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Effect of humic acid as an additive to growing media to enhance the production of eggplant and tomato transplants

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SUMMARY
In two experiments, conducted in 2011 and in 2012, 14 different growing media were prepared from peat moss, vermiculite, and/or perlite with or without 0.5 g l^{-1} humic acid (HA) to determine the optimum growing medium for eggplant (*Solanum melongena* L.) and tomato (*Lycopersicum esculentum* Mill.) transplants. None of the media tested affected the percentages of seedling emergence or survival in either crop, although the rates of emergence were affected. Higher dry weights, relative water contents, membrane stability indices, and N, P, and K uptake values were recorded for eggplant and tomato transplants in all HA-supplemented media than in the corresponding HA-free media. In terms of efficacy, the best medium among all 14 tested was 0.5 g l^{-1} HA + 50% (v/v) vermiculite + 50% (v/v) perlite, followed by 0.5 g HA + 65% (v/v) vermiculite + 35% (v/v) perlite, then 50% (v/v) vermiculite + 50% (v/v) perlite without HA. The first medium was significantly better for all traits measured than the standard medium currently used for tomato and eggplant transplant production in Middle Eastern countries.

Peat moss, vermiculite, and perlite are common components of the growing media used in nurseries and greenhouses. The standard growth medium for transplants in Middle Eastern countries is a 2:1 (v/v) mix of peat moss and vermiculite. Peat moss has long been the primary component of vegetable transplant media worldwide, due to its high water-holding capacity, low density, and acidity. Perlite is produced from igneous rock under high temperatures (600° - 900°C). Perlite differs from vermiculite in that the finished product is a "closed cell" that has a low cation-exchange capacity (CEC) and does not absorb or hold water. For this reason, it is usually included to improve drainage or to increase aeration. Perlite has a low density (96.11 - 128.15 kg m^{-3}), is chemically inert, pH neutral, sterile, and odourless (Robbins and Evans, 2004).

Vermiculite has a high CEC and high water-holding capacity.It is sterile and has a low density (80.10 - 128.15 kg m^{-3}). The pH of vermiculite varies depending on its source. Most US sources are neutral-to-slightly alkaline, whereas vermiculite from Africa can be more alkaline (pH 9). Vermiculite is used extensively in the greenhouse industry as a component of various media and for propagation.The finer grades of vermiculite are used extensively for seed germination or to top-dress seed flats. Expanded vermiculite should not be pressed or compacted, especially when wet, as this will destroy its desirable physical properties (Robbins and Evans, 2004).

The addition of humic acid (HA) to growing media has been shown to have beneficial effects on plant growth (Türkmen *et al.*, 2004) and on the physical characteristics of growing media in Styrofoam flats in which soils or growing media tend to become compacted. Compaction is often accompanied by reductions in water-holding capacity, drainage, aeration, the rate of water infiltration, and root penetration.

Humic substances (HA and fulvic acid) represent 65 - 70% of the organic matter in many soils. These compounds are produced by the decomposition of plant tissue and are predominantly derived from lignified cell walls. The major functional groups in HA include carboxyl, phenolic hydroxyl, alcoholic hydroxyl, ketone, and quinoid moieties (Russo and Berlyn, 1990). The benefits of HA for plant growth may be related to its positive effects on increased fertiliser-use efficiency and/or reduced medium compaction of the growing medum (Nardi *et al*., 2002). In addition, HA has been claimed to promote plant growth by increasing cell membrane permeability, oxygen uptake, respiration and photosynthesis, nutrient uptake, and root cell elongation (Russo and Berlyn, 1990; Böhme and Thi Lua, 1997; Nardi *et al*., 2002). The effects of HA on the growth of tomato seedlings have been investigated in some growing media (Loffredo *et al*., 1997; Pertuit *et al*., 2001; Atiyeh *et al*., 2002; Nardi *et al*., 2002; Osman and Rady, 2012).

The aim of the present study was to measure the effects of supplementation with 0.5 g l^{-1} HA in a range of transplant growing media containing peat moss, vermiculite, and/or perlite, compared to HA-free media and to the standard growing medium used in the Middle East. Transplant growth [measured in terms of dry mass (DM), the uptake of N, P and K, and their effects on the nutrient status, relative water content, and membrane stability index were compared between HA-containing and HA-free media, including the standard medium used in the Middle East, over two successive seasons (2011 and 2012).

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MATERIALS AND METHODS

Source and analyses of humic acid (HA) and other media components

The HA, added at 0.5 g l^{-1} to each growing medium, was produced by Alpha Chemika, Mumbai, India.

