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Pivotal Role of Humic Acid against Powdery and Downy Mildews of Cucumber under Plastic House Conditions

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ABSTARCT

Evaluating the efficacy of humic acid in controlling powdery (*Sphaerotheca fuliginea* Schlecht., Pollacci) and downy (*Pseudoperonospora cubensis* Berk. and Curt.) mildews of cucumber and enhancing the cucumber yield components was explored through experiments of plastic houses under pathogens pressure during two growing seasons 2016/2017 and 2017/2018. Three concentrations 5, 10 and 15 ml/l of humic acid were sprayed five times with seven days interval against powdery and downy mildews compared to bio-agents (*Bacillus subtilis* and *Trichoderma asperellum*) and chemical registered fungicides Ridomil Gold MZ 68 WG with downy mildew and Topas 100 EC with powdery mildew. Maximum reduction percent in disease severity of both powdery (85.23 and 80.88) and downy (70.36 and 81.14) mildew diseases was recorded with the third concentration (15 ml/l) of humic acid compared to other used concentrations during both seasons respectively. The significant reduction effect on powdery and downy mildew diseases was increased with increasing of humic acid concentrations. All treatments increased peroxidase and polyphenol oxidase activity compared with control treatments at all periods of activity. Enzymes of peroxidase and polyphenol oxidase showed the highest activity when induced by *T. asperellum* as well as humic acid at the third concentration. The highest activity was induced by *T. asperellum* and humic acid at concentration of 15 ml/l during the growing season 2016/2017. Similar results were obtained during the second season 2017/2018. Foliar application of humic acid by different concentrations increased significantly the plant height, chlorophyll content, fresh and dry weights as well as yield components and fresh fruit shelf life. These results established that humic acid could play an essential role either for controlling powdery and downy mildews of cucumber through enzymes induction or advantage of enhancing the plant growth, yield and fresh fruit shelf life.
Keywords: humic acid; bioagent; powdery and downy mildews; cucumber; fruit shelf life

INTRODUCTION

Powdery mildew, caused by *Sphaerotheca fuliginea* (Schlecht.) Pollacci, syn. *Podosphaera xanthii* (Castagne) Braun and Shishkoff, formerly known as *Sphaerotheca fusca* (Blumer) (Miazzi *et al*., 2011), and downy mildew, caused by *Pseudoperonospora cubensis* (Berk. and Curt.) Rostov., are worldwide diseases of cucumber under plastic houses as well as field conditions. Powdery and downy mildews are serious diseases which infect all of upper parts of the cucumber plants (*Cucumis sativus* L.) which lead to significant yield loss (Yang *et al*. 2008). The majority of control strategies of cucumber powdery and downy mildews depend on chemical fungicides and hazardous control ways. Obviously, using of chemical fungicides against plant diseases explored a resistance of target pathogens with phytotoxicity of host plants in addition to the environmental pollutants.

Recently, developing of alternative approaches to chemical fungicides for managing powdery and downy mildew diseases of cucumber is urgently needed (Ketta *et al*., 2016; Ketta *et al*., 2017; Kamel *et al*., 2017). This need was grown and more attention was taken to develop alternative ways which are eco-friendly and non-toxic for humans, animals and beneficial microorganisms. Different bio-agents, natural compounds and plant extracts were used in integrated pest management programs for controlling the powdery mildew attacking crops under greenhouses (Dik *et al*., 2002). Among bio-control agents, species of *Trichoderma* are effective and promising bio-agents, because of their antagonistic effect against various plant pathogens (Chet *et al*., 1997). Moreover, *Pseudomonas* and *Bacillus* spp. were used as antagonistic bacteria against several plant diseases (Abdel-Kader *et al*., 2012). Organic fertilizers contain some components that increase product quality, root system development and tolerance to diseases, pests and environmental stresses were estimated by Kamel *et al*., (2014). Humic substances are considered as organic fertilizers, because they contain a lot of minerals necessarily for plant growth improvement, such as Nitrogen, Phosphorus, Potassium and some elements *i.e*, Zinc, Ferric, Cupper and Magnesium (Ajalli *et al*., 2013). Humic substances have been applied successfully as stimulants or soil conditioners (Scheuerell and Mahaffee 2006). Additionally, stimulation of the soil microorganisms and plant growth via increasing of cell division and uptake of water and nutrients were recorded (Chen *et al*., 2004). Most of effective treatments suppressed *Botrytis cinerea* (causal agent of gray mould disease of geranium plants) were compost tea extract and humic acid (Scheuerell and Mahaffee 2006). However, humic acid reduced *Alternaria* leaf spot disease in bean plants when applied as foliar sprays in different concentrations (Abd El-Kareem, 2007). Therefore, recent studies have been aimed to use new approaches for controlling powdery and downy mildews of cucumber such as organic fertilizers, which have the advantages of being less harmful to humans, animals, beneficial microorganisms and surrounding environment and being biodegradable by microflora (Sánchez-Rodríguez *et al*., 2002).

