, are produced by microorganisms, and there have been suggestions that the promotion of microbial activity in organic matter by earthworms would result in production of significant quantities of plant growth regulators (Tomati et al., 1983, 1988, 1990; Tomati and Galli, 1995; Krishnamoorthy and Vajranabhiah, 1986; Edwards, 1998). Earthworm activity accelerates the humification of organic matter, and their influence in increasing microbial populations enhances the presence of auxins and gibberellin-like substances as well as humic acids (Casenave de Sanfilippo et al., 1990). Moreover, humic acids have also been shown to stimulate plant growth in auxin, gibberellin and cytokinin bioassays (Phuong and Tichy, 1976).

Vermicomposts originating from animal manures, sewage sludges or paper-mill sludges have been shown to contain large amounts of humic substances (Albanell et al., 1988; Petrussi et al., 1988; Hervas et al., 1989; Senesi et al., 1992; Garcia et al., 1995; Masciandaro et al., 1997; Elvira et al., 1998). Studies of the positive effects of these humic substances on plant growth, when full requirements for mineral nutrition, have resulted in consistently positive effects on growth independent of nutrition (Chen and Aviad, 1990). For instance, in controlled experiments, humic substances increased dry matter yields of corn and oat seedlings (Lee and Bartlett, 1976; Albuzio et al., 1994); numbers and lengths of tobacco roots (Mylonas and Mccants, 1980); dry weights of shoots, roots, and nodules of soybean, peanut, and clover plants (Tan and Tantiwiramanond, 1983); vegetative growth of chicory plants (Valdrighi et al., 1996); and induced shoot and root formation in tropical crops grown in tissue culture (Goenadi and Sudharama, 1995). The typical growth response curves that have been reported to result from treating plants with humic substances show progressively increased growth with increasing concentrations of humic substances, but there is usually a decrease in growth at higher concentrations of the humic materials (Chen and Aviad, 1990). Hypotheses accounting for this stimulatory effect of humic substances at low concentrations are numerous, the most convincing of which hypothesizes a "direct" action on the plants, which is hormonal in nature, together with an "indirect action" on the metabolism of soil microorganisms, the dynamics of uptake of soil nutrients, and soil physical conditions (Cacco and Dell'Agnola, 1984; Nardi et al., 1988; Albuzio et al., 1989; Casenave de Sanfilippo et al., 1990; Chen and Aviad, 1990; Muscolo et al., 1993, 1996, 1999). Other mechanisms which have been suggested to account for promotion of plant growth by humic substances include: enhanced uptake of metallic ions and increases in cell permeability (Chen and Aviad, 1990).

During the last decade, the biological activities of humic substances, particularly those derived from earthworm feces, have begun to be investigated (Dell'Agnola and Nardi, 1987; Nardi et al., 1988; Muscolo et al., 1993, 1996, 1999). Dell'Agnola and Nardi (1987) reported hormone-like or plant-growth regulator effects, of depolycondensed humic fractions obtained from the feces of the earthworms *Apporectodea rosea* (Eisen) and *Apporectodea caliginosa* (Sav), on plants. Nardi et al. (1988) reported that humic materials produced in the feces of *A. rosea* and *A. caliginosa* exhibited auxin-, gibberellin-, and cytokinin-like activities. Treating carrots cells with humic substances obtained from the feces of the earthworm *A. rosea* increased their growth and induced morphological changes similar to those produced by auxins (Muscolo et al., 1999). It seems very likely that vermicomposts, which consist of an amalgamate of humified earthworm feces and organic matter, stimulated plant growth beyond that produced by mineral nutrients because of the effects of the humic substances present in the vermicomposts or due to plant growth regulators associated with the humic acids.

