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Effects of Humic Acid Extracted from Organic Waste Composts on Turnip Culture (*Brassica rapa subsp. rapa*) in a Sandy Soil

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ABSTRACT

Adding humic acid to soil can improve soil structure and fertility, which can lead to better plant growth and higher crop yields. Extracting humic acid from compost is a sustainable and environmentally friendly way to obtain a valuable organic material. Humic acid (HA) can be extracted from compost relatively easily and at a low cost, making it an attractive option for farmers. In this study, we investigated the use of sugarcane bagasse (SB) and immature horse manure (IHM) as bulking agents for the composting of separated municipal solid waste (SMSW) and the extraction and characterization of humic acid from the mature composts produced. Fertilizing solutions containing different concentrations of humic acid were prepared and used to evaluate their effects on turnip crop growth and various biochemical parameters during cultivation. The results showed that the humic acid extracted from the composts had high yields and were rich in elemental carbon. The application of humic acid at both low and high concentrations resulted in a significant improvement in all the parameters measured except for the total protein in the roots, which did not differ significantly between the humic acid concentrations. The yield, root diameter and fresh weight increased significantly, and the leaf area was proportional to the humic acid concentration of the solution used. The highest increase in chlorophyll a content was observed in the treatment of humic acid extracted from composts C2 and C3 at a concentration of 0.1 gL^{-1}, with an increase of 31% and 37%, respectively, compared to the control. The use of humic acid provided by co-compost can be considered a successful management strategy for degraded sandy soils and sustainable agriculture production in sandy poor soils worldwide.

Keywords: compost, humic acid, turnip, growth, chlorophyll foliar, protein, total soluble sugar.

INTRODUCTION

The demand for mineral fertilizers has been increasing worldwide [Vanotti et al., 2019], especially in countries like Morocco where agriculture is a significant part of the economy and the primary plant nutrients (N, P_2O_5, and K_2O) are expensive. In 2015, the global demand for these nutrients reached 184 Mt, and it was expected to exceed 200 Mt in 2020 [FAO. 2017]. However, the production of these nutrients is costly and has significant environmental consequences, such as the depletion of non-renewable resources and energy consumption [Basosi et al., 2014]. Meanwhile, large quantities of organic waste are being dumped in landfills, causing health and environmental problems. Morocco produces a significant amount of municipal solid waste (MSW), and it is estimated that this will increase to 9.30 million tonnes by 2030 [M.E.W.E. 2015; Saghir et al., 2019]. Composting has emerged as a viable solution to separated municipal solid waste (SMSW) treatment, and various bulking agents, such as poultry manure [Aylaj et al., 2018], cattle manure [Bayındır et al., 2022], food waste [Angeriz-Campoy et al., 2023], and bio-based plastic film [Gadaleta et al., 2021], have been used to transform MSW into organic fertilizers. However, the use of sugarcane bagasse (SB) and immature horse manure (IHM) as bulking agents for composting MSW processing wastes has not been extensively explored, despite their potential benefits. The use of these materials increases the porosity of the organic mixture, improves the availability of oxygen, maintains the water content of the composting mass, and reduces the loss of static pressure in the process using forced aeration.

Humic acid (HA) is a natural stimulator substance that is a rich source of macronutrients, such as nitrogen, phosphorus, and sulfur, which can enhance root initiation, stimulate plant growth, and improve soil fertility, especially in sandy soil [Shafi et al., 2020; Yuan et al., b2022]. Sandy soil is poor-quality soil with low organic matter and clay content, which limits agricultural productivity [Jaiarree et al., 2014; Zhou., 2019]. The use of HA in sandy soils has been gaining attention due to its global availability, low cost, and positive effect on soil aggregate stability and crop yields [Ma et al., 2022].

Turnip (Brassica rapa var. rapa) is a biennial root crop that is widely cultivated in Morocco and is one of the oldest crops in the world. It has a high variety of functional compounds that have been shown to have medicinal properties, such as anti-hypoxic and detoxifying effects, as well as potential benefits for diabetes, liver injury, immune system regulation, and cancer prevention [Wang et al., 2016; Fu et al., 2016; Chu et al., 2017; Hua et al., 2021]. However, turnip cultivation requires a high amount of fertilizer to increase yield.

The aim of this study was to investigate the management of SMSW through composting with immature horse manure and sugarcane bagasse and to extract and characterize humic acids from the co-compost. We will use alkaline extractants and analyze the structural and spectral characteristics of the resulting HAs using a UV spectrophotometer. Our goal is to determine the optimal combination of SMSW, IHM, and SB to produce high-quality HA with desirable properties. We also aimed to determine the optimal type and level of HA for improving the growth and yield of turnip in sandy soil. We hypothesized that the addition of HA to sandy soil would improve the growth and yield of turnip.

MATERIALS AND METHODS

Experimental conditions

Phytotoxicity tests of the humic acids used

Phytotoxicity tests were conducted using cress seeds directly in Petri dishes. Cress (*Lepidium sativum*) was selected due to its good response to toxic materials and rapid and easy germination. Nine boxes were prepared, with eight boxes for the eight solutions prepared by the Humic Acids and one box for the control. The experimental design followed a randomized complete block with five repetitions. Ten seeds were soaked in 3 ml of test solutions and placed in a 10 cm diameter Petri dish lined with Whatman N1 filter paper. The Petri dishes were then placed in a growth chamber at 25 °C, with distilled water used as the control. After seven days, the number of germinated seeds was determined, and root elongation was measured. The germination index (GI) [Zucconi. 1983] was calculated as a percentage of the control, using the following equation:

$$GI = (GX\% \times RL\%) \times 100 \quad (1)$$

where: GX% – number of seeds germinated in a sample / number of seeds germinated in the control × 100;
RL% – mean root length in a sample / mean root length in the control × 100.

Culture conditions and treatments

In this experiment, turnip plants (*Brassica rapa subsp. rapa*, variety Marteau) were grown in pots under greenhouse conditions at the Faculty of Sciences of El Jadida, Morocco. Turnip culture was chosen for its importance as a widely used crop [Takuno et al., 2007].

The seeds were germinated in plastic pots (0.173 m^2 surface area, 41 cm length, 16.5 cm width, and 15 cm height) filled with 8 kg of soil. Nine points were performed in each pot and in each point three seeds were placed and after germination, the most developed plant was kept, resulting in nine plants per pot. The pots were irrigated with potable water using drip irrigation, and soil humidity was maintained at approximately 60%. The pot experiment was conducted in a greenhouse conditions with natural lighting (temperature range of 20–25 °C and humidity of 67%).