The aim of the present study was to investigate humic acid efficacy under different concentrations to control powdery and downy mildew diseases of cucumber compared to bio-agents and chemical fungicides. Moreover, current work was aimed to enhance the quantity and quality of cucumber grown under plastic house conditions.

MATERIALS AND METHODS

Experiment materials

Evaluation of humic acid as eco-friendly natural product against cucumber powdery and downy mildew diseases was carried out under natural infestation by used cucumber cv. Dp 164 (DP Elite Zaden). The used treatments were applied as foliar sprays under protected cultivation system conditions in commercial plastic houses at Sakha Agriculture Research Station, Ministry of Agriculture and Soil Reclamation during two growing seasons 2016/2017 and 2017/2018. The efficacy of different treatments against downy and powdery mildews infection were applied as follows: humic acid in concentrations (5, 10 and 15 ml/l), *Bacillus subtilis* (Bacterial bio-agent), *Trichoderma asperellum* (Fungal bio-agent), Fungicides, Topas 100 EC against powdery mildew and Ridomil Gold MZ 68 WG against downy mildew and control treatment (untreated).

Humic acid was obtained from commercial humate Leonardite by Microbiology Dept., Soils, Water and Environment Research Institute, Agriculture Research Center, Giza, Egypt. Humic acid (Table 1) was considered as the main factor at three concentrations (5, 10 and 15 ml/ liter of water).

Table 1. Analysis of humic acid.

N (%)	P %	K %	pH	$N\text{-}NH_4$ (mg/L)	$N\text{-}NO_3$ (mg/L)	P_2O_5 (mg/L)	K_2O (mg/L)	MgO (mg/L)
2.8	0.4	10.0	8.2	460	850	890	8600	620
Cu (mg/L)	Zn (mg/L)	S (mg/L)	Fe (mg/L)	Mn (mg/L)	B (mg/L)	Mo (mg/L)	Co (mg/L)	
4.5	11.5	400	15	10	2.3	2	1	

Bio-control agents used in the present work were *B. subtilis* and *T. asperellum*. Bacterial bio-agent *B. subtilis* was obtained from Microbiology Dept., Soils, Water and Environment Research Institute, Agriculture Research Center, Giza, Egypt and cultured for growth on nutrient broth medium for 5 incubation days under 28±2°C, while *T. asperellum* was obtained from Plant Pathology Research Institute, Agriculture Research Center (ARC), Giza, Egypt and cultured for growth on potato dextrose broth medium (PD broth) for 15 incubation days under 30±2°C. Bacterial suspension was used against downy and powdery mildews in concentration 2.6×10^6 CFU/ml, while 2×10^9 spore/ml was used with *T. asperellum*.