2. Methods

We used two separate greenhouse experiments to assess the effects of humic acids derived from vermicomposts on plant biomass production. In both studies, humic acids were extracted using the classic alkali/acid fractionation procedure (Valdrighi et al., 1996). The vermicompost was digested in 0.1 N KOH (1:10 w/v) for 24 h at room temperature. The undigested bulk residue from each vermicompost was then separated from the solute fraction by centrifugation at 5000 rpm for 30 min followed by vacuum filtration through a glass filter paper. The filtered supernatant was acidified to pH 2 with 6.0 N H_2SO_4 and kept in a cold room in the dark for 24 h in order to obtain flocculation of humic acids. After acidification, the humic precipitate (humate) was collected by centrifuging at 5000 rpm equivalent to $4.19 \times 10^5 g$ Relative Centrifugal Force (RCF) for 30 min, washed three times with distilled water to remove residual H_2SO_4, freeze-dried, then ground with a mortar and a pestle into a brown powder.

In the first experiment, humic acids were extracted from a commercially produced pig manure vermicompost. This was provided by Vermicycle Organics (Charlotte, NC) and consisted of separated pig solids processed by earthworms (*Eisenia fetida* Savigny) in indoor beds. The humates, extracted from pig manure vermicompost, were mixed with a commercial container medium, Metro-Mix 360 (Scotts, Marysville, OH), to provide a range of 0, 50, 100, 150, 200, 250, 500, 1000, 2000, and 4000 mg of humate per kg of dry weight of the container medium. Metro-Mix 360 is a soilless greenhouse container medium prepared from vermiculite, Canadian sphagnum peat moss, bark ash and sand. It contains 31.78% organic C, 93 μg/g NH_4, 77 μg/g NO_3, 0.43% total N, 0.15% total P, and 1.59% total K. Three tomato seeds ('Rutgers') were sown in plastic pots (7.5 cm in diameter), each containing 50 g of the humate/Metro-Mix 360 potting material, with 10 replicate pots per humate concentration. After sowing, the pots were placed in a mist house until the seeds germinated. After germination, seedlings were thinned to one plant per pot and the pots were moved into an environmentally controlled glasshouse. Seedlings were watered daily with 20–10–20 (100 ppm N) Peters Professional plant nutrient solution to eliminate nutrient limitations. Peters Professional is a water-soluble fertilizer that is recommended for continuous liquid feed programs of plants, and contains 7.77% NH_4–N, 12.23% NO_3–N, 10% P_2O_5, 20% K_2O, 0.15% Mg, 0.02% B, 0.01% Cu, 0.1% Fe, 0.056% Mn, 0.01% Mo, and 0.0162% Zn. Twenty-one days later, plant heights (distances from the potting mixture level to the top leaf node) and total leaf areas of each of the seedlings were measured. Plants were then removed from the potting mixtures, separated into shoot and root portions, and oven-dried at 60 °C for three days to determine their dry weights. Dried leaves were detached from the stems, ground with a ball mill and analyzed for tissue N concentration on a Carlo Erba NA 1500 C/N analyzer.

In the second experiment, humates were extracted from the same pig manure vermicompost, and also from a food waste vermicompost using the same extraction methods. The commercially produced food (fruit and vegetable) waste-based vermicompost was provided by Oregon Soil Corporation (Portland, Oregon) and consisted of waste pre-consumer supermarket produce processed in a continuous flow automated vermicomposting reactor (Edwards, 1998) by *E. fetida*. Each batch of humates was mixed with vermiculite, to provide a range with 0, 50, 125, 250, 500, 1000, and 4000 mg of humate per kg of dry weight of the container medium, vermiculite. Vermiculite (42% SiO_2, 13% Al_2O_3, 26% MgO, 3% CaO, 4% K_2O, 10% Fe_2O_3, 2% TiO_2) was chosen for its ability to hold water and its inert chemical properties, minimizing the interaction of humates with the container medium. Cucumber ('Long Green') seeds were sown in plastic pots (7.5 cm in diameter) in a glasshouse, each pot containing 80 g of the humate/vermiculite potting material mixtures. There were 15 replicate pots per humate concentration for the two humate sources. Seedlings were watered daily with Peters Professional plant nutrient solution as in the first experiment. Sixteen days after germination, plants were removed from the potting mixtures, separated into shoot and root portions, and oven-dried to determine their dry weights. The basic chemical characteristics of both types of humates are summarized in Table 1.

