

Effects of Humic Substances Isolated from Earthworm Faeces

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ABSTRACT

Earthworm biomass and activity in soil are highly related to the nature of vegetable organic wastes and are controlled by climatic conditions, food composition, physical and chemical soil characteristics. Earthworms strongly affect soil fertility by increasing the availability of nutrients, improving soil structure and water-holding capacity, but the main effect of earthworms in soil concerns the control of the humification process. It has been suggested that earthworms can increase the velocity of organic residue decomposition and also produce faeces rich in bioactive humus. While the role played by earthworms in the growth and productivity of plants was previously related to their effects on soil structure, recent work has suggested that these effects may be due to the production of faeces that contain more than 40% of humic substances endowed with hormone-like activity. This mini-review summarizes the progress made in recent years on the effects of humic substances isolated from earthworm faeces. Some specific issues will focus on the effects of earthworms on humification processes, plant growth and physiological processes in plant tissues.

Keywords: biological activity, hormone-like activity, humus, plant growth

Abbreviations: HA, humic acid; HS, humic substance; IAA, indole-3-acetic acid; LMS, low apparent molecular size; OM, organic matter; PGR, plant growth regulator; SHC, soluble humic complex

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INTRODUCTION

Earthworms as one of the major soil macro fauna constitute an important group of secondary decomposers. They are considered key biological agents in the degradation of organic matter and wastes (Syres *et al.* 1979; Albanell *et al.* 1988; Jambakar 1992). Earthworms are commonly referred to as ecosystem engineers for their ability to modify soils and plant communities (Lavelle *et al.* 1997; Hale *et al.* 2005). Through the incorporation of surface litter, casting, burrowing and other activities, earthworms can significantly alter soil physical properties (Edwards and Shipitalo 1998), soil nutrients (Edwards and Bohlen 1996), soil biological communities (Doube and Brown 1998), plant parasitic nematodes (Arancon *et al.* 2002) and above-ground plant communities (Pearce *et al.* 1994; Wurst *et al.* 2005). Earthworms have long been recognized by farmers as beneficial to soil chemical physical and biological properties (Singh and Pillai 1973; Edward and Lofty 1977). These beneficial effects have been attributed to improvements in soil 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 (PGRs) (Tomati and Galli 1995; Doube *et al.* 1997). Many researchers have also demonstrated that these effects can increase plant growth and crop yields in both natural and managed ecosystems (Edwards and Bohlen 1996; Edwards 1998). Earthworms fragment organic substrates, stimulate microbial activity greatly and

increase the rates of mineralization, rapidly converting organic matter 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.* 1998; Atiyeh *et al.* 2000b). Vermicompost is a product of biodegradation and stabilization of organic materials by interaction between earthworms and microorganisms (Subler *et al.* 1998). It is a finely-divided, peat-like material, with high porosity, aeration, drainage, water holding capacity and microbial activity, which make it an excellent soil conditioner (Edwards and Burrows 1988; Edwards 1998; Atiyeh *et al.* 2001). There is a close relationship between humus formation which results from earthworm activity and vegetable species and litter composition. It has been found that the relationship between earthworms and some species of plants depends on chemical composition of vegetable residues. The kind of biomass and activity of earthworms are highly related to the nature of organic residues in the soil (Kale 1998). Animal residues, agricultural- and even urban-wastes can be used as organic matter sources, although the last types can cause problems due to their heavy metal content, which can, in some cases, be rather high (Garcia *et al.* 1995). The preference for some organic substrates depends also on preliminary microbiological transformation of organic residues, but that utilization comes after a partial microbial degradation. In fact earthworms show a particular preference for animal dejections, in addition *Lumbricus terrestris* prefers cellulose attacked by mycetes. This preference is attributed to trophic utiliza-

Table 1 The effects of vermicompost-derived humic acid on the growth of a variety of crops assessed in the greenhouse and to a lesser degree in field crops: cucumber (*Cucumis sativum*); maize (*Zea mays*); strawberry (*Fragaria ananassa* Duch); tomato (*Lycopersicum esculentum*); pepper (*Capsicum frutescens*); petunia (*Petunia hybrida*); rice (*Oryza sativa*).