At 15 days after planting, *Brassica rapa L* plants were treated with HA extracted from four types of organic waste composts at two concentrations (0.1 and 1 gL^{-1}). 10 ml of the HA extract (equivalent to 2.8 m^3ha^{-1}) were injected at the bottom of each pot using a pipette to ensure that the solution of HA stayed near the roots. The dose for the extracted humic acid was stipulated at an equivalent dose of 2.8 m^3ha^{-1} for a turnip field with an average of 520231 plants/ha, resulting in 1.1 ml per plant.

The HA treatment was applied on the 15th, 30th, 36th, and 45th days after planting. The tubers were harvested after 60 days from turnip plant transplanting, when the plants reached physiological maturity.

Four types of composts were used, derived from composting of four mixtures: (1) a mixture of separated municipal solid wastes (SMSW), immature horse manure (IHM), and sugarcane bagasse (SB) at a ratio of 14:3:3 (weight-weight^{-1}, ww^{-1}) (C1), (2) a mix composed of the same organic matrices but at a ratio of 18:1:1 ww^{-1} (C2), (3) a mixture of (MSW) and IHM at a ratio of 9:1 ww^{-1} (C3), and (4) a mix of SMSW and SB at a ratio of 9:1 ww^{-1} (C4). Two concentration levels of HA were prepared for each HA extracted from the compost mixes (0.1 $gL^{-1}$and 1 gL^{-1}, equivalent to 2.8 $Kg_{ww}ha^{-1}$ and 28 $Kg_{ww}ha^{-1}$). A soil with no HA injected (no biostimulant) was used as control (0 gL^{-1} of HA). In total, nine treatments were used, taking into consideration four compost types and two different concentration levels of HA, plus a blank control. The experiment was carried out in a split plot design with three replicates, resulting in a total of 27 experimental pots.

Composting process

During the composting process, organic municipal solid wastes were mechanically separated to select the suitable organic fraction for composting. To adjust the C/N ratio of the mix, immature horse manure and sugarcane bagasse were used as bulking agents. The composting was conducted in a high-rate reactor vessel (measuring 40 cm in length and 20 cm in diameter) and lasted for 22 days, after which a maturation stage of 180 days was carried out in a plastic container. During the initial stage in the reactor vessel, the four composts reached their maximum temperatures between 58 and 69 °C on the ninth day of composting. Thereafter, the temperature decreased gradually and reached the mesophilic level, indicating the final stage of composting.

Methods of analysis

Soil and compost samples were subjected to physical-chemical analysis based on standard methods by AFNOR (1987). Soil texture was determined using an automatic reference particle size meter (Malvern Instruments Ltd) as described by El Kadiri et al. (2017). Soil pH and electrical conductivity (EC) were measured in a soil-water suspension (1:5 ratio) using a glass electrode pH meter and EC meter, following the procedure outlined by Pansu and Gautheyrou (2007).

Chemical extraction of humic acid

The chemical extraction of humic acid was performed on four HA samples obtained from four mature compost mixes resulting from aerobic fermentation in a laboratory-scale reactor. The extraction and fractionation method was based on the solubility properties of humic acids in basic media and fulvic acids in acidic or basic media.

The extraction of humic substances was carried out following the method described by Nardi et al. (1994). Approximately 4g of dry compost was weighed, crushed, and sieved. The sample was placed in a 500 mL Erlenmeyer flask and 40 mL of potassium hydroxide (KOH) was added. The flask was shaken for 6 hours and left to rest for a further 24 hours in a refrigerator. The suspension was then transferred to a centrifuge tube and centrifuged for 30 minutes at 3000 rpm to obtain two fractions. The AH and AF were found in the supernatant recovered from each extract [Amir et al., 2010]. The solubility of HA in the basic medium was explained by the formation of salts due to the interaction of HA with the cations of the bases used [Schnitzer. 1978].

Fractionation and separation of HA and FA

The HA recovered from the supernatant are in the form of humic acid salts. The fractionation of humic extracts was achieved by acidifying the media [Baglieri et al. 2014]. For this, HCl (6 N) was added until the pH reached around 2. The sample was then centrifuged at 3000 rpm for 30 minutes, and the supernatant was eliminated. After centrifugation, a light yellow supernatant was formed by the FA and a residue was formed by the HA. The fulvic acid fraction was soluble in both alkaline and dilute acid solutions. HA that were not soluble in acid media precipitated.

The precipitates were re-extracted repeatedly to solubilize and remove the FA. The residues left in the centrifuge tubes after the reextraction were oven dried at 68 °C before being put into solutions.

Characterization of the HA fractions

Two stock solutions of HA with final concentrations of 0.1 and 1 gL^{-1} were prepared from each of the four compost mixe. The HA fractions were characterized using CHNOS elemental analysis and UV-Vis spectroscopy. Thermo Finnigan EA 1110 CHNS (Waltham, MS, USA) was used for solid-state elemental composition analysis, with high temperature combustion to ensure accurate results. Physico-chemical parameters such as pH, C.E., humidity, O.C., N, and O.M. were measured according to AFNOR standards.

The specific absorbances of HA were measured by dissolving 12.5 mg in one liter of low-normality bicarbonate, following protocols established by Swift (1996), Chen et al. (1977), and Owen (1996). The absorbance of HA was measured using a UV-2450 spectrophotometer (SHIMADZU). The Welt ratio (E4/E6) was calculated by dividing the absorbance at 465 nm by the absorbance at 665 nm. The Welt ratio is commonly used as a humification index and is used to classify HA into four different types. A low Welt ratio indicates an increase in the degree of humification, condensation, and abundance of aromatic compounds [El Herradi et al., 2014]. A higher ratio (>5) implies that there are more FA than HA and that the decomposition is less advanced [El Herradi et al. 2014; Cunha et al. 2009]. Conversely, a lower ratio indicates more mature composts used in HA extraction [El Herradi et al. 2014]. The classification of HA is based on the value of ΔlogK, which is the difference in the logarithms of the absorbances at 400 nm and 600 nm [Kumada. 1967; Cunha et al. 2009], with three classifications established in 1967 [Kumada. 1988].