Data were analyzed statistically by one-way ANOVA in a general linear model using SAS (SAS Institute, 1990). For each source of humates, the means of each plant growth parameter that was measured were separated statistically using Tukey's multiple range test, with significance defined as $P \leqslant 0.05$.

3. Results

The incorporation of 150, 200, 250 and 500 mg/kg of pig manure vermicompost humates into Metro-Mix 360 increased the heights and the leaf areas of tomato seedlings grown in these mixtures significantly,

Table 1
Elemental analysis of humates extracted from food waste and pig manure vermicomposts

Vermicompost type	N (mg/g)	P (mg/g)	K (mg/g)	Ca (mg/g)	Fe (mg/g)	Mg (mg/g)
Pig manure	46.6	2.19	15.46	0.77	0.72	0.14
Food waste	47.2	1.01	25.61	2.91	4.41	0.27

compared to those grown in Metro-Mix 360 controls (Fig. 1), with the greatest plant heights (19.45 cm) occurring in potting mixtures containing 200 mg/kg humates, whereas greatest leaf areas (269 cm^2) occurred in potting mixtures containing 500 mg/kg humates. The dry weights of shoots of tomato seedlings (Fig. 2) grown in mixtures containing 200, 250, and 500 mg/kg humates were 47.0%, 37.4%, and 43.4% greater than those of seedlings grown in Metro-Mix 360 controls. The roots of tomato seedlings (Fig. 2), grown in mixtures containing 250, 500, and 1000 mg/kg pig manure vermicompost humates, weighed (dry weights) 77.5%, 79.3%, and 72.1%, respectively, more than those of seedlings grown in the controls with no humates (0.11 g). Tomato seedlings grown in the potting mixtures with 500 mg/kg humates had the greatest root dry weights (0.20 g). Growth (height, leaf area, shoot and root dry weights) of tomato seedlings in potting mixtures containing more than 500 mg/kg of pig manure vermicompost humates was often reduced and was similar to those of tomato seedlings grown in Metro-Mix 360 controls with no humates. The root to shoot ratios of tomato seedlings grown in all humate/Metro-Mix 360 potting mixtures, with the exception of those containing 50, 200 and 2000 mg/kg humates, were significantly greater than those of plants grown in the controls with no humates (Fig. 2). The root to shoot ratios of tomato seedlings grown in the potting mixtures containing 4000 mg/kg were the largest, and were 1.5 times greater than those of plants

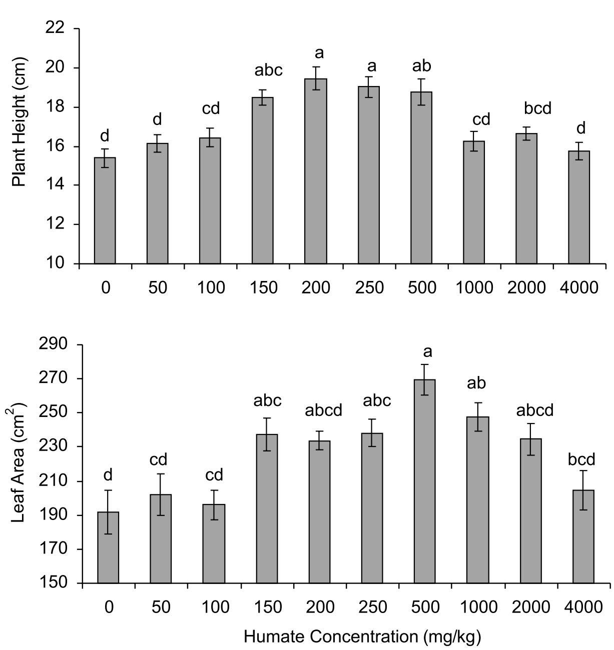

Fig. 1. Average plant heights and leaf areas of tomato seedlings (mean ± standard error) grown in a standard commercial potting medium (Metro-Mix 360) containing different concentrations of vermicompost-derived humic acids, with all nutrients needed supplied. Columns followed by the same letter do not differ significantly ($P \leqslant 0.05$).