Peat moss from Latvia (FAVORIT; SAB Syker Agrarberatungs-und Handels GmbH & Co., Syke, Germany), vermiculite, and perlite (ECPV; El-Masryya Co., Cairo, Egypt) were analysed according to the standard procedures recommended by Black *et al*. (1965). The main physico-chemical characteristics of the peat moss, vermiculite, and perlite were, respectively: total porosity, 91, 79, and 70% (v/v); bulk density, 0.07, 0.17, and 0.14 g m^{-3}; air space after drainage, 15, 7, and 29% (v/v); water-holding capacity, 690, 290, and 270 ml l^{-1} medium; pH, 3.2, 8.5, and 7.1; electrical conductivity (EC), 0.18, 0.06, and trace dS m^{-1}; and C:N ratio, 46:1, trace, and trace. The other chemical characteristics of the peat, perlite, and vermiculite used are presented in Table I.

Composition of the growing media

Fourteen media were formulated (seven with and seven without 0.5 g l^{-1} HA) and peat moss as the organic component, and vermiculite and perlite as the inorganic components, as illustrated in Table II.

All growing media were mixed with a fertiliser consisting of 0.415 g l^{-1} ammonium nitrate, 0.500 g l^{-1} calcium superphosphate, 0.333 g l^{-1} potassium sulphate, 0.083 g l^{-1} magnesium sulphate, 0.033 g l^{-1} iron, 0.033 g l^{-1} zinc, 0.033 g l^{-1} manganese, 1.250 g l^{-1} calcium carbonate (to adjust the pH of peat-containing media), and 0.125 g l^{-1} Moncut SC [a 25% (w/w) active flutolanil-containing, wettable powder, fungicide; Central Glass Co. Ltd, Tokyo, Japan].

Cultural practices

Two greenhouse trials were conducted at the Experimental Station of the Faculty of Agriculture, Fayoum University starting on 15 October 2011 and on 17 March 2012. Individual 209-cell Styrofoam flats (2.6 cm 2.6 cm 7.0 cm; 25 cm^3 per inverted pyramid cell; Speedling, El-Amryya, Alexandria, Egypt) each containing one of the various growth media tested were sown (one seed per cell) with tomato (*Lycopersicon esculentum*, Mill. 'Champion' hybrid) or eggplant (*Solanum melongena*, L. 'Black Beauty' hybrid) seed produced by Seeds Semences Semillus (Ball Horticulture Co., Chicago, IL, USA). After sowing and irrigation, eight Styrofoam flats containing each of the 14 media were subjected to average day and night temperatures of 24º ± 3ºC and 16º ± 2ºC, respectively. The relative humidity ranged from 62.0 - 65.1%, and natural day-length ranged from 11 - 12 h.The Styrofoam flats (n = 112) were arranged in a randomised complete block design with four replications (n = 56 flats) for each species in each trial. Each tray was one replicate and was placed on rails in the greenhouse. The trays were rotated daily within each block to avoid any positional bias. All transplants were grown for 4 weeks. The transplants were overhead-irrigated daily, one day with tap water and the next day with a nutrient solution containing 1.750 g l^{-1} N, 0.875 g l^{-1} P, 1.750 g l^{-1} K, 17.5 mg l^{-1} Fe, 8.75 mg l^{-1} Mn, 0.875 mg l^{-1} B, 0.875 mg l^{-1} Cu, 0.875 mg l^{-1} Zn, and 0.35 mg l^{-1} Mo.

Measurements of the total percentage of seedling emergence, the rate of emergence, and seedling survival

The total percentage emergence of tomato and eggplant seedlings was calculated from those that emerged above the level of the growth medium on day-7 after sowing. The rate of emergence was calculated from the number of seedlings that emerged each day until day-7 after sowing. The rate of emergence was calculated according to Murillo-Amador *et al*. (2002) as follows:

$$Rate\ of\ emergence\ (d^{-1}) = \frac{n_1}{t_1} + \frac{n_2}{t_2} + \frac{n_3}{t_3} + \frac{\dots}{\dots} + \frac{n_7}{t_7}$$

where, n_1, n_2, … n_7 were the numbers of the seedlings emerged on day-1 to day-7 (t_1, t_2, … t_7).

The percentage survival of seedlings was measured 28 d after sowing (DAS).

Measurement of transplant dry weights (DWs)

Twenty 28-d-old tomato or eggplant transplants were removed at random from the four replicate Styrofoam flats of each medium (i.e., five transplants from each flat) and dipped in a bucket filled with water. The transplants were moved smoothly to remove any adhering particles of medium. The transplants were then separated into leaves, stems, and roots and placed in an oven at 70ºC to reach a constant DW which was recorded in each case.