Biological parameters

Plant measurements: Leaf area was measured at 15, 30, 45, and 60 days after planting (d.a.p). Chlorophyll a and b content, as well as total soluble sugar content, were also analyzed before (15 d.a.p) and after treatment (30 d.a.p). at the harvest (60 d.a.p), we analyzed the biometric and biochemical parameters in the roots including, tuber length, root length, fresh and dry root weight, root hairs weight, total protein and carbohydrate content.

Two turnip plants per pot were harvested for each treatment and replicate, and rinsed with distilled water. The plants were then subdivided into leaves, tubers, and root hairs and weighed to determine the average fresh material mass. Root hairs were collected from harvested roots after sieving the soil. The length of tuber and non-tuber roots was also determined. Dry matter was measured after biomass drying in an oven at 105 °C.

Leaf area estimation

The leaf area for individual leaves and each plant was calculated by measuring the length (L) and width (l) of the leaf using a caliper, following the procedure of Picard (1990).

Chlorophyll estimation

The chlorophyll a (Chl a) and chlorophyll b (Chl b) were measured using the methodology developed by Fan et al. (2014). Fresh leaf tissue (0.1g) was incubated in 3 ml acetone and ethanol mixture (3:1 ratio) for 48 hours. The absorbances of the extract solution were read at 645 and 663 nm using a spectrophotometer. The concentrations of Chl a and Chl b were calculated using the following equations:

$$\text{Chl a } (\mu g\ g\ fw^{-1}) = 12.7 \times DO\ (663\ nm) - [2.69 \times DO\ (645\ nm) \times V/(1000 \times W)] \quad (2)$$

$$\text{Chl b } (\mu g\ g\ fw^{-1}) = 22.9 \times DO\ (645\ nm) - [4.68 \times DO\ (663\ nm) \times V/(1000 \times W)] \quad (3)$$

where: fw – fresh material;
V – the extraction volume;
DO – the optical density;
W – the weight of fresh material.

Total soluble sugars estimation

The soluble sugars content were extracted and measured following the method of DuBois et al. (1956). Fresh leaf tissue (0.1g) was homogenized in 3 ml of 85% ethanol, and then 1 ml of extract was mixed with 1 ml of phenol (5%) and 5 ml of 1.8 N sulfuric acid. The contents were incubated in a water bath at 40 °C for 15 to 20 minutes. The absorbance was read at 485 nm using a spectrophotometer. The amount of soluble sugar content was calculated from a calibration of glucose solution.

Crude protein estimation

Nitrogen and protein in tubers were determined using the Kjeldahl method, which consists of sample digestion, distillation, and titration

[Keeney and Nelson. 1982]. Crude protein percentage was estimated by multiplying the Kjeldahl nitrogen content (NTK) with a conversion coefficient of 6.25 [Maclean et al., 2003].

Carbohydrate estimation

Carbohydrate was determined in plants using the method reported by Monrose (2009). Approximately 0.1g of dry matter was collected, crushed, and homogenized in distilled water. The sample was placed in boiling water at 40 °C for 15 to 20 minutes. A fraction of the sample was mixed with concentrated sulfuric acid and 5% phenol. The absorbance was recorded using a spectrophotometer at 485 nm, with glucose serving as the standard.

Statistical analysis

Statistical analysis was performed using SPSS v. 22. One-way ANOVA was used to analyze the data, and significant differences among means at a probability level of 5% were evaluated using the Tukey-HSD multiple comparisons and the least significant difference test.

RESULTS

Compost and soil properties

Table 1 show the main chemical properties of the composts used in this study. All composts, especially compost C4, had high levels of organic matter. The pH levels of the composts were within neutral to alkaline range, while the EC displayed high values. The composts were also characterized by a high mineral fraction content.

The germination indices for the composts, presented in Table 2, ranged from 86 to 110%. These results indicate a low phytotoxicity according to Zucconi (1981).

Table 1. Physical and chemical characteristics of composts used in experiment with Turnip plants

Theses	Measure unit	[a] C1	Compost C2	C3	C4
pH		8.00±0.03 [b]	7.91±0.06	7.88±0.04	8.66±0.02
CE	(ds cm^{-1})	3.26±0.02	3.04±0.03	3.50±0.05	3.40±0.03
TOC		30.19±0.22	32.36±0.34	28.72±0.40	36.40±0.73
TKN		2.76±0.05	2.58±0.03	2.94±0.03	2.89±0.03
C/N		10.95±0.22	12.55±0.25	9.77±0.07	12.60±0.27
NH_4^+		0.21±0.03	0.15±0.01	0.22±0.01	0.22±0.02
NO_3^-	% DM	0.26±0.02	0.19±0.01	0.26±0.01	0.24±0.01
MgO		3.56±0.05	4.83±0.03	4.83±0.02	5.08±0.02
CaO		7.88±0.16	9.08±0.09	8.22±0.34	7.97±0.16
K_2O		12.04±0.26	12.55±0.10	10.05±0.03	14.68±0.06
P_2O_5		6.58±0.14	6.69±0.54	5.95±0.49	7.49±0.03
Fe		0.90±0.03	0.99±0.03	1.00±0.09	0.85±0.08
Zn		0.07±0.01	0.16±0.01	0.76±0.09	0.04±0.00

Note: [a] C1 – mixture of separated municipal solid wastes (SMSW), immature horse manure (IHM) and sugarcane bagasse (SB) at a ratio of 14:3:3 weight weight $weight^{-1}$ (www^{-1}); C2 – mixtures of SMSW, IHM and SB at the ratio of 18:1:1 www^{-1}; C3 – mixtures of SMSW and IHM at a ratio 9:1 ww^{-1} and C4 – mixture of SMSW and SB at a ratio of 9:1 ww^{-1} ; [b] means±standarddeviation of three replicates.

