HUMIC SUBSTANCES: A POWERFUL TOOL FOR CONTROLLING FUSARIUM WILT DISEASE AND IMPROVING THE GROWTH OF CUCUMBER PLANTS

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SUMMARY

The effects of humic substances (HS) and their basic components, such as humic acid (HA) and fulvic acid (FA), were evaluated for their ability to control *Fusarium oxysporum* f. sp. *cucumerinum*, as well as to improve vegetative growth of cucumber plants under pathogen pressure, in greenhouse conditions. Three concentrations ranging from 50, 100 and 150 ppm for each HS, HA and FA were tested for their efficacy in reducing pathogen growth. *In vitro*, FA extracted from green compost of plant residues and biogas manure significantly reduced ($P = 0.05$) pathogen growth at a concentration of 150 ppm compared to the control. FA extracted from plant residues at concentrations of 150 ppm reduced disease severity significantly (84.7%) in relation to other treatments. A significant increase in dehydrogenase enzyme activities and total number of microorganisms in soil were obtained by HA extracted from biogas manure with respect to other treatments. All applications significantly improved growth parameters of cucumber plants in the presence of natural inoculum, with the effects of HA treatments being more pronounced.

Keywords: Cucumber, Fulvic acid, Fusarium wilt, Humic acid.

INTRODUCTION

Fusarium wilt of cucumber (*Cucumis sativus* L.) is a destructive disease responsible for great yield losses. It is caused by the soil-inhabiting fungus *Fusarium oxysporum* f. sp. *cucumerinum* (Morsey *et al*., 2009; Jun-Li *et al*., 2010). Disease-management methods range from the use of resistant cultivars, grafting, crop rotation and soil replacement (Yu, 2001). Chemical methods used to control Fusarium wilt are inefficient and have hazardous side effects on living organisms, the environment and human health, and overuse could lead to the development of resistance in fungal species (Minuto *et al*., 2006). Grafting of cucumbers onto resistant rootstocks is an effective way to prevent this disease; however, this could lead to loss of the cucumber's taste (Yang *et al*., 2014).

There is considerable interest in the use of organic amendments, not only to improve crop productivity, but also as crop protection strategy (Loffredo *et al*., 2007; Ismail *et al*., 2011). Humic substances (HS) are recognised by most soil scientists and agronomists as the most important component of healthy, fertile soil. Humic acids (HA) comprise a mixture of weak aliphatic (carbon chains) and aromatic (carbon rings) organic acids that are only soluble in water under alkaline conditions. Fulvic acid (FA) is a mixture of weak aliphatic and aromatic organic acids, which is soluble in water in acidic, neutral and alkaline conditions (Pena-Mendez *et al*., 2005). HAs and FAs and other humates supplemented into soil by organic amendment can influence, either directly or indirectly, a number of physiological and biochemical processes occurring in plants and soil-borne organisms, especially in the rhizosphere (Shusheng *et al*., 2008; Meihua *et al*., 2012). The beneficial effect of HAs and FAs as alternatives to synthesised products in controlling plant diseases, especially Fusarium wilt, is well documented (Alvarez *et al*., 2002; Yigit and Dikilitas, 2008; Abdel-Monaim *et al*., 2012, 2014; Kamel *et al*., 2014). Bio-fertilisation also has been proven effective against Fusarium wilt (Shusheng *et al*., 2008; Meihua *et al*., 2012). From environmental and agricultural points of view, non-chemical control procedures are highly desirable. Therefore, the present study is aimed at assessing the efficacy of different sources of humates in suppressing *F. oxysporum* f. sp. *cucumerinum* and improving vegetative parameters of cucumber plants under greenhouse conditions, in the presence of natural inoculum.

MATERIALS AND METHODS

Isolation and identification of cucumber wilt pathogen. Severe wilt symptoms were observed on cucumber plants cultivated under commercial greenhouse conditions. Symptoms included yellowing and necrosis of old leaves, which progressed to the younger leaves and led to the death of the entire plant. Some plants showed a

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Table 1. Physical, chemical and biological analyses of two compost sources and biogas manure.