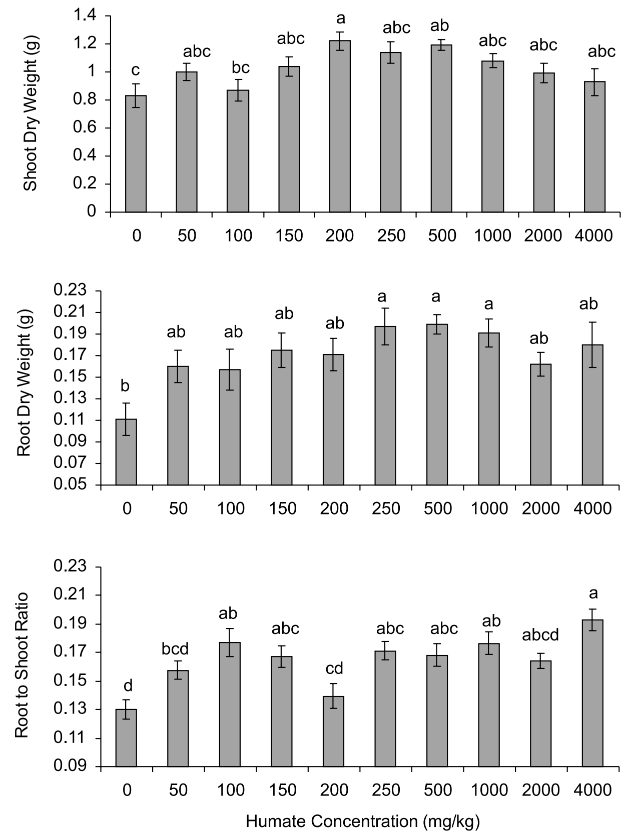

Fig. 2. Average shoot and root dry weights and root to shoot ratios of tomato seedlings (mean ± standard error) grown in a standard commercial potting medium (Metro-Mix 360) containing different concentrations of vermicompost-derived humic acids, with all nutrients needed supplied. Columns followed by the same letter do not differ significantly ($P \leqslant 0.05$).

grown in the Metro-Mix 360 controls with no humates. Only those tomato seedlings grown in the humate/Metro-Mix 360 potting mixtures containing 250 mg/kg pig manure vermicompost humates had greater nitrogen concentrations than those of tomato plants grown only in Metro-Mix 360 (Fig. 3). The lowest nitrogen con-

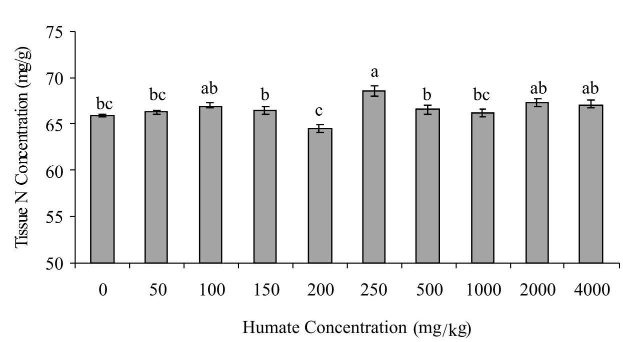

Fig. 3. Average tissue nitrogen concentrations of tomato seedlings (mean ± standard error) grown in a standard commercial potting medium (Metro-Mix 360) containing different concentrations of vermicompost-derived humic acids, with all nutrients needed supplied. Columns followed by the same letter do not differ significantly ($P \leqslant 0.05$).

centrations were in the tomato plants grown in the potting mixtures containing 200 mg/kg humates.