Table 2. Germinatin idex of composts used in experiment with turinp plants

Mix type	control	AH_1[a]	AH_2	AH_3	AH_4	AH_1	AH_2	AH_3	AH_4
Dose	$0gL^{-1}$	$0.1gL^{-1}$[b]				1 gL^{-1}			
GI (%)	98±10[c]	108±12	102±10	103±14	97±17	110±3	104±14	110±8	86±3

Note: aHA_1 – humic acid extracted from compost C1 produced by mixtures of separated municipal solid wastes (SMSW), mixtures of immature horse manure (IHM) and sugarcane bagasse (SB) at a ratio of 14:3:3 weight weight $weight^{-1}$ (www^{-1}); HA_2 – humic acid extracted from compost C2 produced by a mixtures of SMSW, IHM and SB at the ratio of 18:1:1 www^{-1}; HA_3 – humic acid extracted from compost C3 produced by a mixtures of SMSW and IHM at a ratio 9:1 ww^{-1}; HA_4 – humic acid extracted from a compost C4 produced by a mix of SMSW and SB at a ratio of 9:1 ww^{-1}; b – humic acid extracted at 0 gL^{-1} as control and at two dosages 0.1gL^{-1} or 1 gL^{-1}; c – means±standard deviation of three replicates.

Table 3. Physical and chemical characteristics of soil used in experiment with turinp plants

Granulometry (%)			pH_{water}	(dsm $^{-1}$) CE (1:5)	(gkg $^{-1}$ DM) OM	TOC	(mg kg^{-1} DM)			
Clay	Silt	Sand					Total Kjeldahl N	N-NH^{4+}	N-NO^{3-}	P_2O_5 available
0.03	3.3	96.67	8.60	0.33	0.69	0.40	0.03	0.02	0.01	0.11

Note: DM – dray matter.

Table 3 presents the physical and chemical characteristics of the soil used in the turnip plant experiment. The soil had a sandy texture with a predominance of fine sands and was low in organic matter and nutrients.

Characterization of the HA fractions

The physicochemical parameters of the prepared HA solutions are summarized in Table 4. This characterization is based on elemental analysis, UV-Vis spectroscopy, and other physicochemical methods performed in the laboratory. Table 4 shows that the pH of the applied liquid fertilizers is slightly acidic, and the CE and total dissolved solids (TDS) values increase with increasing mass concentration of HA. This explains why the mineral content increases with the increase in the concentration of HA. The results also reveal that the carbon content of the 0.1 and 1 gL^{-1} HA solutions is significant. For solutions containing 1 gL^{-1} of HA, the carbon concentration exceeds 0.5 g, indicating their organic matter richness (Table 4).

The UV-Visible analysis of humic acids is shown in Table 5. This table summarizes the specific absorbances and the Welt ratio, as well as the ΔlogK values for the classification of HA. Based on this characterization, we find that the E4/E6 ratio of all the HAs used is less than 5, indicating that the degree of humification of the composts used in the extraction of HA is advanced and clearly translates the richness of these composts into humic acid compared to fulvic acid.

HA can be classified according to their ΔlogK values. Based on Table 5, we can confirm that HA_1 and HA_3 belong to class B in terms of the importance of humification, and that even HA_2 and HA_4 are mature and can also correspond to class B.

Effect of humic acid on the growth and productivity of turnip plants

To evaluate the effect of HA extracted from four composts on the growth and productivity of turnip plants, both leaf and root were examined.

Table 4. Physical and chemical characteristics of humic acid used in experiment with turinp plants

Dose (gL^{-1})	[AH_1]		[AH_2]		[AH_3]		[AH_4]	
	0.1	1	0.1	1	0.1	1	0.1	1
pH	5.45	5.75	5.52	5.69	5.35	5.73	5.45	5.73
C.E (ds cm^{-1})	0.17	1.6	0.16	1.48	0.2	1.72	0.16	1.39
Salinité (gKg^{-1})	0.1	0.8	0.1	0.7	0.1	0.8	0.1	0.7
TDS (mgL^{-1})	90.6	848	87.3	788	105.2	910	84.2	740
COT (mgL^{-1})	51.9	519	51.9	519	51.6	516	51.9	519
NTK (mgL^{-1})	7.3	73	8.1	81	7.7	77	8.4	84
S (mgL^{-1})	0.45	4.5	0.55	5.5	0.48	4.8	0.52	5.2
P_t (mgL^{-1})	8.08	80.8	6.98	69.8	7.42	74.2	5.67	56.7
K (mgL^{-1})	13.8	138	13.9	139	13.8	138	14.1	141

Table 5. Specific absorbances and the Welt ratio (E4/E6) of the humic acids studied

Parameter	280	Abs (nm) 465	665	E_4/E_6 E_4/E_6	Δlog *K*
AH_1	0.169	16.01 10^{-3}	3.89 10^{-3}	4.12	0.79
AH_2	0.164	15.56 10^{-3}	3.64 10^{-3}	4.27	0.80
AH_3	0.174	21.87 10^{-3}	6.97 10^{-3}	3.14	0.66
AH_4	0.159	15.11 10^{-3}	3.39 10^{-3}	4.46	0.82

The leaf area was measured once before treatment on 15 days after planting (d.a.p) and three times after on 30, 45, and 60 d.a.p. Results of root were evaluated in terms of weighted fresh and dried root biomass and measured length of root parts.

According to the results shown below (Table 6), the average leaf area is almost the same for the pots before the treatment. After HA treatments, the leaf area of the turnip plant varied with sampling time and increased for all HA extracted at a level of 1 gL^{-1} (Table 6). The application of humic acid to sandy soil had a positive effect of biostimulation on leaf area in all cases (increase of 23% to 104%) compared with foliar control of untreated plants (Table 6). Statistical analysis of leaf area showed a significant effect of two concentrations of humic acid extracted ($p \leq 0.01$) on day 30, 45, and 60. Whereas the type of composts HA extract and their interactions between their two concentrations are not significant at the last three samplings, except at 30 and 45 d.a.p, the effect of the type of composts HA-extract are significant ($P \leq 0.05$) in terms of the leaf area (Table 6).

Both HA extracted from compost C1 and compost C2 and supplied to plants at 0.1 gL^{-1} exerted similar increases in leaf area compared to the blank, at 30, 45, and 60 d.a.p being about 123%, 130%, and 124%, respectively. Plants treated with HA from compost C3 and C4 reached the highest average value of leaf area, at all sampling times, but leaf area was higher in plants supplied with a higher dosage of 1 gL^{-1} of HA by about 174% to 204% than in plants treated with a lower dosage of 0.1 gL^{-1} by about 147% to 187%, if compared to the blank.