Physical and chemical analysis			
	Compost from Wastes (commercial)	**Compost from Plant Residues**	**Biogas Manure**
Density kg/m^3	641	609	400
Moisture content %	29.9	48.76	17.7
Dry matter %	70.1	51.24	82.3
pH (1:10)	8.50	5.24	7.51
EC dS/m (1:10)	3.87	1.35	3.75
Ammonia (NH_4) ppm	20.0	9.0	51.7
Nitrate (NO_3) ppm	52.5	11.7	277.3
NH_4/NO_3 ratio	0.38	0.77	0.19
Total nitrogen %	1.02	1.52	1.36
Organic matter %	22.79	70.74	54.80
Organic carbon %	13.22	41.03	31.78
Ash %	77.21	29.26	45.20
C/N ratio	13:1	27:1	23.4:1
Total phosphorus %	0.36	0.35	0.69
Total potassium %	0.63	0.54	0.58
Biological analysis			
Total bacteria *cfu*/g×10^6	96.2	2.5	75.6
Total actinomycets *cfu*/g×10^4	39.6	282	15.9
Total fungi *cfu*/g×10^4	16.6	290	19.5
Total coliform *cfu*/g×10^2	5.6	Nd	Nd
Faecal coliform *cfu*/g×10^2	3.3	Nd	Nd

Cfu, Colony-forming unit; C/N, Carbon/Nitrogen ratio. EC, Electrical Conductivity, Nd = not detected.

distinct pinkish pigmentation on outer tissues of affected roots. Cross sections of the roots of affected plants exhibited an orange discoloration of the vessels. Samples were collected from a greenhouse in the Qallien district, located in the KafrElsheikh Governorate. Isolations were made from diseased roots under sterile conditions. Although the ITS region by itself does not allow for univocal identification of Fusarium species, the combination of molecular and morphological data, and partial satisfaction of Koch's postulates on cucumber plants, reasonably identified the causal agent of the diseases as *Fusarium oxysporum* f. sp. *cucumerinum.* The causal agent was indeed isolated, and once its identity was supported by DNA sequences of the ITS regions (GenBank accession No. KT461496) and shown to be pathogenic to cucumber by soil inoculation, it was re-isolated from the diseased plants.

Plant materials. Trials were carried out on cucumber (*Cucumis sativus* L.) seedlings of cv. beta alpha, prepared at the sale unit of vegetable crops of the Horticult. Research Institute (ARC), Giza, Egypt. The seedlings were grown from surface-disinfected seeds sown in trays containing a mixture of sterilised peat moss and vermiculite (1 : 1 v : v) and, then, kept in the greenhouse at 2 ± 2°C with 60-70% relative humidity for about 30 days.

Source of HS. Biogas manure and green compost plant residues were obtained from the Agricultural Wastes Training Center in Moshtohor, Kalubia Governorate, and commercial compost was obtained from Elebour Wholesale Market in Egypt. The main physical and chemical properties of both were determined according to standard methods described by Jackson (1973) and Page *et al.* (1982) (Table 1). Nutrient agar media (Difco, 1984) was used to estimate the total count of bacteria, Martin's agar medium for fungi and Jensen's medium for actinomycetes (Allen, 1959). The number of total and faecal coliform was determined using Mac Conekey's agar medium, while the number of *Salmonella* and *Shigella* was determined on Salmonella and Shigella agar medium (Difco, 1977). The presence of nematodes was assessed microscopically as described by Taylor and Sasser (1978).

Extraction of HS. Extractions of HS from composts and biogas manure were carried out according to the method described by Sanchez-Monedero *et al.* (2002). HA and FA were separated from the respective HS by acidification, then by centrifugation to allow the HA to form a solid precipitate (while the supernatant is the FA fraction). Then, they were purified according to the method described by Stevenson (1994).

The total phosphorus concentration was determined according to the method described by Murphy and Riley (1962), total potassium concentration by flame photometry (Chapman and Pratt, 1961) and total nitrogen concentration according to Jackson (1973). The total concentrations of acidic, phenolic and carboxylic groups were all determined in extracted HS according to Dragunova (1958). Elemental analysis (C, H, N, S and O_2) of the purified FA was performed using a gas microanalyser (Vario elementor C, H, N, S Germany 2004) as described by Goh and Stevenson (1971).

Treatments. The following treatments were considered: HS, FA and HA from each of the commercial compost, biogas manure and plant residues, as well as Uniform® fungicide (Azoxystrobin: methyl (E)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate 28.2% and Mefenoxam 10.9%) and a control (with pathogen).