Type of vermicompost-derived humic acids	Species	Growth condition	Biological effect	References
Pig manure	Tomato-cucumber	Environmentally controlled glasshouse	Leaf area; root growth; height	Atiyeh <i>et al.</i> 2002
Cattle manure	Maize	Laboratory	Root growth; lateral root	Canellas <i>et al.</i> 2002
Food waste	Cucumber	Environmentally controlled glasshouse	Leaf area; root growth; height	Atiyeh <i>et al.</i> 2002
Cattle waste	Pepper-strawberry-tomato	Greenhouse	Leaf area; root growth; height; fruit	Arancon <i>et al.</i> 2004
Food waste	Pepper-strawberry-tomato	Greenhouse	Leaf area; root growth; height; fruit	Arancon <i>et al.</i> 2004
Food waste	Pepper-strawberry	Greenhouse	Fruit and flowers	Arancon <i>et al.</i> 2006
Sheep manure	Tomato	Greenhouse	Plant height; fruit quality	Gutiérrez-Miceli <i>et al.</i> 2007
Cattle manure food and paper waste	Petunias	Greenhouse	Seedling growth and flowering	Arancon <i>et al.</i> 2006
Cow dung	Rice	Yield	Seedling growth	Tejada <i>et al.</i> 2009
Green forages	Rice	Yield	Seedling growth	Tejada <i>et al.</i> 2009

tion of bacteria and fungi whose cellular constituents undergo rapid lyses after ingestion. According to Satchell and Martin (1984) the high microbiological activity in earthworm faeces could be due to the enrichment after defecation, due in turn to food transformations and integrations in the earthworm gut. On occasion, the use of fresh organic matter (OM) is not advisable since they can cause imbalances in the concentration of nutrients in the soils (due to the high C/N ratios of the wastes) or lead to an excess of toxic organic compounds. It must be borne in mind that these fresh materials contain labile carbon substances which will be rapidly mineralized in the soil (Levi-Minzi *et al.* 1986) and so they must be used with care in soil amendment and regeneration programmes. Organic matter which is added to soils must be sufficiently stable for it to produce beneficial effects. Among the ways of ensuring this stability is to submit the wastes to composting or vermicomposting. Both are biological aerobic processes which transform the fresh organic matter into a more stable and less toxic substance. For animal wastes, vermicomposting, which consists of the bioconversion of organic matter into a more humified material by using worms, is the most efficient process. In comparison with composting, vermicomposting gives a product that is richer in chelating and phytohormonal elements (Tomati *et al.* 1983) and has no further need of processing before its use. The survival of the worms after the vermicomposting process has finished reflects the lack of toxicity in the final product (Beyer *et al.* 1982); the product has a high content of microbial agents and stabilized humic substances. The effects of vermicomposts on the growth of a variety of crops assessed in the greenhouse and to a lesser degree in field crops are reported in **Table 1**. These investigations have demonstrated consistently that vermicomposted organic matter has 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 expected from the mere conversion of mineral nutrients into more plant-available forms (Arancon *et al.* 2008). 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 (Szczec *et al.* 1993; Nakamura 1996), the activity of vesicular arbuscular mycorrhizae is enhanced (Cavender *et al.* 1999), and plant parasitic nematode populations are suppressed (Chan and Griffiths 1988). Earthworm activity accelerates the humification of organic matter, and vermicomposts have been shown to contain large amounts of humic substances (Masciandaro *et al.*

1997; Elvira *et al.* 1998). Additionally, 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 (HAs) (Casenave de Sanfilippo *et al.* 1990), there are many studies showing that PGRs, such as indoleacetic 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 PGRs (Krishnamoorthy and Vajranabhiah 1986; Edwards 1998). They also demonstrated a positive correlation ($r = 0.97$) between earthworm populations and the amounts of auxins and cytokinins occurring in 10 different field soils and they concluded that earthworm activity was linked strongly with PGR production.

Tomati *et al.* (1987, 1988) and Tomati and Galli (1995) tested the vermicompost produced from organic wastes by the action of earthworms as media for growing the ornamental plants and mushrooms. They concluded that the stimulation of plant growth was too great to be explained purely on the basis of the nutrient content of the vermicompost. Moreover the plant growth changes recorded included stimulation of rooting, dwarfing, earlier flowering and lengthening of internodes.

Moreover, HAs have also been shown to stimulate plant growth in auxin, gibberellin and cytokinin bioassays (Phuong and Tichy 1976). Studies on the effects of these humic substances on plant growth, when full requirements for mineral nutrition were supplied, 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 (*Zea mays*) and oat (*Avena sativa*) seedlings (Lee and Bartlett 1976; Albuzio *et al.* 1994); numbers and lengths of tobacco (*Nicotiana tabacum*) roots; dry weights of shoots, roots, and nodules of soybean (*Glycine max*), dry matter production, of peanut (*Arachis hypogaea* L., 'Florunner'), and clover (*Trifolium vesiculosum*, 'Arrow leaf') grown in sand cultures (Tan and Tantiwiranond 1983); vegetative growth of chicory (*Cichorium intybus*) plants (Valdighi *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 a 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, and one of the more suggestive 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 (Albuzio *et al.* 1989; Casenave de Sanfilippo *et al.* 1990; Chen and Aviad 1990).