Chemical fungicides

Two chemical fungicides (Ridomil Gold MZ 68 WG and Topas-100 EC) were used in this study for comparison. Ridomil Gold MZ 68 WG Syngenta, consists of:

a) Metalaxyl-M 4% [methyl N-(methoxyacetyl)-N-(2,6-xylyl)-D-alaninate; methyl (R)-2-{[(2,6- dimethylK humateenyl)methoxyacetyl]amino}propionate]
b) Mancozeb 64% [(1, 2, etahnediylbi (carbamodithioato) (2-) manganese mixture with (1,2- ethanediylbis (carbamodithioato) (2-) zinc] was used in concentration of 250 g/100 L against downy mildew and Topas-100 EC: (Syngenta, Egypt), 10.2% penconazole (1- [2-(2-4 dichlorophenyl) pentyl] – 1H – 1, 2, 4 triazole) was prepared as recommended doses (25 ml/100L) equivalent to 250 ppm against powdery mildew.

Plastic house experiment

Seedlings four weeks-old of cucumber cv. Dp 164 were transplanted into a plastic house divided to units. Each unit contains 12 plants in three rows with 70 cm between each with length 2 m. Twelve cucumber plants (replicates) were used for each treatment. Cucumber plants were irrigated and fertilized with recommended doses. Black polyethylene plastic mulch was used for weed control. All treatments of untreated cucumber plants, treated with humic acid in three concentrations (5, 10 and 15 ml/l); treated plants with *B. subtilis*, treated plants with *T. asperellum* and treated plants with chemical fungicides (Ridomil Gold MZ 68 WG and Topas-100 EC), were mixed with the adhesive surfactant (bio-film 1265, 30 ml/100 l) and applied. Spraying was repeated once per week during the experiment period for five times. Experiments of plastic house were divided into two experiments, one of them with powdery mildew and the second with downy mildew disease of cucumber. Plastic house temperature and relative humidity were recorded.

Disease severity assessment

Cucumber plants naturally infected by powdery and downy mildews were checked every week for 40 days and disease severity was scored as percentage of leaf area per each plant covered with lesions. Disease severity was recorded according to the scale of 0 to 11 described by Horsfall and Barratt (1945) for downy mildew and scale of 0 to 5 (0 = no infection, 1 = 1-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75% and 5 = 76-100%) described by Sen and Kapoor (1974) for powdery mildew. Equation of Descalzo *et al*., (1990) was used to calculate the disease severity as follows:

$$R = [\sum (a \times b) / (N \times K)] \times 100$$

Where, **R: is the disease severity, a: is number of diseased leaves,**
b: is a numerical value of each grade,
N: is the total examined plants and
K: is the highest infection degree in the used scale.

Enzyme extraction and assays

A sample of 0.5 g fresh leaves was immediately homogenized with liquid nitrogen. One gram of powdered sample was extracted with 2 ml of sodium phosphate buffer, 0.1 M (pH 7.0) at 4°C. The homogenates were centrifuged at 4000 xg at 4°C for 20 min. The supernatant was used as a raw extract for enzymatic assay.

Peroxidase assay:

Peroxidase activity (based on oxidation of pyrogallol to pyrogallin in the presence of H_2O_2) was spectrophotometric measured (as optical density 420 nm (Hartee, 1955). The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract, and 0.5 ml of 1% H_2O_2. The reaction mixture was incubated at room temperature. Changes in absorbance at 420 nm were recorded at 60 s intervals for 3 min and the boiled enzyme preparation served as a blank. Enzyme activity was measured according to the protocol of Hammerschmidt *et al*., (1982).

Polyphenol oxidase assay:

The polyphenol oxidase activity was spectrophotometric measured (as optical density 495nm) according to the method described by Mayer *et al*. (1965). The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 200 µl of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 nm.