***In vitro* growth inhibition of *F. oxysporum* f. sp. *cucumerinum*.** The poisoned media technique was used on potato dextrose agar (PDA) to determine if the growth of *F. oxysporum* f. sp. *cucumerinum* was affected by humates. The humate preparations were centrifuged at 6000×*g* for 20 min, and the supernatant was injected into a Spritzen-/Syrine-Filter 0.22 µm fast-flow PES membrane with very low protein binding. Different concentrations of the filtrate (*i.e.*, 50, 100 and 150 ppm) were added to cooled PDA medium (45°C). The medium was dispensed in Petri dishes (diam. 9 cm) and allowed to solidify. Mycelial plugs (5 mm)

Table 2. Characteristics of humic substances, fulvic acid and humic acid extracted from two sources of compost and biogas manure.

Type		% of extraction	Total (mmol/100g HS)			Elementary composition %				
			Acidity	Carboxylic groups	Phenolic groups	C	N	H	S	O2
HS		46.2	1425	605	820	58.5	5.8	5.1	10.3	21.2
FA	Commercial	20.6	800	360	440	44.6	2.3	4.1	2.8	46.2
HA		25.6	625	245	380	52.4	5.1	4.0	9.0	29.5
HS		47.9	1470	510	960	62	6	6.2	3.2	22.6
FA	Biogas manure	17.5	950	300	650	48.6	2.8	4.3	2.3	42.0
HA		30.4	520	210	310	57.1	5.6	5.1	2.1	30.1
HS		36.3	1085	410	675	62	5.1	6.3	4.6	22
FA	Plant residues	28.8	560	210	350	50.0	1.4	3.3	2.0	43.3
HA		7.5	525	200	325	58.0	4.1	5.0	1.0	31.9

Note: HS are the sum of HA + FA data in percentage of extraction, total acidity, carboxylic and phenolic groups only.

of fungal pathogen (7-day-old cultures) were placed in the middle of the plate. Inoculated, filtrate-free plates were included as controls. Four replicates were used for each treatment. All plates were incubated at 25 ± 1°C for about 7 days. The radial growth of the pathogen was measured until the fungus covered the control plates completely. Inhibition of the pathogen compared to the control was calculated by the equation provided by Vincent (1947).

Assessments of cucumber wilt severity under greenhouse conditions. Greenhouse trials were carried out in the Qallien district located in the Kafr Elsheikh Governorate. The efficiency of HS in controlling Fusarium wilt and their effect on cucumber growth parameters were evaluated in naturally infested greenhouse soil. Beta alpha cucumber seedlings were treated prior to transplanting by dipping the roots in the HS at concentrations of 150 ppm or fungicide (Uniform) at a recommended dose of 2 ml/l for 1 h. Additional treatments included drenching the soils at similar concentrations twice (20 and 40 days from transplanting) using 50 ml/plant. Ten plants were used for each replicate. Seedlings (4-week-old) were transplanted on 2 sides of a soil ridge 50 cm apart. These plants were distributed in 3 rows; each row was 0.7 m wide and 2 m long. Plants were fertilised periodically by mineral fertilisers using the recommended dose. Disease severity (DS) was recorded 90 days after transplanting according to Abdou *et al.* (2001), using a rating scale of 0-5 on the basis of root discoloration or leaf yellowing as follows: 0 = neither root discoloration nor leaf yellowing, 1 = 1-25% root discoloration or 1 leaf yellowed, 2 = 26-50% root discoloration or > 1 leaf yellowed, 3 = 51-75% root discoloration plus 1 leaf wilted, 4 = > 76% root discoloration or > 1 leaf wilted, and 5 = completely dead plant. DS was calculated as described by Liu *et al.* (1995) using the following equation:

$$\% \text{ DS} = (\Sigma d / d\,max \times \text{n}) \times 100$$

where DS is the possible disease rating, *d max* is the maximum disease rating and n is the total number of plants examined in each replicate.