TABLE I
Some of the chemical characteristics of the peat, perlite, and vermiculite used in these experiments

Parameter (units)	Peat moss	Parameter (units)	Perlite	Vermiculite
N (g kg^{-1})	10.10	SiO_2 (g kg^{-1})	741.6	444.5
K (g kg^{-1})	0.21	Al_2O_3 (g kg^{-1})	112.9	131.2
P (g kg^{-1})	0.29	Fe_2O_3 (g kg^{-1})	26.9	76.5
Ca (g kg^{-1})	3.21	CaO (g kg^{-1})	9.9	15.6
Mg (g kg^{-1})	0.37	MgO (g kg^{-1})	5.3	219.3
S (g kg^{-1})	1.21	Na_2O (g kg^{-1})	33.9	36.5
Fe (g kg^{-1})	2.14	K_2O (g kg^{-1})	39.2	39.9
NH_{4^-} (g kg^{-1})	0.23			
NO_{3^-} (mg kg^{-1})	5.00			
P_2O_5 (mg kg^{-1})	2.33			
K_2O (mg kg^{-1})	61.24			
Cu (mg kg^{-1})	1.40			
Zn (mg kg^{-1})	3.70			
Mn (mg kg^{-1})	11.32			
Moisture (g kg^{-1})	500			
Ash (g kg^{-1})	50			

TABLE II
Components of the 14 media used in these experiments

Medium No.	Humic acid (g l^{-1} medium)	Peat moss [% (v/v)]	Vermiculite [% (v/v)]	Perlite [% (v/v)]
1	0	50	50	0
2	0	25	50	25
3	0	0	50	50
4	0	0	65	35
5	0	0	35	65
6	0	65	35	0
7	0	35	65	0
8	0.5	50	50	0
9	0.5	25	50	25
10	0.5	0	50	50
11	0.5	0	65	35
12	0.5	0	35	65
13	0.5	65	35	0
14	0.5	35	65	0

TABLE III
Percentage seedling emergence, rate of emergence, and percentage survival of 4-week-old tomato and eggplant transplants produced in seven different media with or without 0.5 g l^{-1} humic acid (HA) in two seasons

Medium components [% (v/v)]				Tomato			Eggplant		
HA (g l^{-1})	Peat	Verm.[#]	Perl.[#]	Percentage emergence	Rate of emergence (d^{-1})	Percentage survival	Percentage of emergence	Rate of emergence (d^{-1})	Percentage survival
2011 season									
0	50	50	0	99.17	5.03def[†]	96.03	93.21	4.10cd	92.75
0	25	50	25	98.44	5.87ab	94.15	93.24	3.99cd	90.50
0	0	50	50	99.77	5.95a	95.30	93.45	4.21abc	94.75
0	0	65	35	98.69	5.78ab	95.00	93.04	4.11bcd	94.50
0	0	35	65	98.44	6.00a	92.00	93.28	4.55a	94.25
0	65	35	0	99.17	5.13de	96.80	93.48	4.20bc	93.75
0	35	65	0	98.75	4.93def	96.05	93.49	3.79de	93.50
0.5	50	50	0	98.45	4.87ef	97.82	93.56	3.96cde	93.50
0.5	25	50	25	98.37	5.63abc	95.92	93.37	3.77de	91.53
0.5	0	50	50	98.50	5.30cd	97.33	93.36	4.10cd	94.50
0.5	0	65	35	99.08	5.54bc	97.27	93.85	4.02cd	95.75
0.5	0	35	65	99.40	5.73ab	95.77	93.87	4.45ab	95.25
0.5	65	35	0	99.11	4.84ef	97.89	93.47	4.11bcd	94.00
0.5	35	65	0	99.25	4.65f	97.89	93.94	3.62e	93.75
2012 season									
0	50	50	0	98.99	5.14def	97.00	93.96	4.17c	93.25
0	25	50	25	98.80	6.05ab	95.50	93.73	4.07cde	91.50
0	0	50	50	99.52	6.08ab	96.00	93.96	4.25bc	95.00
0	0	65	35	99.25	5.96ab	96.60	93.04	4.18c	94.75
0	0	35	65	99.00	6.15a	95.35	93.78	4.63a	94.50
0	65	35	0	99.24	5.29de	97.55	93.48	4.27bc	94.25
0	35	65	0	99.25	5.05ef	96.55	93.69	3.78e	94.00
0.5	50	50	0	99.00	4.96ef	98.14	93.74	4.07cde	94.00
0.5	25	50	25	99.16	5.78abc	96.42	93.87	3.86de	92.53
0.5	0	50	50	99.00	5.47cd	97.83	93.48	4.17c	94.75
0.5	0	65	35	99.33	5.71bc	97.75	93.85	4.08cde	96.25
0.5	0	35	65	99.51	5.90ab	96.47	94.18	4.55a	95.50
0.5	65	35	0	99.00	4.97ef	98.14	93.47	4.16cd	94.25
0.5	35	65	0	99.00	4.77f	98.14	93.94	3.63g	94.25

[†]Mean values in each column followed by different lower-case letters are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$ (n = 20).
[#]Verm., vermiculite; Perl., perlite.