Plant parameters measurement

Growth parameters were selected and used such as plant height, chlorophyll content, number of leaves, leaf area, fresh and dry weight. While, the yield components were assessed at harvesting stage such as number of fruits per plant, fruit weight per fruit, yield per plant and fruit shelf life. Chlorophyll (greenness) was measured by portable leaf chlorophyll meter (Minolta SPAD-502, Japan) with fully expanded cucumber leaves according to (Torres-Netto *et al*., (2005). Plant height was taken from the base of cucumber plants to the top by centimeter unit, while fresh and dry weights were scored in gram unit. Number of fruits and fruit yield per plant were taken by harvesting the fruits at marketable size every 2 or 3 days for seven times.

Statistical analysis

A randomized complete block design was used in the present experiments. Duncan's Multiple Rang Test was applied for comparing means. Pearson correlation coefficient (r) was calculated for each treatment with growth parameters. Based on these data, a correlation matrix was constructed and from this matrix (all treatments with enzymes activity) were clustered by the unweight pair group method based on arithmetic mean (UPGMA). The data were analyzed statistically by using the SPSS18.0 software package.

RESULTS AND DISCUSSION

Effect on powdery mildew

Data presented in Table (2) show that humic acid in different concentrations (5, 10 and 15 ml/l) significantly reduced the disease severity of powdery mildew compared to used bio-agents, chemical fungicide (Topas-100 EC) and untreated cucumber plants. Disease severity at the end of experiment calculated with application of humic acid at concentration (15 ml/l) was very low (5.7%) compared to other concentrations applied. Additionally, disease severity of treated cucumber plants with *B. subtilis*, *T. asperellum* and Topas-100 EC was recorded as (5.3, 5 and 3.3%) respectively, compared to untreated control (38.6%) during 2016/2017 growing season. Similar results were obtained with the second growing season 2017/2018. Plastic house temperature was recorded as follows (22-28 and 24-29°C during 2016/2017 and 2017/2018, respectively) and relative humidity (65-84 and 70-80 % during 2016/2017 and 2017/2018, respectively).

Table 2. Percentage of powdery mildew disease severity in response to application of different treatments on cucumber grown under protected cultivation system.

Treatment	Season 2016/2017		Season 2017/2018	
	Disease severity (%)	Efficacy (%)	Disease severity (%)	Efficacy (%)
B. subtilis	5.3 cd	86.26	7.5 d	82.51
T. asperellum	5.0 de	87.04	7.9 d	81.58
HA (5 ml/l)	12.3 b	68.13	15.6 b	63.63
HA (10 ml/l)	7.3 c	81.08	9.5 c	77.85
HA (15 ml/l)	5.7 cd	85.23	8.2 d	80.88
Topas 100 EC	3.3 e	91.45	6.6 e	83.91
Untreated control	38.6 a	0	42.9 a	0

Values in the same column followed by the same letter (Duncan's test) are not significantly different *P < 0.05*. HA = Humic acid

Effect on downy mildew

Application of different treatments as foliar sprays to cucumber plants grown in plastic house resulted a reduction in downy mildew disease severity comparing with untreated plants. Data in Table (3) showed that at the first season all treatments reduced downy mildew disease severity significantly at reduction percent ranged from 34.66 to 79.57%. Furthermore, throughout the second growing season, the applied treatments showed significant suppressive effect on the downy mildew of cucumber comparing with untreated check control (Table 3). Disease severity calculated with application of humic acid (15 ml/l) was low (7.33%) compared to other concentrations applied. In addition, disease severity of treated cucumber plants with *B. subtilis*, *T. asperellum* and Ridomil Gold was recorded as (10.66, 6.66 and 7%) respectively, compared to untreated control (32.6%) during 2016/2017 growing season. Similar results were obtained with the second growing season 2017/2018.

Presented data revealed the suppressive effect of bio-agents against downy mildew disease as well as Ridomil Gold MZ 68 WG (they recorded 8.7; 7.3 and 5.3 disease severity, respectively) compared with 29.7% for untreated plants. Humic acid applied treatments suppressed disease severity of downy mildew during both seasons and the reduction percent increased by humate concentration increasing.