Regarding tuber size, the HA extracted from different compost supplied to plants at both levels enhanced the tuber size equally. Indeed, compost HA-extract types and their concentrations and also their interaction did not have any significant effect. The value of tuber root length was smallest in the control as 7.49 cm and highest in C3 HA-extract at a dosage of 1 gL^{-1} as 9.83 cm, equivalent to 131% of the control value (Table 7). The classification, according to the Least Significant Difference test at p=0.05, yielded two classes for the type HA-effect, that is, control, HA_4, and HA_2 > HA_4, HA_2, HA_1, and HA_3, and two classes for the concentration level, that is, control > 0.1 and 1 gL^{-1} of HA.

The analysis of variance indicates that there was no significant difference in the no tuber root length and total root length of turnip plants for both humic acid types and levels. However, the interaction effects for both humic acid types and levels seemed to be significant ($p \leq 0.01$) on the no tuber root length and significant ($p \leq 0.05$) on the total root length. Compared with the control

Table 6. Evolution of leaf area (cm^3) of turinp depending on type of compost producing HA and HA levels

Dose	Mix type	Leaf area cm^2 at 15 d.a.p	Leaf area cm^2 at 30 d.a.p	Leaf area cm^2 at 45 d.a.p	Leaf area cm^2 at 60 d.a.p
[b] $0gl^{-1}$	Control	2.75±0.21[c]	21.38±1.63f[d]	23.66±2.52c	27.37±3.36c
$0.1gl^{-1}$	[a]HA_1	3.21±0.36	26.40±0.70e	30.77±1.52b	34.07±0.57b
	HA_2	4.87±0.89	40.03±1.40a	43.34±4.72b	45.69±2.61b
	HA_3	4.42±0.68	36.20±1.42c	39.01±2.01b	40.23±5.19b
	HA_4	3.86±0.69	31.97±2.37d	34.52±2.54b	38.52±1.84b
$1gl^{-1}$	HA_1	4.41±0.69	35.82±2.91c	42.82±2.74a	43.98±3.68a
	HA_2	4.53±1.11	38.85±2.39b	46.69±0.73a	47.58±0.70a
	HA_3	4.79±1.30	40.85±3.57a	48.19±4.65a	49.40±3.67a
	HA_4	4.55±1.21	38.72±3.55b	41.51±2.39a	43.19±2.99a
Dose		NS	**	**	**
Compost		NS	*	*	NS
Dose*compost		NS	NS	NS	NS

Note: aHA_1 – humic acid extracted from compost C1 produced by mixtures of separated municipal solid wastes (SMSW), mixtures of immature horse manure (IHM) and sugarcane bagasse (SB) at a ratio of 14:3:3 weight weight $weight^{-1}$ (www^{-1}); HA_2 – humic acid extracted from compost C2 produced by a mixtures of SMSW, IHM and SB at the ratio of 18:1:1 www^{-1}; HA_3 – humic acid extracted from compost C3 produced by a mixtures of SMSW and IHM at a ratio 9:1 ww^{-1}; HA_4 – humic acid extracted from a compost C4 produced by a mix of SMSW and SB at a ratio of 9:1 ww^{-1}; b – humic acid extracted at 0 gL^{-1} as control and at two dosages 0.1gL^{-1} or 1gL^{-1}; c – means±standard deviation of three replicates; d – values followed by the same letters in a column are not significantly different at p = 0.05 according to least significant difference (LSD).

Table 7. Turnip growth depending on type and concentrations of HA at 60 days after planting

Dose	Mix type	Root tuber length cm	Root no tuber length cm	Total root length cm	Fresh root weight %	Dry root weight %	Total dry root hairs weight g/ plant
[b]0gL^{-1}	Control	[c]7.49±0.37b[d]	4.26±0.42b	11.75±0.74b	76.10±2.67b	26.43±0.64a	0.11g
0.1gL^{-1}	[a]HA$_1$	8.89±0.31a	5.30±0.08a	14.19±0.38a	89.93±3.48a	14.16±0.35d	0.18e
	HA$_2$	9.53±0.5ab7	6.37±0.14a	15.90±0.59a	94.04±2.12a	7.42±0.32d	0.17f
	HA$_3$	9.39±0.57a	6.21±0.28a	15.59±0.83a	86.88±1.13a	12.63±0.29e	0.24b
	HA$_4$	9.30± 0.30ab	7.92±0.66a	17.23±0.94a	89.13±3.64a	12.73±0.17d	0.24±0.01b
1gL^{-1}	HA$_1$	9.68±0.29a	7.01±0.51a	16.70±0.25a	92.97±1.77a	7.92±0.13c	0.23c
	HA$_2$	8.42±0.51ab	7.37±0.39a	15.79±0.52a	87.84±1.76a	16.27±0.80c	0.20d
	HA$_3$	9.83±0.89a	6.09±0.60a	15.93±1.47a	84.15±1.82a	20.64±0.89b	0.30±0.01a
	HA$_4$	8.43±0.44ab	4.94±0.87a	13.38±1.28a	95.45±2.17a	8.79±0.12c	0.31±0.01a
Source of variation							
Dose		NS	NS	NS	NS	***	***
Compost		NS	NS	NS	*	***	***
Dose*Compost		NS	**	*	NS	***	NS

Note: aHA$_1$ – humic acid extracted from compost C1 produced by mixtures of separated municipal solid wastes (SMSW), mixtures of immature horse manure (IHM) and sugarcane bagasse (SB) at a ratio of 14:3:3 weight weight^{-1} (ww^{-1}); HA$_2$ – humic acid extracted from compost C2 produced by a mixtures of SMSW, IHM and SB at the ratio of 18:1:1 www^{-1}; HA$_3$ – humic acid extracted from compost C3 produced by a mixtures of SMSW and IHM at a ratio 9:1 ww^{-1}; HA$_4$ – humic acid extracted from a compost C4 produced by a mix of SMSW and SB at a ratio of 9:1 ww^{-1}; b – humic acid extracted at 0 gL^{-1} as control and at two dosages 0.1gL^{-1} or 1gL^{-1}; c – means±standard deviation of three replicates; d – values followed by the same letters in a column are not significantly different at p = 0.05 according to Least Significant Difference (LSD).

sandy soil, humic acid addition increased no tuber root length, total root length, and fresh root weight (Table 7), which are 130 %, 105 % and 106 % of the control (as an average of all traetments), respectivly.