Cacco and Dell’Agnola (1984) tested the biological activity of soluble humic complexes (SHC) through auxin and cytokinin bioassays. Their results showed that ‘pea (*Pisum sativum*) split stem curvature’ (auxin test) and ‘cucumber’ tests (cytokinin test) did not show any quantifiable activity of soluble humic complexes. ‘Cress test’ and ‘senescence test’ offered good evidence of a hormonal-like activity 100 times lower than that of IAA (1 mg IAA.g⁻¹ SHC) and 10 times lower than that of N⁶ BA (100 mg N⁶ BA.g⁻¹ SHC). At a high concentration of SHC, toxic effects were evident, indicating the presence of inhibitory substances which counteracted the hormone-like activity of humic complexes. Nardi *et al.* (1988, 1994) reported that humic materials produced in the faeces of *A. rosea* and *A. caliginosa* exhibited auxin-, gibberellin-, and cytokinin-like activities. Subsequently, Muscolo *et al.* (1996) studied the biological activity of earthworm humic substances on carrot cells in culture.

Carrot cells were grown in cultures supplemented with two hormones [2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (6BAP)] and two humic fractions extracted from earthworm faeces, one with high acidity and a low apparent molecular size (LMS) (<3500 Da) and the other with low acidity and a large molecular size. 2,4-D stimulated growth through an effect on cell enlargement, while the strongly acidic humic fraction (0.2 mg L⁻¹) and the weakly acidic fraction (1 mg L⁻¹) were both less effective. With 4–16 h of pre-incubation, the highly acid humic fraction, mainly alone, induced the best increase in protein content; the effect of the weakly acid humic fraction and the hormones was generally less important. The two humic fractions also differed in their influence on glutamate dehydrogenase activity. After 2 h of pretreatment, the highly acidic fraction increased glutamate dehydrogenase activity, while the other fraction did not affect it. After 4–16 h of pre-incubation, the activity of this enzyme was still not influenced by these humic fractions. The presence of the two hormones did not interfere with the humic matter effects. Glutamine synthetase activity was not affected by a pre-incubation of up to 4 h with the two humic fractions, but it was stimulated after 8–16 h of pre-incubation. A 2,4-D+6BAP mixture stimulated glutamine synthetase activity (from +12 to +50%). Again, the presence of the hormones did not interfere with the effects induced by the humic fractions. After 16 h of pre-incubation, phosphoenolpyruvate carboxylase activity was increased by the highly acidic humic fraction (+93%) and by both humic fractions together (+34%). An explanation of the different incubation times necessary for the humic fractions to exert stimulatory effects on these enzymes is proposed here. The regulatory properties of the strongly acidic humic fraction appeared to depend on the combination of high acidity (especially carboxylic C) with low molecular size. Other mechanisms which have been suggested to account for promotion of plant growth by humic substances involve: enhanced uptake of metallic ions and increases in cell permeability (Chen and Aviad 1990).

The effect of earthworms on humification processes

Earthworm activity can increase crop growth through increased nutrient uptake, improved physical properties of the soil, better mixing of the soil, and increased water infiltration rates (Kladivko and Timmenga 1990). A significant effect of their activities is through the humification process. By grinding and mixing mineral constituents and organic wastes with substances secreted by the gut, earthworms stimulate biological activity, modify the composition of microbial communities and speed up the humification of orga-