In the second experiment with cucumber plants (Fig. 4), the shoot dry weights of seedlings grown in mixtures containing up to 500 mg/kg pig manure vermicompost-derived humates were not significantly different from those of seedlings grown in vermiculite controls. However, with applications of pig manure vermicompost-derived humate concentrations greater than 500 mg/kg, the shoot dry weights of the cucumber seedlings were significantly less than those of seedlings grown in the vermiculite controls. Only the roots of cucumber seedlings grown in mixtures containing 500 mg/kg of pig manure vermicompost-derived humates were significantly greater (0.147 g) than those of seedlings grown in the controls with no humates (0.124 g). For the cucumber seedlings grown in food waste-derived humates (Fig. 5), significant increases in shoot and root dry weights over those of seedlings grown in vermiculite controls were observed in mixtures containing 50 mg/kg of food wastes-derived humates (28.6% and 18.5% increase, respectively).

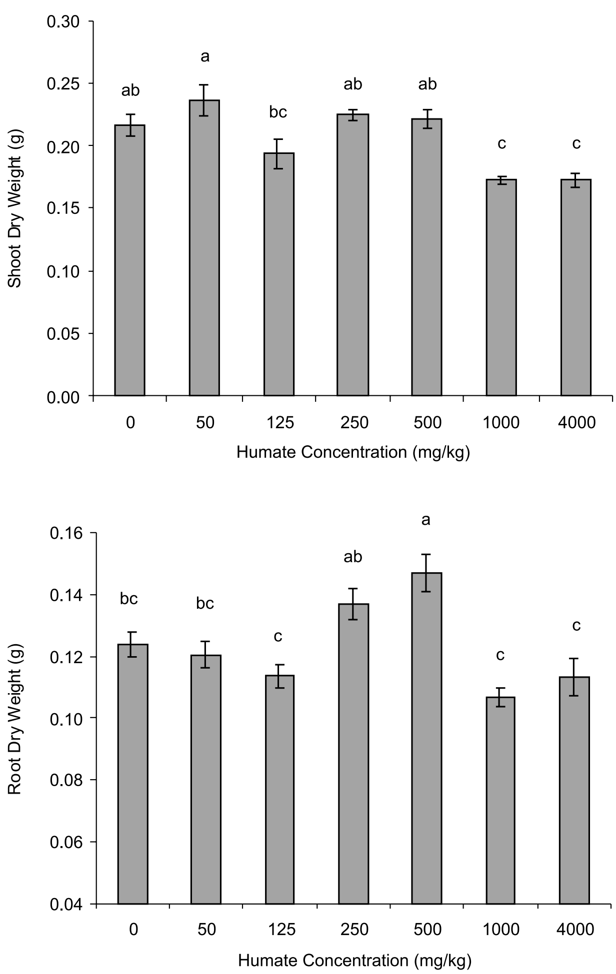

Fig. 4. Shoot and root dry weights of cucumber seedlings (mean ± standard error) grown in vermiculite containing different concentrations of pig manure vermicompost-derived humic acids, with all nutrients supplied. Columns followed by the same letter do not differ significantly ($P \leqslant 0.05$).