Determination of relative water contents (RWC) and membrane stability indices (MSI)

Values of RWC were determined using fresh, 2 cm-diameter, fully-expanded leaf discs (n = 20 per medium *ex* five flats) after excluding the midrib. The 20 fresh leaf discs were weighed (fresh mass; FM) and immediately floated on double-distilled water in a Petri dish for 24 h, in the dark, to saturate them with water. Any adhering water was blotted dry and the turgid mass (TM) was measured. The dry mass (DM) was recorded after dehydrating the discs at 70°C for 48 h. The RWC was then calculated using the formula of Hayat *et al*. (2007):

$$RWC\ (\%) = [(FM - DM) / (TM - DM)] \times 100$$

The membrane stability index (MSI) was estimated as described by Rady (2011) using duplicate 0.2 g samples of fully-expanded leaf tissue (five transplants per flat). Each sample was placed in a test-tube containing 10 ml of double-distilled water and heated at 40°C in a water bath for 30 min. The EC of the solution (EC_1) was recorded using a conductivity bridge (Starlac Industries, Ambala, Haryana, India). A second sample was boiled at 100°C for 10 min, and its EC was measured (EC_2). The MSI was calculated using the formula:

$$MSI\ (\%) = [1 - (EC_1 / EC_2)] \times 100$$

Determination of nitrogen (N), phosphorus (P) and potassium (K) concentrations

Dried, powdered, eggplant and tomato transplants (five per flat) were used to determine N, P, and K concentrations (in mg g^{-1} DW), to calculate the transfer factor for each nutrient. N concentrations were determined by the Ningbo Medical Instruments Co. (Ningbo, P. R. China) using a micro-Kjeldahl apparatus, as described by the AOAC (1995). P concentrations were measured using the molybdenum-reduced molybdophosphoric blue colour method (Jackson, 1967) in sulphuric acid (with reduction to exclude arsenate). Sulphomolybdic acid (molybdenum blue), diluted sulphomolybdic acid, and 8% (w/v) sodium bisulphite-H_2SO_4 were used as standard reagents. K concentrations were measured using a Perkin-Elmer Model 52-A flame photometer (Glenbrook, Stamford, CT, USA) as described by Page *et al*. (1982).

Transfer factors for N, P and K from each medium to the transplants

The transfer factor (TF) is the ratio of the concentration of a mineral nutrient in transplant tissues to its concentration in the growing medium. The TF of each nutrient was calculated based on the method described by Harrison and Chirgawi (1989), according to the formula:

$$TF = Pn\ (\text{mg g}^{-1}\ \text{DW}) / Sn\ (\text{mg g}^{-1}\ \text{DW})$$

TABLE IV

Dry weights of 4-week-old tomato transplants produced in seven different media with or without 0.5 g l^{-1} humic acid (HA) in two seasons

Medium components [% (v/v)]				Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Total transplant
HA (g l^{-1})	Peat	Verm.[#]	Perl.[#]	transplant^{-1}	transplant^{-1}	transplant^{-1}	dry weight (g)
2011 season							
0	50	50	0	0.37ef[†]	0.21de	0.35ef	0.92hi
0	25	50	25	0.40de	0.22cde	0.41cd	1.01de
0	0	50	50	0.53b	0.24bc	0.47ab	1.23bc
0	0	65	35	0.50b	0.23cd	0.44bc	1.17cd
0	0	35	65	0.43cd	0.20e	0.35ef	0.98gh
0	65	35	0	0.40de	0.23cd	0.36e	0.99fgh
0	35	65	0	0.33f	0.17f	0.28g	0.77j
0.5	50	50	0	0.42cd	0.23cd	0.37de	1.01fg
0.5	25	50	25	0.43cd	0.24bc	0.44bc	1.10de
0.5	0	50	50	0.61a	0.27a	0.50a	1.38a
0.5	0	65	35	0.58a	0.26ab	0.47ab	1.31ab
0.5	0	35	65	0.45c	0.22cde	0.39de	1.06efg
0.5	65	35	0	0.43cd	0.26ab	0.39de	1.07ef
0.5	35	65	0	0.36ef	0.20e	0.31fg	0.87i
2012 season							
0	50	50	0	0.38f	0.21e	0.36fg	0.95gh
0	25	50	25	0.41ef	0.22de	0.42cde	1.05ef
0	0	50	50	0.54b	0.24cd	0.48ab	1.27bc
0	0	65	35	0.52bc	0.23cde	0.46bc	1.21cd
0	0	35	65	0.44d	0.22de	0.36fg	1.02fg
0	65	35	0	0.41ef	0.24cd	0.39ef	1.05ef
0	35	65	0	0.34g	0.17f	0.29h	0.79i
0.5	50	50	0	0.42df	0.24cd	0.39ef	1.06ef
0.5	25	50	25	0.44d	0.25bc	0.45bcd	1.14de
0.5	0	50	50	0.64a	0.28a	0.52a	1.44a
0.5	0	65	35	0.60a	0.27ab	0.49ab	1.36ab
0.5	0	35	65	0.47cd	0.23cde	0.40def	1.11ef
0.5	65	35	0	0.44d	0.28a	0.40def	1.10ef
0.5	35	65	0	0.37fg	0.21e	0.33gh	0.90h

[†]Mean values in each column followed by different lower-case letters are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$ (n = 20).