nic matter. The value of the castings of earthworms, especially in pasture soils, has been the subject of much work and discussion. Casting, in combination with other earthworm activities, plays an important part in the darkening of mineral soil (melanisation) by admixture of organic matter and in the build-up of humified layers. An experiment carried out by von Hensen many years ago will serve to illustrate this point (Lee 1951). Von Hensen took a vessel 46 cm in diameter, filled it with sand and put a layer of leaves on the top. He then introduced two earthworms into the vessel. Leaves were soon dragged down into the sand to a depth of up to 15.2 cm and after six weeks there was 1.0 cm. of humus formed in an even layer over the surface of the sand. The amount of soil cast by earthworms at the surface in pasture lands has been estimated many times. Darwin (1881) gives a figure of 18.75–45.30 tons per ha per year, giving a total depth of 2.54 cm in 10 years. Evans (1948) states that in a 300-year-old pasture 62.5 tons per ha per year are cast by earthworms at the soil surface. In forest soils the presence of earthworms accelerates the formation of mull, since by dragging leaves from the surface into their burrows they deepen the zone of humification. The general suitability to earthworms of overall environmental conditions in New Zealand has permitted specialisation of endemic species so as to take advantage of a great variety of soil conditions. They are found in the soils of forests, scrubland, mountain tops and plains and in many other ecological niches including swamps, rotten logs and under the bark of trees at heights of up to 3000 m above the ground. Certain species are confined entirely to the topsoil while others are confined to the subsoil. The great specialisation of most species to suit well defined environmental conditions makes their destruction inevitable as soon as their environment is changed by the conversion of virgin lands into pasture. The only species which survive such changes are those that live in the subsoil and are only slightly affected by a change to pasture conditions and a few, apparently little specialised species, that seem able to adapt themselves to the change in their environment and then to maintain themselves in the face of strong competition from introduced species. When native vegetation is removed and pasture is planted the first introduced earthworm species to appear in the soil is usually *Octolasion cyaneum*, a large sluggish species which spreads fairly rapidly and becomes dominant, sometimes with a few survivors of the endemic fauna as minor elements of the fauna (Lee 1951). *O. cyaneum* is followed fairly rapidly by smaller and more active species of the genera *Lumbricus* and *Allolobophora*. The common introduced species appear to have a digestive system capable of coping with a wide variety of plant residues, but find their optimum conditions in pasture soils (Baker *et al.* 2006). By their great rate of reproduction, immense activity and consequent heavy drain on the food supplies the common species of *Lumbricus* and *Allolobophora* soon gain undisputed possession of the soils of pasture lands (Gerard 1967). It is an interesting fact that when pasture land reverts into scrub or forest the whole process is reversed and *O. cyaneum*, or sometimes an endemic species, again becomes dominant. The humic matter produced by earthworms improves seed germination and plant growth and enhances plant nutrient absorption capacity (McColl *et al.* 1982). As shown in **Table 2** earthworms impede the polymerization and polycondensation of humic compounds (Dell’Agnola and Ferrari 1975; Dell’Agnola and Nardi 1987) and promote the accumulation of humic fractions of medium molecular weight. This is due to the dynamic cycle that prevents a final equilibrium in order to obtain humic products of a low molecular weight that are the results of the hydrolysis of vegetable polymers. The role played by these changes in humic extracts has still to be clarified. Talashilkar *et al.* (1999) studied the influence of two species of earthworms viz. *Eisenia foetida* and *Eudrilus eugeniae* on the changes in various chemical parameters governing the compost maturity of local grass, mango leaves and farm wastes. The results showed a decrease in C:N ratio, while HA, cation exchange capacity and water

Table 2 Chemical and microbial analysis of organic substrates of different origin.

	Substrates		
	Humified straw-lucerne meals	Casting of <i>Eisenia foetida</i>	Faeces of <i>Allolobophora</i> sp.
pH	8.5	7.5	8.1
Organic carbon %	40	24.4	24.8
C/N	23.0	9.7	18.0
HS mw < 9000 %	27.3	13.5	27.2
HS mw 9000-100.000 %	2.8	58.2	47.6
HS mw >100.000 %	69.9	28.3	25.2
Microfungi*	5.4×10^5	6.8×10^8	8.7×10^7
Actinomycetes*	1.8×10^5	8.4×10^8	6.2×10^6

*In C.F.U. \times g⁻¹dry matter

Source Dell'Agnoia and Nardi 1987

soluble carbohydrates increased up to 150 days of composting. Compost maturation was achieved up to a period of 120 and 150 days in farm wastes and mango leaves, respectively, while more than 150 days would be required to reach the maturity in case of local grass. Inoculation of both the species of earthworms reduced the period of composting by 13 days only. Vermicomposting of all the residues resulted in significant reduction in C:N ratio and increase in other parameters like HA content, cation exchange capacity and water soluble carbohydrate content of all the residues after 150 days of composting over the residues uninoculated with earthworms. However, significant differences in the said parameters governing the process of composting were not recorded due to inoculation of two different species of earthworms. Petrusi *et al.* (1988) and Concheri *et al.* (1992) suggested that the biological transformation of organic matter by earthworms favours the concentration of nutrients and promote the formation of humic matter. The higher value of the C/N index in the starting mixtures promoted the biodegradation of organic matter and lignin. The quantity of humic carbon (Ch) is always higher at the end of the first phase compared to the humic carbon at the end of second phase; on the contrary the ratio between humic carbon and fulvic carbon (Ch/Cf) was higher at the end of the second phase. The distribution of different molecular weight fractions during the humification processes followed a common trend: an increase of the highest and a decrease of the lowest molecular weight substances. The results obtained by Concheri *et al.* (1992) showed that at the end of the second phase the yield of humus having high molecular weight was generally higher than that of the first phase. Canellas *et al.* (2002) showed with ¹³C NMR analysis that the HA from earthworm curiously, had a very large peak in the region δ_C -44 to -57, which is not typical of ¹³C NMR spectra from pedogenic HA and, thus, revealed the low humification stage of HAs isolated from earthworm because of an increase in C bonded to mono- and di-O. The signals at δ_C -44, -53, and -55 were attributed to sp³ carbon atoms, including C bound to N in amino acids; at δ_C -57, they were because of OCH₃ groups bound to the hybridization state of the atom C (orbital sp² bound). The signal around δ_C -65 was attributed to carbonic C of primary alcohols and polysaccharides at δ_C -70.6 indicated sp³ C atoms bound to N. The signals at around δ_C -100 suggested sp³ carbon atoms bound to two atoms of oxygen (anomeric carbon), as founded in carbohydrates. The peak centered at δ_C -130 was because of aromatic carbons. The high field peaks at δ_C -150 to -160 were because of carbon bonded to phenolic OH groups. The δ_C -160 to -190 regions showed signals because of the presence of differently substituted carbonyl-C atoms. Quantitatively, the spectra revealed 8.7% carboxyl, 7.4% phenolic, 41.9% aromatic, 31.5% peptide and carbohydrate, and 17.5% other aliphatic carbons. In comparison with typical values reported for an average soil HA (Schnitzer 1991), the earthworm compost HA had a low content of C in alkyl chains and carboxylic functions and a high content of aromatic and mono- or di-O-C alkyl and N-C atoms. However, this HA earthworm compost fraction is endowed with the characteristic structural network described for most HAs