Dehydrogenase activity (DHA) and microorganism counts. Fresh soil from the rhizosphere of the cucumber plants was collected. The samples initially were homogenised using a mortar and sieved through with 2 mm grid. The pH and the dry matter content of the test samples were determined. Tubes with a diameter of 1.8 cm and minimum 20 ml capacity were used. Approximately 2 g aliquots of naturally moist soil were weighed and placed in the tubes. Two ml of substrate solution (2,3,5-triphenyltetrazolium chloride) was added to each sample tube, and 2 ml of Tris buffer solution [Tris(hydroxmthyl) aminomethan 0.1M] instead of the substrate solution was added to the blank tubes. Two replicates of each sample, blank and calibration solution were prepared. Tubes were shaken and incubated at 25 ± 1°C in the dark for 24 h. Then, the tubes were centrifuged at 4500 rpm for 10 min. Triphenyl tetrazolium chloride is converted to red triphenyl formazan salts (TPF) by DHA in microbial cells. The enzyme activity was calibrated against a TPF dilution curve, determined as absorbance at 485 nm and expressed as µg TPF/100g of soil/day as described by Carbonell *et al.* (2000).

Plant growth parameters. Shoot and root length (cm), number of leaves, branches and flowers per plant, dry weight per plant (g) were assessed. Total chlorophyll was quantified using the SPAD-501 portable leaf chlorophyll meter (Minolta Corp) for greenness measurements in the 5th apical fully expanded leaf (Yadava, 1986).

Statistical analysis of data. Data were subjected to analysis of variance (ANOVA) using the XLSTAT-Pro software (AddinSoft, Inc., NY, USA). Homogeneity between groups was tested first, and the mean values of the main effect were compared using the least significant difference (LSD) test at $P < 0.05$.

RESULTS

Characteristics of HS. The data presented in Table 2 show that the extracted biogas manure yielded the highest

Table 3. Effect of humic substances, fulvic acid and humic acid extracted from two sources of composts and biogas manure on the growth of *Fusarium oxysporum* f. sp. *cucumerinum in vitro***.**

Treatments (T)		Inhibition percentages			
		Concentrations (C)			
		50 ppm	100 ppm	150 ppm	Mean (T)
HS	Commercial	31.11	37.78	52.22	40.37
FA		46.67	52.78	63.33	54.26
HA		30.00	36.67	48.89	38.52
HS	Biogas manure	43.33	57.58	70.00	56.97
FA		47.78	80.00	87.78	71.85
HA		41.11	56.67	66.66	54.81
HS	Plant residues	31.11	37.78	64.44	44.44
FA		41.11	54.44	74.44	56.66
HA		33.33	34.44	57.78	41.85
Uniform®		88.89	88.89	88.89	88.89
Control		0.00	0.00	0.00	0.00
Mean (C)		39.49	48.82	61.31	–

LSD at 0.05 Treatment (T) = 2.38, Concentration (C) = 1.24 and T×C = 4.12.

amount of HS (47.9%) and a high amount of HA (30.7%) compared to other two kinds of compost. The biogas manure, however, produced the lowest yield of FA (17.5%). Moreover, the total acidity in HS extracted from commercial compost was extremely high (1425 mmol/100 g). This might be attributed to the components of the wastes composted. Furthermore, total acidity of FA was constantly higher than HA in all samples. For the carboxylic and phenolic groups, the total values for HS from all sources were higher relative to HA and FA. Elementary composition, in particular oxygen, was higher in FA than HA, which may lead to the assumption that FA is a better oxidising agent than HA.

HS affects the growth of *F. oxysporum* f. sp. *cucumerinum in vitro*. Three types of organic sources were evaluated *in vitro* for their ability to suppress growth of *F. oxysporum* f. sp. *cucumerinum*, using the poisoned growth-medium technique. Generally, FA extracted from the three organic sources significantly inhibited growth of *F. oxysporum* f. sp. *cucumerinum* at the three concentrations tested, compared to HS and HA from the same sources, with FA from biogas manure being the most effective; all tested substances were most effective at the highest concentration (150 ppm, Table 3). A significant reduction (88.89%; $P < 0.05$) of pathogen growth also was obtained with the fungicide Uniform at all concentrations compared to the control but not compared to FA at a concentration of 150 ppm (Table 3).