[#]Verm., vermiculite; Perl., perlite.

where *Pn* was the concentration of each nutrient in the transplant, and Sn was the concentration of the same nutrient in the growth medium before sowing (determined according to Page *et al.*, 1982) plus that added to the nutrient solution throughout the duration of the experiment (28 d).

Statistical analysis

All values (in 20 replicate samples per treatment) of the measured parameters for the eggplant or tomato transplants were subjected to statistical analysis following the standard procedures described by Gomez and Gomez (1984). Fisher's test was applied to assess the least significant difference (LSD) of each treatment at a probability level of 95% ($P \leq 0.05$).

RESULTS

Effect of adding HA on the rate of seedling emergence, on the percentage of seedling emergence, and on the survival of eggplant and tomato seedlings

All seven media supplemented with 0.5 g l^{-1} HA showed no differences in the emergence or survival percentages of tomato and eggplant seedlings, but 0.5 g l^{-1} HA significantly affected the rate of emergence when compared to the corresponding HA-free media (Table III). The same trend was observed in both seasons (2011 and 2012). The rates of seedling emergence varied among the 14 media from 4.65 - 6.00 d^{-1} and from 4.77 - 6.15 d^{-1} for tomato seedlings, and from 3.62 - 4.55 d^{-1} and from 3.63 - 4.63 d^{-1} among the 14 media for eggplant seedlings in 2011 and 2012, respectively. Perlite-supplemented media gave the highest rates of emergence compared to peat moss- or vermiculite-supplemented media. The rates of seedling emergence of tomato and eggplant seedlings in all 14 media therefore ranged from 4 - 7 d^{-1}, as previously reported (Styer and Koranski, 1997).

Effect of adding HA on the dry weights (DWs) of tomato and eggplant transplants

Leaf DWs, stem DWs, root DWs, and total DWs of tomato and eggplant transplants showed significantly higher values in HA-supplemented media than in HA-free media (Table IV; Table V). The medium consisting of 50% (v/v) vermiculite + 50% (v/v) perlite + 0.5 g l^{-1} HA gave the highest transplant DWs compared to all other HA-supplemented media. Yields in this medium exceeded those in the standard medium currently used in the Middle East [i.e., 65% (v/v) peat moss + 35% (v/v) vermiculite] by 39.4% and 37.1% for the DWs of tomato transplants, and by 30.8% and 31.5% for the DWs of eggplant transplants, in 2011 and 2012, respectively. The medium containing 65% (v/v) vermiculite + 35% (v/v) perlite + 0.5 g l^{-1} HA was second-best with respect to transplant DWs.

Effect of HA on the relative water contents (RWCs) and membrane stability indices (MSIs) of tomato and eggplant transplants

Both the RWCs and MSIs of tomato and eggplant transplants showed significantly higher values in HA-

TABLE V

Dry weights of 4-week-old eggplant transplants produced in seven different media with or without 0.5 g l^{-1} humic acid (HA) in two seasons

Medium components [% (v/v)]				Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Total transplant
HA (g l^{-1})	Peat	Verm.[#]	Perl.[#]	transplant^{-1}	transplant^{-1}	transplant^{-1}	dry weight (g)
2011 season							
0	50	50	0	0.42def[†]	0.25bcd	0.29e	0.96gh
0	25	50	25	0.44cdef	0.29ab	0.35cd	1.08def
0	0	50	50	0.56a	0.30a	0.42a	1.28ab
0	0	65	35	0.53ab	0.28abc	0.41ab	1.22bc
0	0	35	65	0.45cde	0.28abc	0.41ab	1.14cde
0	65	35	0	0.44cdef	0.28abc	0.32de	1.04fg
0	35	65	0	0.38f	0.22d	0.24f	0.84i
0.5	50	50	0	0.46cde	0.29ab	0.31de	1.05efg
0.5	25	50	25	0.47cde	0.32a	0.37bc	1.16cd
0.5	0	50	50	0.59a	0.32a	0.45a	1.36a
0.5	0	65	35	0.57a	0.31a	0.43a	1.31ab
0.5	0	35	65	0.49bc	0.30a	0.44a	1.22bc
0.5	65	35	0	0.48bcd	0.31a	0.35cd	1.14cde
0.5	35	65	0	0.41ef	0.24cd	0.28ef	0.93hi
2012 season							
0	50	50	0	0.43ef	0.26cd	0.30f	1.00fg
0	25	50	25	0.46def	0.30abc	0.36de	1.12de
0	0	50	50	0.58ab	0.31ab	0.43abc	1.33ab
0	65	35		0.55abc	0.29bc	0.42bc	1.26bc
0	35	65		0.47de	0.29bc	0.43abc	1.18cde
0	65	35	0	0.46def	0.29bc	0.33ef	1.08ef
0	35	65	0	0.39f	0.23d	0.25g	0.88h
0.5	50	50	0	0.47de	0.29bc	0.32ef	1.09gh
0.5	25	50	25	0.49cde	0.33ab	0.39cd	1.21cd
0.5	0	50	50	0.62a	0.34a	0.47a	1.42a
0.5	0	65	35	0.59a	0.32ab	0.45ab	1.36ab
0.5	0	35	65	0.51bcd	0.31ab	0.46ab	1.27bc
0.5	65	35	0	0.50cd	0.32ab	0.36de	1.18cde
0.5	35	65	0	0.42ef	0.26cd	0.28fg	0.96gh

[†]Mean values in each column followed by different lower-case letters are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$ (n = 20).