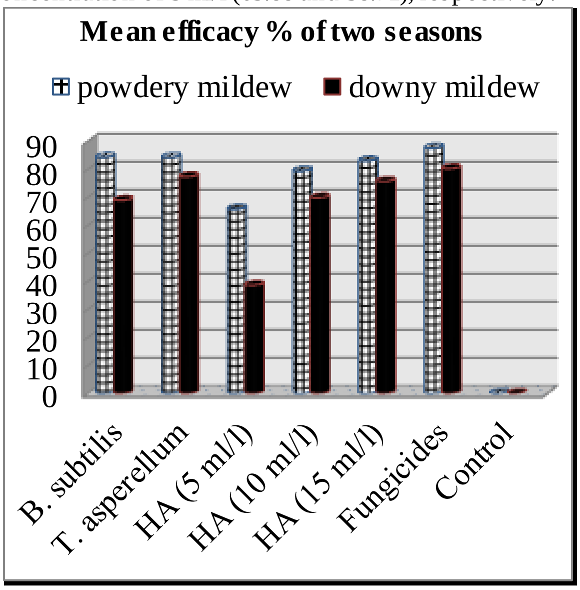
Table 3. Percentage of downy mildew disease severity in response to application of different treatments against foliar disease of cucumber grown under protected cultivation system.

Treatment	Season 2016/2017		Season 2017/2018	
	Disease severity (%)	Efficacy (%)	Disease severity (%)	Efficacy (%)
B. subtilis	10.66 c	67.30	8.7 c	70.77
T. asperellum	6.66 e	79.57	7.3 d	75.42
HA (5 ml/l)	21.3 b	34.66	17.0 b	42.76
HA (10 ml/l)	9.66 d	67.8	8.3 c	72.05
HA (15 ml/l)	7.33 e	70.36	5.6 e	81.14
Ridomil Gold MZ 68 WG	7.0 e	78.52	5.3 e	82.15
Untreated control	32.6 a	-	29.7 a	-

Values in the same column followed by the same letter (Duncan's test) are not significantly different *P < 0.05*. HA = Humic acid

Mean efficacy of used treatments

The illustrated data in Fig. (1) showed that, the highest reduction in disease severity calculated as 87.68, 84.38, 84.31, and 83.1% for powdery mildew at the treatment, [Topas 100 EC, *B. subtilis; T. asperellum;* and humic acid (15 ml/l), respectively]. Applied treatments showed similar effect on the disease severity of downy mildew throughout the growing seasons, Ridomil Gold, *T. asperellum*, and humic acid (15 ml/l). The least reduction of disease severity (mean of two seasons) of both powdery and downy mildews was recorded with the humic acid under concentration of 5 ml/l (65.88 and 38.71), respectively.



HA = Humic acid

Fig. 1. Mean efficacy of reduction (%) in downy and powdery mildew disease severity in response to application of different treatments through two seasons of cucumber grown under protected cultivation system.

Obtained results about the effect of humic acid in controlling powdery and downy mildew diseases are similar to those obtained by Kamel *et al*., (2014) whose reported that fulvic acid extracted from humic substances have been used effectively for controlling the powdery and downy mildew diseases of cucumber and improved growth parameters of cucumber plants.

Effect on defense related enzyme activities

Peroxidase activity:

Results in Table (4) reveal that all treatments increased peroxidase activity compared with control treatment at all periods of activity (converted to values of Δ OD/t). The highest activity of peroxidase was induced by *T. asperellum*, humic acid at the second and third concentration and *B. subtilis* (0.096, 0.096, 0.095 and 0.096 Δ OD/t, respectively) during the growing season 2016/2017. Similar results were recorded with the second season 2017/2018. Also the tested fungicides increased the enzyme activity over the control.

Table 4. Effect of treatments on peroxidase enzyme activity in treated cucumber plants.