isolated from different sources of organic matter (Clapp and Hayes 1999). This was consistent with the notion that the same pathways for the formation of HA may operate in all environments resulting in a substance with defined identity (Stevenson 1994). The additional value of HS from vermicompost compared most HAs isolated from different sources should be due to its content of auxin. Canellas *et al.* (2002) showed the presence of IAA in HAs extracted from vermicompost.

The effect of earthworm on plant growth and metabolism

During the last years, some reports have appeared in the literature, which clearly suggest that application of vermicompost along with chemical fertilizers result in increased yield and fruit quality (Arancon *et al.* 2004, 2006; Gutiérrez-Micelia *et al.* 2007) mainly due to production of PGRs by microorganisms during the process of vermicomposting (Muscolo *et al.* 1999; Atiyeh *et al.* 2002). However, Singh *et al.* (2008) reported that in field-grown strawberries there is production of albino and malformed fruit in large number, which affect fruit yield and quality. Hence, it could be hypothesized that with the use of vermicompost in strawberry, the production of albino and malformed fruit may be reduced due to the presence of plant-growth-influencing substances and suppression of *Botrytis* rot in strawberry. Considering these points, systematic studies were conducted to determine the effect of different doses of vermicompost along with chemical fertilizers on growth, occurrence of albinism, fruit malformation disorders, and yield and fruit quality of 'Chandler' strawberry. Earthworm activity can increase crop growth through increased nutrient uptake, improved physical properties of the soil, better mixing of the soil, and increased water infiltration rates (Kladivko and Timmenga 1990). The small number of field experiments reported in the literature have shown that amending soils with vermicomposts can increase the growth and yield of some crops such as cowpeas (*Vigna unguiculata*) (Mba 1996), chicory (*Cichorium intybus*) (Valdrighi *et al.* 1996) and grapes (*Vitis vinifera*) (Buckerfield and Webster 1998). Some field experiments have involved amending soils with vermicomposts in conjunction or combination with conventional fertilization programs. Amending soils with vermicompost at 2 kg/plant and together with 75% of the recommended rate of inorganic fertilizers promoted shoot production of bananas (*Musa acuminata* Colla) (Athani *et al.* 1999). Arancon *et al.* (2003) tested the effects of HAs extracted from cattle, food and paper-waste vermicomposts on growth of tomatoes (*Lycopersicon esculentum*), pepper and strawberry, evidencing a significant increase in plant heights, leaf areas, shoot dry weights, root dry weights of peppers, tomatoes and in numbers of fruits of strawberries. Then, Arancon *et al.* (2004) assessed the effects of the application of different types and rates of vermicomposts, on the growth and yields of field-grown strawberries, under field conditions independent of nutrients. They postulated that the positive effects of vermicomposts on strawberry growth and yield were not due to nutrients but due to the availa-