[#]Verm., vermiculite; Perl., perlite.

supplemented media than in HA-free media (Table VI). The highest values of RWC and MSI were recorded in the medium consisting of 50% vermiculite + 50% perlite + 0.5 g l^{-1} HA. This medium exceeded the standard medium used in the Middle East [i.e., 65% (v/v) peat moss + 35% (v/v) vermiculite] by 8.5% and 5.7% for the RWC values of tomato transplants, by 5.1% and 8.5% for the RWC values of eggplant transplants, by 17.5% and 16.8% for the MSI values of tomato transplants, and by 15.3% and 15.8% for the MSIs of eggplant transplants in 2011 and 2012, respectively. The medium consisting of 65% (v/v) vermiculite + 35% (v/v) perlite + 0.5 g l^{-1} HA was second-best for both RWC and MSI values in both species and in both seasons.

Effect of adding HA on the transfer factors (TFs) for N, P and K in tomato and eggplant transplants

The TF values for N, P, and K from the various media to the tomato and eggplant transplants are presented in Table VII for 2011 and 2012. All seven media supplemented with HA gave significant higher TF values for N, P, and K than the seven corresponding HA-free media. The medium containing 50% (v/v) vermiculite + 50% (v/v) perlite + 0.5 g l^{-1} HA gave the highest TF values for N, P, and K compared to all other HA-supplemented media. TF values in this medium exceeded the standard medium [65% (v/v) peat moss + 35% (v/v) vermiculite] used in the Middle East by 10.5% and 9.1% for N in tomato transplants, by 9.0% and 10.2% for N in eggplant transplants, by 23.8% and 17.4% for P in tomato transplants, by 22.7% and 27.3% for P in eggplant transplants, by 10.2% and 12.1% for K in tomato transplants, and by 11.3% and 11.1% for K in eggplant transplants in 2011 and 2012, respectively. The medium consisting of 65% (v/v) vermiculite + 35% (v/v) perlite + 0.5 g l^{-1} HA was second-best for TF values for N, P and K.

DISCUSSION

The application of 0.5 g l^{-1} HA to each of seven different growth media was found to produce healthier and stronger tomato and eggplant transplants, in higher yields (data not shown). There were significant positive effects for all growth media supplemented with 0.5 g l^{-1} HA compared to HA-free media for both tomato and eggplant transplants. The benefits of HA as an additive to the media may be due to its effects on increased fertiliser-use efficiency, and/or reductions in medium compaction, leading to improvements in overall transplant biomass (DM). The presence of HA increased the overall fertility, stability, and functionality of each medium. These positive effects did not occur in the HA-free media, which gave the poorest quality transplants due to their higher water-holding capacity accompanied by lower air-space after drainage, resulting in a shortage of oxygen, which has negative effects on the respiration of roots.

Perlite-containing media showed relatively higher rates of seedling emergence, which may be attributed to their reduced water-holding capacity and increased air-

TABLE VI

Relative water content [RWC (%)] and membrane stability index [MSI (%)] of 4-week-old tomato and eggplant transplants produced in seven different media with or without 0.5 g l⁻¹ humic acid (HA) in two seasons