Severity of cucumber wilt disease under greenhouse conditions. In this trial, the capability of the three sources of organic materials to reduce *F. oxysporum* f. sp. *cucumerinum* infection severity was evaluated under naturally infested greenhouse conditions. Reduced DS, compared to the control, was obtained using FA, HS and HA (Table 4). Treatment with HA extracted from biogas manure compost provided more DS reduction (76.9%) than HS (61.7%). In addition, the lowest DS was attributed to the treatment with FA extracted from plant residue compost (84% reduction), a better performance than Uniform fungicide (80.7% reduction, Table 4).

Table 4. Effect of humic substances, fulvic acid and humic acid extracted from two sources of composts and biogas manure on *Fusarium oxysporum* f. sp. *cucumerinum* on cucumber plants under greenhouse conditions (naturally infested soil).

Treatments		Disease Severity %	Reduction %
HS	Commercial	$10.7^{c} \pm 0.05$	69.2
FA		$8.00^{d} \pm 0.11$	78.9
HA		$13.3^{b} \pm 0.11$	61.7
HS	Biogas manure	$13.3^{b} \pm 0.11$	61.7
FA		$6.70^{e} \pm 0.05$	80.7
HA		$8.00^{d} \pm 0.11$	76.9
HS	Plant residues	$10.7^{c} \pm 0.05$	69.2
FA		$5.30^{f} \pm 0.11$	84.7
HA		$13.3^{b} \pm 0.11$	61.7
Uniform®			80.7
Control		$37.7^{a} \pm 0.05$	–
L.S.D. ($P = 0.05$)		0.25	

Data within columns are the mean of $n = 12 \pm$ standard error (SE). Means in columns followed by the same letters are not significantly different according to Fisher LSD test at $P = 0.05$.

Table 5. Effect of humic substances, fulvic acid and humic acid extracted from two sources of composts and biogas manure on dehydrogenase activity.

Treatments		Dehydrogenase (µg TPF*/100g soil/day)		
		Mean ± SE Initial	Mean ± SE Final	% Increase
HS	Commercial	$36^{de} \pm 0.19$	$55.2^{e} \pm 0.59$	53
FA		$33^{f} \pm 0.94$	$40^{g} \pm 0.37$	21
HA		$35.1^{e} \pm 0.35$	$45^{f} \pm 0.03$	28
HS	Biogas manure	$38.1^{bc} \pm 0.45$	$60.7^{d} \pm 0.59$	59
FA		$35.8^{de} \pm 0.41$	$54.2^{e} \pm 0.09$	51
HA		$43.1^{a} \pm 0.31$	$71.2^{a} \pm 0.49$	65
HS	Plant residues	$39^{b} \pm 0.24$	$63.5^{c} \pm 0.94$	63
FA		$37.1^{cd} \pm 0.31$	$60.2^{d} \pm 0.96$	62
HA		$41.1^{a} \pm 0.30$	$67.3^{b} \pm 0.68$	64
Uniform®		$29^{h} \pm 0.24$	$33.2^{i} \pm 0.36$	14
Control		$30^{g} \pm 0.29$	$35^{h} \pm 0.25$	17
L.S.D (P=0.05)		1.491	2.428	

* Triphenyl formazan.

Data within columns are the mean of n=12 ± standard error (SE). Means in columns followed by the same letters are not significantly different according to Fisher LSD test at $P = 0.05$.

Dehydrogenase activity (DHA) and microorganism count. Table 5 shows that basal DHA levels were increased by all treatments (HS, HA and FA). HA extracted from biogas manure was extremely efficient in increasing DHA in the rhizosphere of cucumber plants. At time zero of the

experiment, the DHA was 43.1 µg TPF/100 g of soil/day. This value was raised to 71.2 by the end of the experiment. The fungicide-treated samples displayed the least activity. It is worth mentioning that HA was more effective than HS or FA in inducing DHA. This may be due to its higher content of free functional groups and to the ability of the latter to chelate elements, acting as food source for microorganisms.

The total numbers of microorganisms (bacteria, fungi and actinomycetes) correlated with the increase of DHA. HA extracted from biogas manure was efficient in increasing the total number of bacteria, fungi and actinomycetes in soil. Also, HS was associated with a higher total number of microorganisms than FA. The fungicide treatment was the lowest in this regard, as shown in Table 6.