bility of plant growth-influencing materials, such as PGRs and HAs, produced by the greatly increased microbial populations resulting from earthworm activity. Subsequently Arancon *et al.* (2006) designed experiments to evaluate the effects of HAs extracted from vermicompost and compare them with the action of commercial HA in combination with a commercial PGR, IAA which is commonly found in vermicomposts. In the experiments, HAs were extracted from cattle, food and paper waste vermicomposts and they were applied to a plant growth medium to pepper and strawberry plants in greenhouse. They demonstrated the numbers of pepper flowers and fruits increased significantly in response to treatment with HA, IAA and a combination of HA and IAA. Peppers treated with HAs extracted from food waste vermicomposts produced significantly more fruits and flowers than those treated with commercially-produced HAs and the numbers of strawberry's fruits increased significantly in presence of vermicompost HAs. Muhammad *et al.* (2007) tested vermicompost produced from a green waste compost feedstock and assessed for its potential use in a high value horticultural market. Replicated plant growth trials were undertaken with lettuce (*Lactuca sativa* L.) using pure worm cast (vermicompost), green waste-derived compost and mixtures of the two, i.e. 50/50 (v/v) and 20/80 (v/v) of worm casts and green waste feedstock. The highest plant fresh and dry weights were observed for the two compost mixtures, i.e. 50/50 and 20/80 (VC/FS). The enhanced plant weights were independent of the nutrient contents of the substrates and to some extent the root development. In recent work, Eriksen-Hamel and Whalen (2008) determined the effects of an earthworm community, dominated by *A. caliginosa* and *L. terrestris*, on plant growth in soybean and maize agroecosystems. The research suggested that earthworms contribute positively to plant growth in some agroecosystems only when populations are high (>300 ind. m^{-2}) and when favourable weather conditions (warm and wet climate) exist. Earthworms can be found in most parts of the world except the driest and the coldest regions, because they are strongly dependent on the soil water potential (Edwards and Bohlen 1996). A higher earthworm population, principally consisting of the endogenous species *A. caliginosa*, was associated with greater mineral-N and microbial biomass N concentrations in surface (0–15 cm) soils, similarly, soybean grain and grain-N yield was significantly greater in enclosures with the largest earthworm populations than the control which had no earthworms added. In addition, when climate conditions were less favourable, there was no effect of earthworms on soil N pools or maize plants, probably due to poor survival of earthworms. The interactions between earthworms and microorganisms can produce significant quantities of plant growth hormones and HAs which act as plant regulators. Valdrighi *et al.* (1996) compared the effects on plant productivity and soil microorganisms of HAs extracted from vermicompost and those of the surfactant Tween 80 alone using a pot experiment carried out with chicory (*Cichorium intybus*). The responses of chicory plants to amendments with natural and synthetic surface active substances, represented by either potassium humates from compost stabilised green waste or Tween 80, are reported from a pot trial. Results are evaluated in terms of plant biomass production and behaviour of soil microbial populations following different treatments. Amendments with HAs stimulated vegetative growth of chicory. They also caused significant variations in the numbers of bacterial heterotrophic and autotrophic nitrifiers in the soil. The study suggested that the mechanism through which HAs affect both plant and soil microbes may chiefly involve enhancement of cell membrane permeability to nutrients.

Atiyeh *et al.* (2002) studied some effects of HAS, formed during the breakdown of organic wastes by earthworms (vermicomposting), on plant growth. In the first experiment, HAs 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 HAs, 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 HAs 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 HAs, but often decreased significantly when the concentrations of HAs derived in the container medium exceeded 500-1000 mg/kg. These growth responses were most probably due to hormone-like activity of HAs from the vermicomposts or could have been due to plant growth hormones adsorbed onto the humates. These findings showed a biological mechanism by which vermicomposts 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 HAs 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). There is a possible explanation for the hormone-like mode of action of vermicompost HAs. PGRs such as indole acetic acid, gibberellins and cytokinins may be relatively transient in soils; however, they may become adsorbed on to humates and act in conjunction with them to influence plant growth. Successively, Arancon *et al.* (2006) evaluated the effects of HAs extracted from vermicompost on plant growth. They evaluated the effects of HAs extracted from vermicompost and compared them with the action of commercial HA in combination with a commercial plant growth hormone, IAA which is a commonly found in vermicomposts. In the first experiments, HAs were extracted from cattle, food and paper waste vermicomposts. They were applied to a plant growth medium, Metro-Mix360 (MM360), at rates of 0, 250 or 500 mg humates kg^{-1} dry wt. of MM360, to marigold, pepper, and strawberry plants in the greenhouse. Substitution of humates ranging from 250 to 1000 mg kg^{-1} MM360 increased the growth of marigold and pepper roots, and increased the growth of roots and numbers of fruits of strawberries significantly. In other experiments, HAs extracted from food waste vermicomposts were applied at a rate of 500 mg kg^{-1} dry wt. of MM360, singly or in combination with IAA at a rate of 10–5 μM , to pepper seedlings. This experiment was designed to compare the differences in effects between the most effective dosage rate of HA from food waste, a phytohormone (IAA), and a commercial source of HA. The numbers of pepper flowers and fruits increased significantly in response to treatment with HA, IAA and a combination of HA and IAA. Peppers treated with HAs extracted from food waste vermicomposts produced significantly more fruits and flowers than those treated with commercially-produced HAs.