Medium components [% (v/v)]				Tomato		Eggplant	
HA (g l⁻¹)	Peat	Verm.[#]	Perl.[#]	RWC (%)	MSI (%)	RWC (%)	MIS(%)
2011 season							
0	50	50	0	84.6c[†]	54.1de	89.4b	55.7gh
0	25	50	25	87.5bc	56.6cde	89.5b	59.5cdefg
0	0	50	50	90.3ab	61.8bc	90.3ab	60.8abcd
0	0	65	35	87.7bc	59.2cd	89.6b	60.4bcde
0	0	35	65	87.4bc	51.5e	89.4b	57.3defgh
0	65	35	0	85.9c	59.2cd	89.7b	56.3efgh
0	35	65	0	85.8c	48.5f	89.8b	53.0h
0.5	50	50	0	84.5c	59.2cd	90.3ab	59.1cdefg
0.5	25	50	25	87.4bc	61.8bc	90.4ab	63.2abc
0.5	0	50	50	93.2a	69.5a	94.3a	64.9a
0.5	0	65	35	90.6ab	66.9ab	91.9ab	64.3ab
0.5	0	35	65	87.8bc	56.6cde	89.9b	60.6bcd
0.5	65	35	0	87.5bc	66.7ab	89.3b	59.8cdef
0.5	35	65	0	84.3c	51.6e	88.0b	56.1fgh
2012 season							
0	50	50	0	88.5b	50.1e	85.4c	52.4fg
0	25	50	25	88.5b	52.6de	88.5bc	55.2ef
0	0	50	50	89.4ab	57.3cd	91.3ab	61.4abc
0	0	65	35	88.6b	55.0cde	88.6bc	60.0bcd
0	0	35	65	88.5b	52.6de	88.4bc	58.9bcd
0	65	35	0	88.4b	57.3cd	86.7c	56.8de
0	35	65	0	87.9b	47.1f	86.8c	50.9g
0.5	50	50	0	90.0ab	57.3cd	85.3c	55.3ef
0.5	25	50	25	89.6ab	59.7bc	88.4bc	61.1abc
0.5	0	50	50	93.4a	66.9a	94.1a	65.8a
0.5	0	65	35	90.2ab	64.5ab	91.4ab	62.0ab
0.5	0	35	65	90.0ab	54.9cde	88.8bc	60.4abcd
0.5	65	35	0	89.0ab	64.3ab	88.3bc	58.2cde
0.5	35	65	0	88.5b	50.2e	85.0c	52.9fg

[†]Mean values in each column followed by different lower-case letters are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$ (n = 20).
[#]Verm., vermiculite; Perl., perlite.

spaces after drainage, compared to peat moss and/or vermiculite. Adding HA to the perlite-containing media may have compensated for any physico-chemical defects of perlite. The rates of emergence for tomato and eggplant seedlings recorded here were within the range of 4 - 7 d^{-1} shown by Styer and Koranski (1997).

All seven HA-supplemented media produced higher tomato and eggplant DWs than the corresponding HA-free medium (Table IV;Table V).This result agreed with those reported by Chen and Aviad (1990), Tattini *et al.* (1991), and Türkmen *et al*. (2004) in tomato and olive. Zhang and Schmidt (2000), Nardi *et al.* (2002), and Zhang and Ervin (2004) reported that HAs contain cytokinins and have an auxin-like activity which resulted in increased concentrations of endogenous cytokinins and auxins which can improve root and shoot growth. The enhanced growth of transplants in HA-supplemented media may therefore be caused by HA-acting as a hormone-like substance (Nardi *et al*., 1996). Rady and Osman (2011) also stated that HA may stimulate plant growth by acting as a hormone-like substance, and by its promotion of respiration, photosynthesis, oxidative phosphorylation, protein synthesis, and anti-oxidant and other enzymatic reactions (Zhang and Schmidt, 1999; 2000; Zhang *et al*., 2003). Among the humic fractions, those of low molecular size (< 3,500 Da) can induce morphological changes similar to those caused by indole-3-acetic acid (IAA; Muscolo *et al*., 1993), and thereby improve transplant metabolism (Muscolo and Nardi, 1999). Has also have a positive effect on the growth of various micro-organisms which may excrete a range of vitamins, growth substances, and antibiotics that can further promote the growth of transplants (Nardi *et al*., 2002).

Higher RWC and MSI values were observed in tomato and eggplant transplants grown in HA-supplemented media than in HA-free media (Table VI). This may be attributed to the low molecular weight fraction of HA which can reach the plasma membrane where it exerts its positive effects (Nardi *et al*., 2002), improving the RWC and MSI, and promoting nutrient uptake (Varanini and Pinton, 1995) by enhancing the permeability of root cell membranes (Valdrighi *et al*., 1996).

The transfer (uptake) of N, P, and K from the medium into tomato and eggplant transplants is considered key to the strength of the young transplant.Transfer factors (TFs) were calculated for N, P, and K to quantify relative differences in the bio-availability of these nutrients to transplants, and to identify the efficiency of transplant roots to absorb a given nutrient and translocate it to the shoots.These TF values were based on the uptake of a nutrient by the roots, not the foliar absorption of nutrients. Variations in TF values among transplants produced in the 14 different media may be attributed, in part, to differences in the concentrations of the nutrients in the various media. However, all HA-supplemented media had higher TF values than HA-free media (Table VII). This may indicate that HA-supplementation improved the chemical properties of

TABLE VII
Transfer factor (TF) ratios of N, P and K in 4-week-old tomato and eggplant transplants produced in seven different media with or without 0.5 g l^{-1} humic acid (HA) in two seasons