Based on the results presented in Table 7, the root hairs of the treated plants exhibited a higher dry weight compared to the control for all treatments, particularly at the dose of 1 gL^{-1}. The data indicates that the fresh root weight was only statistically influenced ($p \leq 0.05$) by the types of humic acid, with no significant difference observed for the levels of humic acid and their interaction with compost HA-extract types.

The analysis of variance revealed a significant difference ($p<0.001$) in the dry root weight and total dry root hairs of turnip plants when considering the humic acid type, dose, and their interaction, except for the dry root where the interaction effect of hairs was not found to be statistically significant (Table 7).

Effects on chlorophyll a and b and total soluble sugars of leafs of turnip plants

According to the Table 8, it can be observed that the contents of chlorophyll a and b were almost equal before the application of HA. After 15 days of treatment (at day 30), plants treated with C1HA-extract, C2HA-extract and C3HA-extract showed an increase in total chlorophyll a and b content. The chlorophyll b content was significantly ($p<0.05$) affected by HA levels, whereas no significant differences were observed in chlorophyll a content. The type of compost HA-extract and its interaction with concentration significantly ($p<0.001$) affected both chlorophyll a and b.

At an application level of 0.1 gL^{-1} of HA$_4$ extracted from compost C4, there was a lower average change in chlorophyll a and b contents compared to those without HA application. However, the chlorophyll b increased with an increase in the level (1 gL^{-1}) of the same HA supply with an average of 85.29 μg g fw^{-1} as compared to control plants (74.04 μg g fw^{-1}). It is important to note that the highest value of chlorophyll a content was observed in the treatment of HA extracted from composts C2 and C3 at a level of 0.1 gL^{-1}, with an increase of 31% and 37%, respectively, compared to control plants. The treatment of HA$_1$ and HA$_2$ extracted from composts C1 and C2 at a level of 0.1 gL^{-1} improved the highest increase in chlorophyll b content by 20% and 29%, respectively, compared to control plants (Table 8).

Table 8. Evolution of Chlorophyll content of turinp depending on type of compost producing HA and HA doses

Dose	Mix type	Chl a-15d.a.p	Chl a-30d.a.p	Chl b-15d.a.p	Chl b-30d.a.p
		(μg g fw^{-1})			
[b] 0gl^{-1}	Control	[c]34.69±0.76a[d]	44.91±0.77c	60.23±0.85a	74.04±0.24c
0.1gl^{-1}	[a]HA_1	31.86±0.53b	51.28±0.99ab	55.23±1.28b	89.06±3.09ab
	HA_2	28.93±1.02f	58.91±1.75a	50.90±2.20d	95.31±1.54a
	HA_3	26.03±1.01g	61.31±2.56a	42.81±0.99g	87.73±2.12b
	HA_4	39.80±0.90a	43.46±1.77bc	66.58±0.90a	68.86±1.68c
1gl^{-1}	HA_1	25.95±0.34e	54.23±0.94ab	45.35±0.77f	88.56±2.00ab
	HA_2	31.45±0.34b	57.21±1.23a	53.35±2.63c	91.27±1.72a
	HA_3	29.60±0.77d	52.44±1.00b	52.13±2.25c	87.19±0.28b
	HA_4	30.58±0.65c	56.02±1.12b	50.71±0.68e	85.29±1.57cb
Dose		***	NS	**	*
Compost		***	***	***	***
Dose*Compost		***	***	***	***

Note: aHA_1 – humic acid extracted from compost C1 produced by mixtures of separated municipal solid wastes (SMSW), mixtures of immature horse manure (IHM) and sugarcane bagasse (SB) at a ratio of 14:3:3 weight weight weight^{-1} (www^{-1}); HA_2 – humic acid extracted from compost C2 produced by a mixtures of SMSW, IHM and SB at the ratio of 18:1:1 www^{-1}; HA_3 – humic acid extracted from compost C3 produced by a mixtures of SMSW and IHM at a ratio 9:1 ww^{-1}; HA_4 – humic acid extracted from a compost C4 produced by a mix of SMSW and SB at a ratio of 9:1 ww^{-1}; fw – fresh leaf weight.

The content of total soluble sugars in leaves was also increased by HA application, with a greater effect at an HA level of 0.1 gL^{-1} than at a level of 1 gL^{-1}, except for HA_1 extracted from compost C1. The results presented in the Table 9 of the total soluble sugars content in leaves illustrate that the effect of the level and type of HA application, as well as their interactions, are significant ($P \leq 0.001$), but no variation was observed between plants supplied with HA from composts C2, C3, and C4 at a level of 0.1 gL^{-1}. However, the HA obtained from compost C1 at both dosages of 0.1

Table 9. Evolution of Total soluble sugars content of turnip depending on type of compost producing HA and HA levels

Dose	Mix type	Total soluble sugars at 15d.a.p (mg g fw^{-1})	Total soluble sugars at 30d.a.p (mg g fw^{-1})
[b] 0gl^{-1}	Control	0.11f[d]	3.67±0.08f
0.1gl^{-1}	[a]HA_1	[c] 0.24±0.10d	4.60±0.11b
	HA_2	0.29±0.10b	4.35±0.07c
	HA_3	0.13f	4.58±0.11c
	HA_4	0.16f	4.01±0.10c
1gl^{-1}	HA_1	0.49±0.10a	5.80±0.10a
	HA_2	0.21±0.10e	3.47±0.14d
	HA_3	0.27±0.10c	3.25±0.01e
	HA_4	0.23d	3.26±0.04d
Dose		***	***
Compost		***	***
Dose*compost		***	***

Note: aHA_1 – humic acid extracted from compost C1 produced by mixtures of separated municipal solid wastes (SMSW), mixtures of immature horse manure (IHM) and sugarcane bagasse (SB) at a ratio of 14:3:3 weight weight weight^{-1} (www^{-1}); HA_2 – humic acid extracted from compost C2 produced by a mixtures of SMSW, IHM and SB at the ratio of 18:1:1 www^{-1}; HA_3 – humic acid extracted from compost C3 produced by a mixtures of SMSW and IHM at a ratio 9:1 ww^{-1}; HA_4 – humic acid extracted from a compost C4 produced by a mix of SMSW and SB at a ratio of 9:1 ww^{-1}; b – humic acid extracted at 0 gL^{-1} as control and at two dosages 0.1gL^{-1} or 1gL^{-1}; c –means±standard deviation of three replicates; d – values followed by the same letters in a column are not significantly different at p = 0.05 according to Least Significant Difference (LSD); fw – fresh leaf weight.

gL^{-1} and 1 gL^{-1} achieved a more pronounced accumulation of total soluble sugars, being about 0.25–0.58-fold higher than the control, respectively (Table 9). HA extracted from composts C2, C3, and C4 exerted a general positive effect on the content of total soluble sugars when furnished to plants at a lower level (0.1 gL^{-1}), as they were 19%, 25%, and 9% higher than the control, respectively. Despite the fact that they were almost ineffective at a higher level (1 gL^{-1}), they were 5% to 11% less than the control plants (Table 9).