Hormone-like effect of earthworm humic substances

It seems very likely that vermicomposts, which consist of an amalgamate of humified earthworm faeces and organic matter, stimulated plant growth beyond that produced by mineral nutrients because of the effects of the humic sub-

stances present in the vermicomposts or due to PGRs associated with the HA. These plant growth-regulating materials are produced by action of microbes like fungi, bacteria, actinomycetes (Tomati *et al.* 1987; Edwards 1998), etc., and earthworms. During the last 20 years, the biological activities of humic substances, particularly those derived from earthworm faeces have begun to be investigated. Dell'Agola and Nardi (1987) reported hormone-like or PGR effects of depolycondensed humic fractions obtained from the faeces of the earthworms *Aporrectodea rosea* (Eisen) and *Aporrectodea caliginosa* (Sav), on plants. Earthworm humic substances affected plant growth by increasing anion and cation uptake, protein synthesis and the action of nitrate metabolism enzymes. Successively, Nardi *et al.* (1988) tested humic materials produced in the faeces of *Allolobophora rosea* and *Allolobophora caliginosa* and found a hormone like activity in particular auxin-, gibberellin-, and cytokinin-like activities. In this context, new information arising from more recent papers has further supported this hypothesis. The stimulation of earthworm humic substance was probably due to the hormone-like activity and, in particular, the auxin-like activity of humic matter. In fact humic substances induced, in *Nicotiana plumbaginifolia*, morphogenetic and biological changes similar to those produced by IAA (Muscolo *et al.* 1993) and, like IAA, humic matter affected peroxidase and esterase enzymes which were involved in organogenesis and may be indicators of somatic embryogenesis. In particular, leaf explants of *N. plumbaginifolia* were compared in cultures supplemented with hormones or humic substances (extracted from faeces of *A. caliginosa*) of various molecular complexity and concentration. The results showed that the humic substances (low, high molecular weight and total) at the concentration of 1 mg C L⁻¹ produced greater leaf explants than those grown in the control. Furthermore, humic fractions like gibberellic alone induced a rhizogenic activity in leaf explants. Quantitative differences were also observed in the peroxidase activity induced in *N. plumbaginifolia* by humic matter. In addition, the *Nicotiana* sp. tissue treated with humic fractions revealed, in the esterase enzyme pattern, the appearance of the 2a band, which was attributable to IAA, since its profile was consistent with those obtained from tissues treated with IAA. These differences demonstrate that humic substances exhibited a PGR-like behaviour, but no evidence of a relationship between biological activities and chemical characteristics of humus substances was found. Subsequently, it has been shown that only LMS humic fractions extracted from earthworm faeces induced morphological changes similar to those caused by IAA (Muscolo *et al.* 1993). The LMS humic fraction increased both peroxidase and IAA oxidase activity, albeit IAA increased IAA oxidase, but inhibited peroxidase activity. Again, Nardi *et al.* (1994), utilizing two inhibitors of auxin (TIBA, 2,3,5-triiodobenzoic acid and PCIB, 4-chlorophenoxy-isobutyric acid), demonstrated that the IAA and LMS fractions induced root growth of *N. plumbaginifolia*, while TIBA or PCIB alone inhibited it. The presence of TIBA plus LMS humic fractions or TIBA plus IAA inhibited root growth, while explants, grown in the presence of IAA plus PCIB or LMS fractions plus PCIB, showed roots similar to those obtained with IAA or LMS humic fractions, respectively. These results thus confirm that the LMS humic fraction of earthworm faeces is the fraction endowed with auxin-like activity, although the pathways followed by the IAA and the LMS fraction in inducing their effects may be somewhat different. Muscolo *et al.* (1996) tested the hormone-like activity of two humic fractions extracted from earthworm faeces with different chemical characteristic on carrot cells culture. The results showed that the stimulation of the enzymes involved in nitrate assimilation in carrot cells revealed a relationship between molecular size, carboxyl and phenolic C content and biological activity of the different humic fractions. The effect of the strongly acid humic fraction confirmed both its auxin-like activity and the effectiveness of the combination of high acidity and low molecular