Treatment	Activity of peroxidase enzyme Δ OD/t	
	Season 2016/2017	Season 2017/2018
B. subtilis	0.096 ab	0.094 b
T. asperellum	0.096 ab	0.094 b
HA (5 ml/l)	0.091 b	0.089 c
HA (10 ml/l)	0.096 ab	0.097 a
HA (15 ml/l)	0.095 ab	0.096 a
Topas 100 EC	0.079 d	0.080 d
Ridomil Gold MZ 68 WG	0.086 c	0.066 f
Control (Untreated)	0.073 e	0.076 e

Values in the same column followed by the same letter (Duncan's test) are not significantly different *P < 0.05*. HA = Humic acid

Polyphenol oxidase activity:

Results in Table (5) show that all treatments increased polyphenol oxidase activity (converted to values of Δ OD/t), with some exceptions, compared to control treatment. The highest activity was induced by *B. subtilis*, Topas 100, Ridomil Gold, *T. asperellum* and humic acid at concentration of 15 ml/l (0.07, 0.033, 0.023, 0.019 and 0.015 Δ OD/t, respectively) during the growing season 2016/2017. Similar results were found during the second season 2017/2018. The exception was recorded with fungicides Topas-100 and Ridomil gold which followed the bio-agents.

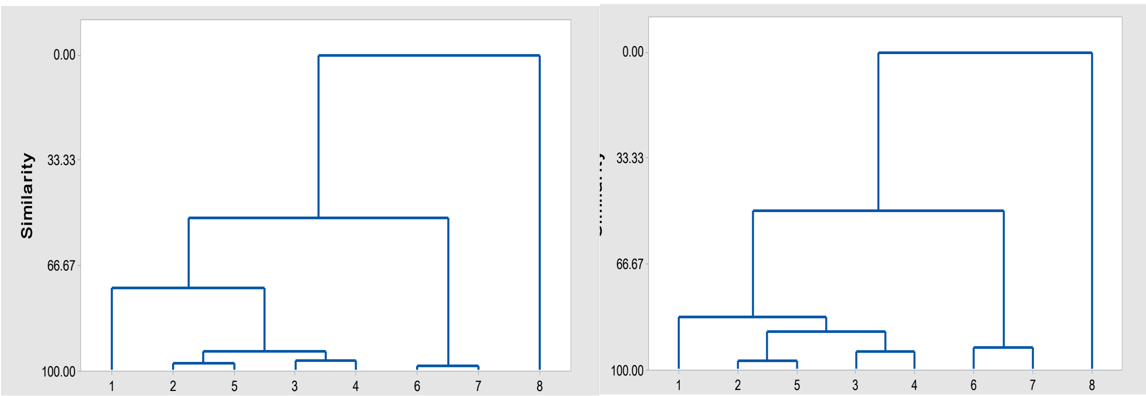
Table 5. Effect of treatments on polyphenol oxidase enzyme activity in treated cucumber plants.

Treatment	Activity of polyphenol oxidase enzyme Δ OD/t	
	Season 2016/2017	Season 2017/2018
B. subtilis	0.07 a	0.07 a
T. asperellum	0.019 d	0.023 c
HA (5 ml/l)	0.011 f	0.013 d
HA (10 ml/l)	0.016 de	0.013 d
HA (15 ml/l)	0.015 e	0.016 cd
Topas 100 EC	0.033 b	0.031 b
Ridomil Gold MZ 68 WG	0.023 c	0.012 d
Control (Untreated)	0.002 g	0.002 e

Values in the same column followed by the same letter (Duncan's test) are not significantly different *P < 0.05*. HA = Humic acid

The tested treatments (Fig. 2) were divided into two unrelated groups at distance = 25. The first group included the fungicides (Ridomil gold and Topas 100). The second group included *B. subtilis*, *T. asperellum* and humic acid (5, 10 and 15 ml/l). It is worth noted that *B. subtilis* was placed in groups although and the rest treatments in other group.