Medium components [% (v/v)]				Tomato			Eggplant		
HA (g l^{-1})	Peat	Verm.[#]	Perl.[#]	TF of N	TF of P	TF of K	TF of N	TF of P	TF of K
2011 season									
0	50	50	0	0.85f[†]	0.21ef	0.59cd	0.89de	0.22de	0.58ef
0	25	50	25	0.88cdef	0.21ef	0.58d	0.90cde	0.22de	0.59def
0	0	50	50	0.91bc	0.24bc	0.61bc	0.93bc	0.25b	0.62bc
0	0	65	35	0.90bcd	0.23cd	0.60bcd	0.93bc	0.24bc	0.61bcd
0	0	35	65	0.89bcde	0.21ef	0.59cd	0.90cde	0.22de	0.59def
0	65	35	0	0.86ef	0.21ef	0.59cd	0.89de	0.22de	0.57f
0	35	65	0	0.85f	0.19g	0.58d	0.87e	0.20f	0.58ef
0.5	50	50	0	0.87def	0.22de	0.60bcd	0.90cde	0.24bc	0.59def
0.5	25	50	25	0.90bcd	0.22de	0.59cd	0.93bc	0.23cd	0.61bcd
0.5	0	50	50	0.95a	0.26a	0.65a	0.97a	0.27a	0.65a
0.5	0	65	35	0.92ab	0.25ab	0.62b	0.94ab	0.25b	0.63ab
0.5	0	35	65	0.91bc	0.23cd	0.61bc	0.92bcd	0.24bc	0.61bcd
0.5	65	35	0	0.88cdef	0.23cd	0.60bcd	0.90cde	0.24bc	0.60cde
0.5	35	65	0	0.88cdef	0.20fg	0.60bcd	0.88e	0.21ef	0.59def
2012 season									
0	50	50	0	0.87e	0.22cd	0.58ef	0.87e	0.22c	0.59e
0	25	50	25	0.89cde	0.22cd	0.59def	0.88de	0.22c	0.60de
0	0	50	50	0.92bc	0.24b	0.63ab	0.93b	0.25b	0.63bc
0	0	65	35	0.91bcd	0.24b	0.62bc	0.92bc	0.24b	0.62bcd
0	0	35	65	0.88de	0.22cd	0.59def	0.90bcde	0.22c	0.61cde
0	65	35	0	0.88de	0.23bc	0.58ef	0.88de	0.22c	0.60de
0	35	65	0	0.87e	0.19e	0.57f	0.87e	0.19d	0.59e
0.5	50	50	0	0.89cde	0.23bc	0.60cde	0.89cde	0.24b	0.61cde
0.5	25	50	25	0.92bc	0.24b	0.61bcd	0.92bc	0.24b	0.62bcd
0.5	0	50	50	0.96a	0.27a	0.65a	0.97a	0.28a	0.66a
0.5	0	65	35	0.93ab	0.26a	0.63ab	0.93b	0.25b	0.64ab
0.5	0	35	65	0.90bcde	0.24b	0.61bcd	0.92bc	0.25b	0.62bcd
0.5	65	35	0	0.91bcd	0.24b	0.59def	0.91bcd	0.24b	0.62bcd
0.5	35	65	0	0.89cde	0.21de	0.59def	0.88de	0.21cd	0.61cde

[†]Mean values in each column followed by different lower-case letters are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$ (n = 20).
[#]Verm., vermiculite; Perl., perlite.

all seven media by increasing the number of micro-organisms which can enhance nutrient cycling (Sayed *et al.*, 2007) and increasing the bio-availability of mineral nutrients to the transplant roots. HA also promoted the absorption of nutrients by preventing their precipitation in the nutrient solution (Osman and Rady, 2012).

In terms of efficacy, the best growth medium was 0.5 g l^{-1} HA + 50% (v/v) vermiculite + 50% (v/v) perlite, followed by 0.5 g l^{-1} HA + 65% (v/v) vermiculite + 35% (v/v) perlite, then 50% (v/v) vermiculite + 50% (v/v) perlite without HA.The medium consisting of 50% (v/v) vermiculite + 50% (v/v) perlite + 0.5 g l^{-1} HA was found to be superior among all seven HA-supplemented media tested. This medium produced stronger and healthier eggplant and tomato transplants than the standard medium currently used in Middle Eastern countries.

CONCLUSIONS

Humic acid (HA) showed promise as an additive to each of the seven different media tested for eggplant and tomato transplant production. The medium which produced the strongest and healthiest eggplant and tomato transplants consisted of 50% (v/v) vermiculite + 50% (v/v) perlite + 0.5 g l^{-1} HA. This medium provided the optimum drainage, aeration, water-holding capacity, and fertility to overcome the problem of medium compaction in Styrofoam flats. These functions appeared to be provided largely by the low molecular weight HA fraction (< 3,500 Da), which could reach the plasma membranes of root cells and be translocated to influence plant cell metabolism. We hypothesise that components of HA have several targets, depending on their chelating capacity and cytokinin- and/or IAA-like activities. Extensive studies will be required to elucidate and analyse the positive effects of HAs on plant metabolism. These studies should focus on the bio-availability of HAs in the soil solution, in the rhizosphere, and on the relationships between the activities of these substances and the metabolism of soil micro-organisms, and their effects on plant metabolism.

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