size in stimulating the biological activity of plant system. In contrast the weakly acidic fraction with high molecular weight (>3500 Da) was not effective in stimulating plant metabolism (Muscolo *et al.* 1999). In an attempt to evaluate the possible interaction of the LMS earthworm humic fraction with plasma membranes (target of IAA) of carrot cells, Muscolo *et al.* (2007a) labelled with fluorescein isothiocyanate (FITC) IAA, HMS and LMS. The results showed that fluorescent plasma membrane staining was only observed in IAA- and LMS fraction-treated cell cultures. Prior treatment of carrot cells with unconjugated IAA or LMS humic fractions blocked the fluorescein staining of both the FITC-IAA and FITC-LMS humic fraction, giving indirect evidence of the possible binding site of LMS humic fraction to the IAA cell membrane receptors. It is important to emphasize that the interaction of HS with cellular membrane is not due only to the possible presence of auxin components in this preparation. In fact, using different approaches, the IAA content was identified in the 0.5% (w/v) to 3.7% range in the earthworm LMS humic fraction, according to the different sensitivities of the assays and the methodology used (Muscolo *et al.* 1998). Results of Russel *et al.* (2006) seem to corroborate the above findings. They have shown that IAA and LMS fractions had the same effect on the stomatal opening in pea leaves. This inducing effect appears to be mediated by phospholipase A₂ (PLA₂) and protein kinase C (PKC), both enzymes involved in the signal transduction pathway leading to the response of plants to IAA (Scherer and Andre 1989; Nemeth *et al.* 1998). In addition Canellas *et al.* (2002) investigated the effects of HAs isolated from cattle manure earthworm (*E. foetida*) compost on the earliest stages of lateral root development and on the plasma membrane H⁺-ATPase activity. The HAs enhanced the root growth of maize (*Zea mays*) seedlings in conjunction with a marked proliferation of sites of lateral root emergence. HAs also stimulated the plasma membrane H⁺-ATPase activity, apparently associated with an ability to promote expression of this enzyme. In addition, structural analysis revealed the presence of exchangeable auxin groups in the macrostructure of the earthworm compost HA. These results confirm the hormonal activity that has been postulated for these humic substances. Quagiotti *et al.* (2004), performed a detailed characterization of earthworm low molecular size humic substances and studied the effects of these substances on the nitrate influx in roots, tissue nitrate content, and expression of maize genes putatively involved in nitrate uptake in maize (*Zea mays* L.). The results showed that the humic fraction with low molecular size was endowed with the characteristic structural network described for most humic substances so far isolated and confirmed the presence of IAA in the low molecular size humic fraction. The results also show that the LMS fraction of humic substances stimulates the uptake of nitrate by roots and the accumulation of the anion at the leaf level. Moreover, the analysis of the expression of genes encoding two putative maize nitrate transporters (*ZmNrt2.1* and *ZmNrt1.1*) and of two maize H⁺-ATPase isoforms (*Mha1* and *Mha2*) show that these substances may exert direct effects on gene transcription in roots, as shown for the *Mha2* gene, and long-distance effects in shoots, as observed for the *ZmNrt2.1* gene Canellas *et al.* (2008) explored the molecular mass dynamics and bioactivity of vermicompost HS as a function of the presence of organic acids and the effects of the HS fractions on the profile of organic acids exudation by maize roots. HAs were extracted and purified from vermicompost of bovine manure with earthworm (*E. foetida*), and their results showed that the plants treated with 50 mg C L⁻¹ of HS exhibited strong changes in their root development as well as in their pattern of organic acid exudation. It was possible to observe an increase in the exudation of oxalic, citric and tartaric acids with the HA treatment. Furthermore, the HS present in the nutrient solutions of maize plants exhibited structural changes during the time of cultivation. They showed that maize seedlings treated with vermicompost HS exhibited both enhanced root growth and changed profile of

organic acids exudation. These results could explain the biological activity of HS with high molecular weight. The enhanced citric, oxalic and tartaric acids exudation could change the conformation assembling of HS adsorbed on cell wall and small units with biological activity could become available in the apoplast and gain access to biochemical targets on plasma membrane, such as the H⁺-ATPases (Canellas *et al.* 2002; Zandonadi *et al.* 2007), or even in the cytoplasm. Muscolo *et al.* (2007b) tested two humic substances, derived from earthworm faeces and forest soil. The two humic substances, each separated in fractions with low (<3500 Da) and high (>3500 Da) relative molecular mass were compared for their effects on *Pinus nigra* J.F. Arnold callus. Growth of callus, and the activities of the key enzymes involved in N metabolism were investigated. Callus was grown on basal Murashige and Skoog medium plus humic matters with or without 2,4-dichlorophenoxyacetic acid (2,4-D). A different biological activity between the earthworm and forest humic fractions was also observed. Both earthworm humic fractions had a positive effect on callus growth and N metabolism similarly to the auxin 2,4-D. On the contrary, forest humic fractions had an inhibitory effect. This study showed that the heterotrophic activity of soil biota and earthworm in particular may produce humic substances biologically active reach of metabolites such as PGRs.

CONCLUSION

It is clear from the above discussion that vermicompost may positively influence higher plant metabolism. This function seems to be carried out more readily by humic fractions with low molecular weight because they are able to reach the plasma membrane of root cells and then to be translocated. Unfortunately, the as yet unknown nature of HS prevents us from drawing more conclusive results concerning the effects of HS on plant growth. We can only affirm that HS from vermicompost appear to influence the metabolism of plant cells at different levels. Their effects may, therefore, be different and be additive, overlapping, or, in some cases, mechanistic related. This apparently puzzling situation can be however, rationalized by hypothesizing that vermicompost have several targets that can be explained partly by their chelating capacity and partly by their hormone-like activity. This is not surprising, considering the complex and differentiated nature of vermicompost. Therefore, more research is necessary to explain the positive effects of humic substances from vermicompost on higher plants. In particular these studies have to be, primarily, focused on the following topics: (1) the link between earthworm humic substance activity and the presence in the soil solution of active metabolites of various microbes; and (2) the use of more characterized earthworm humic substances in experiments on plant metabolism.

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