Effects on total protein and total carbohydrate of roots of turnip plants

At harvest (60 d.a.p), we measured the total protein and total carbohydrate content of turnip roots treated with compost HA-extract at two different dosages. Compared to the control, all compost HA-extract treatments resulted in increased total protein and total carbohydrate content (Table 10). The analysis of variance showed no significant difference in total protein content between the HA levels, but there was a significant difference ($p \leq 0.05$) between compost types of HA-extract and their interaction with the HA levels.

At 0.1 gL^{-1}, all compost HA-extract treatments increased the total carbohydrate content of roots by approximately 12 to 23%, while the higher dosage (1 gL^{-1}) produced a similar effect to the 0.1 gL^{-1} level of HA application, with increases ranging from 10 to 26% compared to the control (Table 10). All four HA-extracts derived from the four composts at both dosages produced similar increases in total carbohydrate content of the roots, ranging between 10 to 26%, compared to plants untreated with HA.

DISCUSSION

The results of this study showed that the addition of HA extracted from compost produced by composting a mixture of SMSW, IHM, and SB at different ratios enhanced the growth of turnip plants in terms of leaf area, root biomass, and length at all sampling times in sandy soils. Biostimulation of plant growth with humic substances has been reported in several studies, which demonstrated their capacity to improve soil fertility and promote crop growth in various crops [Yang et al., 2019a; Shafi et al., 2020], including turnip root [Aisha et al. 2014], chicory [Gholami et al. 2018], medical cannabis [Bernstein et al., 2019], forage sorghum [Adam Ali et al., 2022], and maize [Yuan et al., 2022].

Table 10. Effect of two levels of HA extracted from four types of compost on root total protein and root total carbohydrates in turnip plants at 60 days after planting

Dose	Mix type	Total protein (%)	Total carbohydrate (%)
^{b}0gl^{-1}	Control	0.64±0.01c	3.91±0.09b
0.1gl^{-1}	^{a}HA$_1$	0.77±0.02b	4.40±0.01a
	HA$_2$	0.85±0.01a	4.80±0.06a
	HA$_3$	0.77±0.03b	4.74±0.20a
	HA$_4$	0.81±0.02a	4.39±0.07a
1gl^{-1}	HA$_1$	0.81±0.01a	4.91±0.10a
	HA$_2$	0.75±0.03b	4.34±0.10a
	HA$_3$	0.71±0.03b	4.31±0.21a
	HA$_4$	0.80±0.02a	4.75±0.11a
Dose		NS	NS
Compost		*	NS
Dose*compost		*	***

Note: aHA$_1$ – humic acid extracted from compost C1 produced by mixtures of separated municipal solid wastes (SMSW), mixtures of immature horse manure (IHM) and sugarcane bagasse (SB) at a ratio of 14:3:3 weight weight weight^{-1} (www^{-1}); HA$_2$ – humic acid extracted from compost C2 produced by a mixtures of SMSW, IHM and SB at the ratio of 18:1:1 www^{-1}; HA$_3$ – humic acid extracted from compost C3 produced by a mixtures of SMSW and IHM at a ratio 9:1 ww^{-1}; HA$_4$ – humic acid extracted from a compost C4 produced by a mix of SMSW and SB at a ratio of 9:1 ww^{-1}; b – humic acid extracted at 0 gL^{-1} as control and at two dosages 0.1gL^{-1} or 1gL^{-1}; c – means±standard deviation of three replicates; d – values followed by the same letters in a column are not significantly different at p = 0.05 according to Least Significant Difference (LSD); fw – fresh leaf weight.

Water is crucial to improve plant growth and soil productivity, especially in sandy soils, which have low clay and organic matter content and retain little water from irrigation [Yao et al., 2013]. The presence of HA increased the proportion of macro-aggregates, decreased soil bulk density, and improved water content [Zhou et al., 2019]. HA can increase the permeability of cell membranes, resulting in increased water and nutrient absorption, increased root uptake capacity for water and nutrients, and improved plant growth and fresh weight. Previous studies have shown that the biostimulation of HA from composts is better compared to that from leonardite [Fascella et al., 2018], and the fulvic-like acids biostimulant effect of digestate water extracts in hydroponic cultures of *Lactuca sativa* has also been reported [Guilayn et al., 2020].

In this study, the absence of a nutrient solution and the use of sandy soil poor in organic matter and nutrients meant that the plants grew with limited nutrients. However, the effects of biostimulants on plant growth observed in this study were associated with the supplementary addition of macro and trace nutrients caused by compost extracts. Our findings support previous studies indicating that the application of HA has a significant effect on changing plant morphology, primarily improving root initiation, root biomass, and the stimulation of root hair formation, which results in an increase and accumulation of nutrient uptake by plants [Rouphael et al., 2017; Colla et al., 2017].

Furthermore, humic acid has been reported to up-regulate the gene expression and action of enzymes catalyzing key steps of nitrogen assimilation, cell respiration processes, and hormone-like activities by dint of their content in signaling molecules such as peptides, hormone-like substances, amino acids, and phenols [Crawford and Arst. 1993; Chen et al., 2022; Nardi et al., 2021]. In this context, the hormone-like activity of humic substances has been associated with the presence and activity of auxin-like and gibberellin-like substances and the concentration of indoleacetic acid.

Therefore, the pre-treated organic material derived from combined SMSW, IHM, and SB, and the extraction of HA, improved turnip growth in this study, which may be attributed, at least in part, to the HA-mediated improvement of sandy soil productivity due to their positive effects on nutrition and activity of enzymes involved in N metabolism and glycolysis.