Growth parameters. This study showed that all treatments enhanced the growth parameters of cucumber plants in relation to the untreated, infected control (Table 7). Treatment with HA extracted from plant residue compost caused significant increase in height of cucumber plants ($P<0.05$) relative to other treatments and to the control plants. The number of leaves produced by plants treated with HA and FA from biogas manure, HA from plant residue compost and fungicide did not differ significantly among each other, but still differed from the control plants. Moreover, according to the LSD test, there was no significant difference among the number of flowers formed by plants treated with either HS, HA and FA extracted from plant residue compost or from biogas manure, and fungicide. In relation to the control and fungicide treatments, only the treatment with HA extracted from plant residue compost significantly increased root length and the number of root branches. There was no significant difference in total chlorophyll values obtained in all treatments with the exception of plants treated with HS extracted from plant residue compost, whose values were significantly higher than those of the fungicide-treated and control plants. The highest dry weight value was obtained for plants treated with HA extracted from biogas manure.

Table 6. Effect of humic substances, fulvic acid and humic acid extracted from two sources of composts and biogas manure on total microorganism counts.

Treatments		Total microorganisms counts (cfu g^{-1} soil)			
		Bacteria (10^6)	Fungi (10^4)	Actinomycetes (10^4)	Mean (T)
HS		75	20	65	53.33
FA	Commercial	70	18	54	47.33
HA		65	15	41	40.33
HS		78	23	77	59.33
FA	Biogas manure	72	18	59	49.67
HA		110	28	91	76.33
HS		95	26	83	68.00
FA	Plant residues	84	25	80	63.00
HA		102	27	87	72.00
Uniform®		42	2	32	25.33
Control		55	13	37	35.00
Mean (M)		77.09	19.54	64.19	

LSD at $P=0.05$: Treatments (T) = 5.72, Microorganisms (M) = 2.99, T×M = 9.92.

DISCUSSION

DHA provides correlative information on the biological activity and microbial populations in soil (Kumar *et al*., 2013). In the current study, all organic sources significantly increased the DHA in the soil, with HA extracted from biogas manure being the most effective. Similarly, Borowska and Koper (2011) found that fertilisation with manure resulted in an increase of dehydrogenases and catalase activities in soil with increasing doses of manure. In the present study, very low DHA activity was observed with Uniform fungicide treatments using the recommended dosage. This finding also was supported by Baruah and

Table 7. Effect of humic substances fulvic acid and humic acid extracted from two sources of composts and biogas manure on growth parameters and total chlorophyll of cucumber plants under greenhouse conditions (naturally infected soil).

Treatments		Root length (cm)	No. Root branches	Plant height (cm)	Number of leaves	Number of flower	Dry weight	Total chlorophyll
HS		$16.0^{de}\pm1.15$	$6.0^{c}\pm0.57$	$103.0^{de}\pm1.15$	$28.00^{d}\pm2.30$	$26.33^{b}\pm1.20$	$3.13^{de}\pm0.43$	$33.66^{bcd}\pm0.66$
FA	Commercial	$14.0^{ef}\pm0.57$	$5.3^{c}\pm0.88$	$102.5^{e}\pm0.86$	$30.66^{bcd}\pm3.52$	$29.33^{a}\pm1.20$	$3.5^{bcd}\pm0.47$	$33.00^{cd}\pm0.57$
HA		$18.6^{bc}\pm0.88$	$6.7^{c}\pm0.33$	$109.0^{bc}\pm1.15$	$30.66^{bcd}\pm1.33$	$26.00^{b}\pm1.15$	$3.33^{cd}\pm0.33$	$32.66^{cd}\pm1.33$
HS		$15.4^{ef}\pm0.69$	$5.00^{c}\pm0.57$	$95.00^{f}\pm1.52$	$25.33^{de}\pm3.52$	$21.66^{c}\pm0.88$	$4.5^{ab}\pm0.28$	$36.00^{abc}\pm1.52$
FA	Biogas manure	$20.4^{bc}\pm0.75$	$10.0^{ab}\pm0.57$	$110.0^{b}\pm4.16$	$36.66^{ab}\pm2.40$	$30.00^{a}\pm1.73$	$4.33^{abc}\pm0.33$	$33.33^{cd}\pm0.33$
HA		$18.0^{cd}\pm0.72$	$9.0^{b}\pm0.57$	$109.0^{bc}\pm1.52$	$35.66^{abc}\pm1.20$	$29.66^{a}\pm0.88$	$4.66^{a}\pm0.33$	$36.00^{abc}\pm1.52$
HS		$20.8^{b}\pm0.49$	$6.0^{c}\pm0.57$	$107.5^{cde}\pm1.25$	$29.00^{cd}\pm2.51$	$23.33^{c}\pm0.66$	$3.66^{abcd}\pm0.33$	$38.00^{a}\pm1.15$
FA	Plant residues	$20.4^{bc}\pm0.69$	$6.0^{c}\pm0.57$	$108.0^{bcd}\pm1.52$	$25.00^{de}\pm2.88$	$30.33^{a}\pm0.88$	$3.33^{cd}\pm0.33$	$37.00^{ab}\pm0.57$
HA		$25.5^{a}\pm0.28$	$11.0^{a}\pm0.57$	$119.0^{a}\pm1.52$	$39.00^{a}\pm0.57$	$28.33^{a}\pm1.45$	$3.5^{bcd}\pm0.28$	$38.33^{a}\pm1.20$
Uniform®		$21.0^{b}\pm1.52$	$5.3^{c}\pm0.88$	$110.5^{bc}\pm0.88$	$37.33^{ab}\pm0.88$	$28.66^{a}\pm0.88$	$4.26^{abc}\pm0.46$	$31.00^{d}\pm0.57$
Control		$13.2^{f}\pm0.24$	$2.7^{d}\pm0.33$	$89.50^{g}\pm1.04$	$19.00^{e}\pm2.64$	$17.66^{d}\pm1.20$	$2.23^{e}\pm0.24$	$25.33^{e}\pm0.88$
LSD ($P=0.05$)		2.46	1.79	4.85	0.687	1.87	0.97	3.13