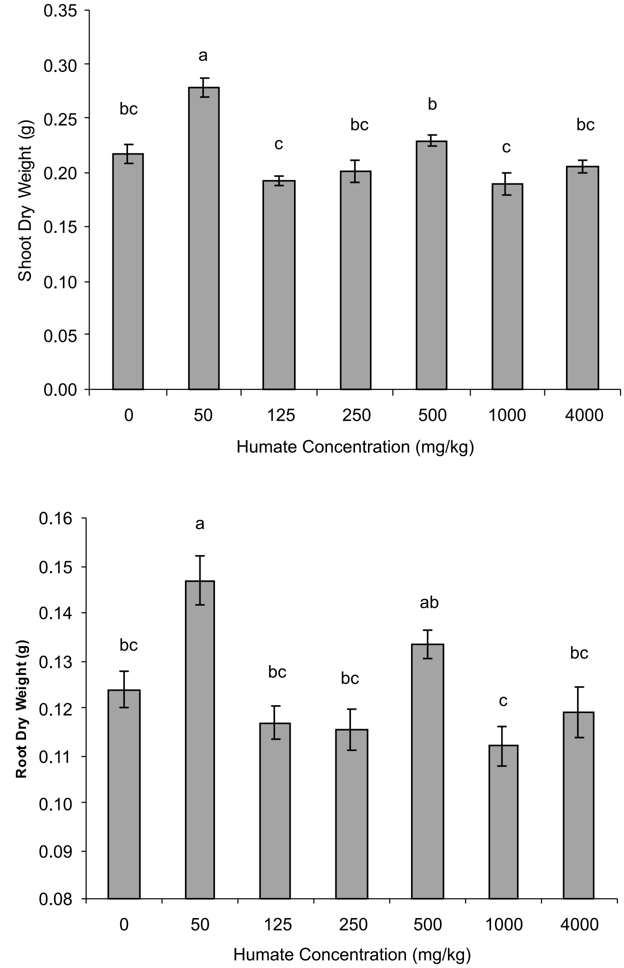

Fig. 5. Shoot and root dry weights of cucumber seedlings (mean ± standard error) grown in vermiculite containing concentrations of food waste vermicompost-derived humic acids, with all nutrients supplied. Columns followed by the same letter do not differ significantly ($P \leqslant 0.05$).

4. Discussion

The incorporation of humic acids, that had been extracted from either pig manure vermicompost or food wastes vermicompost, into soilless plant growth media, when all needed plant nutrients were supplied, increased the growth of both tomato and cucumber plants significantly, results which are in good agreement with those reported by Chen and Aviad (1990) and Tattini et al. (1991). Plant growth was increased consistently in the first experiment by treatments of the plants with 50–500 mg/kg humic acids, but decreased significantly when the concentrations of humic acids in the container medium exceeded 500–1000 mg/kg. These results differ but mainly in scale from those of Valdrighi et al. (1996) who reported that humic acids derived from green composts had a positive effect on the growth of chicory plants when incorporated into the potting soil at concentrations equal to or greater than 1000 mg/kg, although this difference in dose responses may relate to the plant species involved. Additionally, differences in the type of composting process and the kind of organic matter from which the humic acids were extracted may explain discrepancies between their experiments and ours. In our experiments, humic acids, particularly those extracted from pig manure vermicompost, appeared to have greater effects upon the root growth of the plants than on the aboveground parts of the plants. Incorporation of pig manure vermicompost-derived humates, into the soilless container medium, increased the weights of roots of tomato seedlings to a greater extent than those of the shoots. Furthermore, the root to shoot ratios of tomato seedlings increased significantly with increasing concentrations of humic acids in the soilless container medium, indicating greater resource allocation towards the roots than the shoots. Chen and Aviad (1990) also reported that the effects of humic substances on plants are often greater on roots than on shoots. Stimulation of root growth, increased proliferation of root hairs, and enhancement of root initiation by humic acids have been reported commonly by several other researchers (Lee and Bartlett, 1976; Mylonas and Mccants, 1980; Chen and Aviad, 1990; Tattini et al., 1991).