Mutlu and Tas (2022) found results similar to our findings for another culture of durum wheat (*Triticum durum L*). They showed that the application of the highest dose of humic acid led to the highest chlorophyll content value, while the control treatment had the lowest value. Additionally, humic acid may have contributed to the improvement of photosynthesis activity and increase in total chlorophyll content by promoting amino acid presence, accelerating nitrogen and nitrate absorption, increasing nitrogen metabolism, and promoting protein production [Alfatlawi and Alrubaiee. 2020].

Regarding turnip, our results showed that total soluble sugar contents were influenced by the levels of HA treatments extracted by compost C2, C3, and C4 compared to the control (Table 9). Interestingly, a low level (0.1 gL^{-1}) of HA application significantly increased total soluble sugar contents compared to the high level (1 gL^{-1} equivalent to 28 $Kg_{ww}ha^{-1}$) and the control. On the other hand, HA_1 extracted by compost C1 increased sugar contents with an increase in the level of HA application (Table 9). This could be explained by the fact that compost C1 had a higher proportion of sugarcane bagasse and immature horse manure in its initial composition compared to the other composts.

Numerous reports have demonstrated that HA can increase the total sugar content of various plants, including turnip roots [Aisha et al. 2014], cotton (Bakry et al., 2013), sunflower [AL-Abody et al., 2021], peas (Pisum sativum L.) [Khan et al. 2013], chicory [Gholami et al., 2018], tomato [Suliman et al., 2020; Lu et al., 2023], sugar beet, potato [Tan. 2014], pepper [Karakurt et al. 2009], and Acacia saligna [El-Khateeb et al., 2011]. Additionally, HA treatments have been shown to increase the root total sugar value of radish (Raphanus sativus L.) [Barzegar et al., 2021]. In strawberries, the total sugar content of fruits improved significantly following foliar and soil drench application of HA [Eshghi and Garazhian. 2015]. Denre et al. (2013) demonstrated that foliar application of HA significantly influenced the total sugar content of green pepper fruits. Furthermore, Zahid et al. (2020) reported that the HA treatment significantly increased the total sugar of oyster mushrooms, ranging from 5.8% to 12.1%. This increase could be attributed to the promising role of HA in supplying plants with essential macro and micronutrients, which enhances photosynthesis and carbohydrate synthesis [Tan. 2014].

Previous studies have also shown that HA facilitates the transfer of glucose via cell membranes in sugar beet, onion, and sunflower [Tan. 2014].

Studies have indicated that HA can enhance mineral nutrient absorption by the root and affect primary metabolites in plants. For instance, leaf phosphorus levels can improve the function of the RuBisCO enzyme, allowing for more photosynthesis and carbohydrate biosynthesis [Peng et al., 2021; Luo et al., 2021]. Moreover, phosphorus is present in the structures of three coenzymes : coenzyme A, nicotinamide adenine dinucleotide phosphate, and adenosine 3′-phosphate, which are all essential for the production of glucose compounds.

We also examined how HA treatment affects the total protein and carbohydrate contents of the root. Our results showed that the protein and carbohydrate levels increased with two levels of HA application across all composts (Table 10). Numerous studies have discussed the significant effects of HA on carbohydrate and protein content in various plant roots, including ginger rhizomes [Taibo et al., 2007], tomato [Suliman et al., 2020; Lu et al. 2023], turnip roots [Aisha et al., 2014], and radish [Barzegar et al., 2021], which are corroborated by our findings. Ma et al. (2022) demonstrated that HA treatment significantly improves soil health parameters, which may have affected the absorption of macro-elements. Moreover, enhanced nutrient absorption by plants could have increased nitrogen uptake [Adani et al., 1998; Yuan et al., 2022] and activity of nitrate reductase and glutamine synthetase as well [Aylaj et al., 2018], which likely raised carbohydrate and protein production [Barzegar et al., 2021; Ma et al., 2022].

CONCLUSIONS

The results of this study confirm the effectiveness of composting municipal solid waste (MSW) with immature horse manure (IHM) and sugarcane bagasse (SB) at different ratios in producing stabilized composts with no phytotoxic effects. Humic acid (HA) extracted from the composts was characterized via UV-Vis spectroscopy, and the results showed high yield and advanced degree of humification for each extracted compost in terms of elemental composition and spectral characteristics. Spectroscopic analyses also demonstrated the richness of the composts in humic acid compared to fulvic acid.

The research results showed that applying humic acid to turnip crop could improve growth and yield. The use of humic acid demonstrated beneficial effects on various growth parameters of turnip plants, particularly when grown in sandy, nutrient-poor soil. The best treatment for leaf area was obtained for plants treated with humic acid extracted from C1 and C2 at 0.1 gL^{-1} HA and from C3 and C4 at level 1 gL^{-1} HA at all sampling times. Leaf area was higher in plants supplied with higher dosage 1 gL^{-1} of HA by about 174% to 204%, than in plants treated with lower dosage 0.1 gL^{-1} by about 147% to 187%, if compared to the blank.

Humic acid addition increased no tuber root length, total root length and fresh root weight, by an average of 130%, 105%, and 106% respectively compared to the control. Additionally, the highest value of chlorophyll a content was observed in the treatment of humic acid extracted from composts C2 and C3 at level 0.1 gL^{-1} with an increase by 31% and 37%, respectively, compared with the control plant. The treatment of HA_1 and HA_2 extracted from composts C1 and C2 at level 0.1gL^{-1} improved the highest increase in chlorophyll b content by 20% and 29%, respectively, compared with plants with no amendment.

Humic acid extracted from composts C2, C3, and C4 had a general positive effect on total soluble sugars content when furnished to plants at a lower level (0.1 gL^{-1}), with an increase of 19%, 25%, and 9% respectively compared to the control. The highest amount of total soluble sugar, total protein, and total carbohydrate content were 4.6 mg g fw^{-1}, 0.85%, and 4.9% respectively, which were 1 µg g fw^{-1}, 0.12%, and 1% higher than the control at harvest. The application of all composts HA-extract at both dosages resulted in an increase in total protein and total carbohydrate compared to the control. Four humic acid derived from four composts at both dosages determined similar increases in total carbohydrate of root ranged between 10 to 26% compared to values measured for plants untreated with humic acid. Furthermore, this experiment demonsrated how the positive effects of humic acid recovered from separated municipal solid waste, immature horse manure and sugarcane bagasse co-composts were underlined if applied on sandy alkaline, poor soil. The use of humic acid provided by municipal waste composts could be a low-cost strategy to improve soil fertility and productivity in sandy dryland soils around the world.

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