Data within columns are the mean of $n=12\pm$ standard error (SE). Means in columns followed by the same letters are not significantly different according to Fisher LSD test at $P=0.005$.

Mishra (1986), who reported that DHA in soil correlates inversely to the pesticide dose. The findings of the current study, along with those by Pascual *et al.* (2000), indicated that samples with low microbial and biological activity (e. g., low microbial carbon and low respiration rate), also displayed the lowest DHA. Therefore, it is evident that the enzymatic activity in the soil is strongly connected to the soil's organic matter content, which provides substrate to support microbial biomass, hence, higher enzyme production (Yuan and Yue, 2012).

In the present study, a significant growth reduction of *F. oxysporum* f. sp. *cucumerinum* was obtained *in vitro* with FA obtained from biogas manure and with fungicide. Moliszewska and Pisarek (1996) showed that FA and HA potentially suppressed *Alternaria alternata* and *Fusarium culmorum* in PDA medium. Previous studies showed that HA potentially could reduce the mycelial growth of different plant pathogenic fungi, i.e., *Phytium ultimum*, *Fusarium culmorum, Alternaria alternata* and *F. oxysporum* (Pascual *et al*., 2000; Loffredo *et al*., 2007, 2008). Our results revealed however that HA was less effective in reducing mycelial growth of *F. oxysporum* f. sp. *cucumerinum* than other treatments.

The application of FA to cucumber plants grown in a naturally infested soil also significantly reduced DS in relation to the controls. A similar reduction was achieved by applying the Uniform fungicide at the recommended dosages. The suppressive action of FA on fungal diseases of plants has been well documented in the past. Alvarez *et al.* (2002) attributed the efficacy of FA against powdery mildew to its ability to induce plant resistance. Zhang (1997) found that the foliar application of FA enhanced the production of some antioxidants such as α-tocopherol, α-carotene, superoxide dismutase and ascorbic acid in turf grass species. In the current study, there was no correlation detected between the inhibition of disease progression by FA and the microbial counts and DHA. Thus, this finding led the hypothesis that the suppressive effect of FA might be attributed to its chemical and functional properties that were not investigated in the study. Hahlbrock and Scheel (1989) revealed that FA mostly consists of a mixture of phenolic compounds that play a major role in plant defence. Moreover, Kamel *et al.* (2014) also stated that FA is rich in sulphur, which is a potential control agent for plant diseases, especially powdery and downy mildews.

Concluding, this study clearly showed the efficacy of HS application to control *F. oxysporum* f. sp. *cucumerinum*, and in improving growth parameters under naturally infested conditions.

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