It is possible that the enhancement in the growth of tomato and cucumber seedlings, after incorporation of humic acids into the soilless container medium, could be attributed at least partially to increased nutrient uptake by the plants. Tomato plants grown in the potting media, mixed with 250 mg/kg humic acids, contained 4.1% more nitrogen in their leaf tissues than in the tissues of plants grown in the container medium without humates. Humic acids have been reported to enhance mineral nutrient uptake by plants, by increasing the permeability of membranes of the root cells (Valdrighi et al., 1996). Tattini et al. (1991) reported that nitrogen uptake rate by the roots of container grown olive plants increased after application of humic acid concentrations in the range of 30–120 mg/pot, with the greater humic acid concentrations decreasing nitrogen uptake. Moreover, the positive influences of humic acids on plant growth and productivity, which seem to be concentration-specific, could be mainly due to hormone-like activities of the humic acids through their involvement in cell respiration, photosynthesis, oxidative phosphorylation, protein synthesis, and various enzymatic reactions (Vaughan et al., 1985; Chen and Aviad, 1990; Muscolo et al., 1993, 1996, 1999). Although humic acids are known to evoke plant growth responses similar to those induced by plant hormones, it has not yet been proved conclusively whether humic acids contain hormone-like components (Muscolo et al., 1993) although there are indications that they might do (Phuong and Tichy, 1976).

Experiments reported by Atiyeh et al. (1999, 2000a, c,d, 2001) showed that the contributions of vermicomposts, when incorporated into soilless greenhouse container media and supplied with all needed nutrients, consistently exceeded the potential that vermicomposts may have to improve nutrient availability in the container medium and improve those physical conditions of the container medium that favor root growth. The plant growth responses, seen in the experiments reported in the current paper, after incorporation of a range of concentrations of humic acids extracted from pig manure vermicompost and food wastes vermicompost into the container medium, were extremely analogous to those of tomato plants (Atiyeh et al., 1999, 2000a,c,d) grown in the same container media substituted with increasing concentrations of these vermicomposts. Relatively small substitutions of vermicomposts or additions of humic acids obtained from vermicomposts, in the container medium, have increased plant growth consistently, to levels considerably beyond that can be attributed to increased uptake of mineral nutrients, with larger substitutions of vermicomposts or humic acids decreasing growth. This suggests that the consistent enhancements in plant growth produced by vermicomposts were due to their humic acid contents, not nutrient changes. Further confirmation of such a mechanism occurring commonly would render the use of vermicomposting for the treatment of organic wastes much more attractive, since vermicomposting accelerates the production of humates. Widespread adoption of vermicomposting would facilitate the reduction of environmental impacts from traditional waste disposal methods such as landfill and incineration, and vermicomposts would become less expensive and much more attractive materials to use in both the horticultural and agricultural industries. Moreover, if the action of earthworms upon organic matter in producing humic materials, which can influence plant growth significantly, is a common phenomenon, this has important connotations for the role of organic matter promoting plant growth in natural as well as agricultural ecosystems.

5. Conclusions

This experiment is the first to pinpoint precisely a biological mechanism by which vermicomposts can influence plant growth positively and produce significant increases in overall plant productivity, independent of nutrient uptake. Mixing the container media with increasing concentrations of vermicompost-derived humic acids increased plant growth, and larger concentrations usually reduced growth, in a pattern similar to the plant growth responses observed after incorporation of vermicomposts into container media with all needed mineral nutrition (Atiyeh et al., 2000a). Plant growth was increased by treatments of the plants with 50–500 mg/kg humic acids, but decreased significantly when the concentrations of humic acids in the container medium exceeded 500–1000 mg/kg. Although some of the growth enhancement by humic acids could have been partially due to increased rates of nitrogen uptake by the plants, most of the results reported exceed those that would result from such a mechanism, very considerably. However, this does not exclude the possibility of other contributory mechanisms by which humic acids could affect plant growth. There is a further alternative explanation for the hormone-like mode of action of humic acids in these experiments. In our laboratory, we have extracted plant growth regulators such as indole acetic acid, gibberellins and cytokinins from vermicomposts in aqueous solution and demonstrated that these can have significant effects on plant growth. Such substances may be relatively transient in soils. However, there seems a strong possibility that such plant growth regulators which are relatively transient may become adsorbed on to humates and act in conjunction with them to influence plant growth. We are currently investigating this issue.

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