Rescaled Distance Cluster Combine



Number of clusters	Peroxidase Similarity level	Distance level	Clusters joined		Polyphenol oxidase Similarity level	Distance level	Clusters joined	
7	97.1149	0.23267	2	5	98.5195	0.10228	6	7
6	94.4040	0.45129	3	4	97.8437	0.14896	2	5
5	93.0055	0.56407	6	7	96.7949	0.22141	3	4
4	87.9376	0.97278	2	3	93.7241	0.43355	2	3
3	83.4388	1.33559	1	2	73.9392	1.80030	1	2
2	49.8197	4.04684	1	6	51.7809	3.33102	1	6
1	0.0000	8.06459	1	8	0.0000	6.90809	1	8

Fig. 2. Phenogram based on average linkage cluster analysis of response patterns from eight treatments to downy and powdery mildews of cucumber based on peroxidase and polyphenol oxidase activity. The tested treatments were: 1- *B. subtilis*, 2- *T. asperellum*, 3- Humic acid (5 ml/l), 4- Humic acid (10 ml/l), 5- Humic acid (15 ml/l), 6- Topas 100; 7- Ridomil Gold and 8- Untreated control.

Effect of different treatments on plant growth parameters

Data in Table (6) shows that the bio-agents and the third concentrations of humic acid caused a significant increase of all the measured growth parameters (plant height, total chlolorophyll, number of leaves, fresh and dry weight of plant). As shown in Table (6) that humic acid (15ml/l) recorded the highest values of plant growth parameters for the two seasons, except fresh and dry weight of plant at the first season. It recorded 296.6 , 42.9 , 41.0 , 362.9, 33.2 and 5.44 for the first season and, 311.6, 43.5, 44.3, 373.2, 40.2 and 6.4, respectively for the second season compared with

248.3, 31.4 , 25.0 , 316.9 , 25.7 and 4.06 at the first season, and 254.7, 32.1, 27.7, 317.3, 27.2 and 4.2 for the control.

The obtained data revealed that humic acid is responsible for enhancement the growth parameters of treated cucumber plants and this is in agreement with Senn, (1991) who reported that natural organic substances such as humates are rich in humic acid and a lot of minerals necessarily for stages of plant growth.

Data summarized in Table (7) showed that correlation between responses of plant growth parameters was not necessarily related to other. For example number of leaves and total chlorophyll, fresh weight and showed significant correlation (r = 0.923**, p= 0.001 and 0.903**, p=0.002, respectively). Another example was the non-significant correlation (r-0.326, p=0.327) between dry weight and plant height.

Effect on different treatments on yield components

Analysis of variance results showed that effects of humic acid were significant on the studied traits at 5% of probability level. However, applications of humic acid at concentrations 10 and 15ml/l increased all measured yield components. Accordingly, humic acid was more efficient enhancing the productivity where, increase of product percent reached to over 90% through the testing seasons compared with untreated control. On the other hand, humic acid have the supreme effect compared with untreated control (Table 8).

Table 6. Effect of different treatments on plant growth parameters of treated cucumber plants.

Treatment	Season 2016/2017						Season 2017/2018					
	Plant height (cm)	Chlorophyll (SPAD unit)	Number of leaves	Leaf area (cm^2)	Fresh weight /plant (g)	Dry weight/ plant (g)	Plant height (cm)	Chlorophyll (SPAD unit)	Number of leaves	Leaf area (cm^2)	Fresh weight /plant (g)	Dry weight/ plant (g)
B. subtilis	270.0 b	41.8 b	39.7 a	321.5 d	33.5 c	5.67bcd	284d	39.2cd	37.6d	321.9f	36.6d	5.9c
T. asperellum	268.3 b	43.4 a	37.7 b	354.4 c	36.2 ab	6.47 a	281de	40.7b	39.3b	345.6e	37.3c	6.7a
HA (5 ml/l)	306.3 a	35.9 e	36.6bc	353.3bc	32.5 c	5.32 d	304b	37.2e	38.0cd	340.8e	33.8e	5.47d
HA (10 ml/l)	303.3 a	38.6 c	39.7 a	361.1ab	34.9 b	5.94abc	306.3ab	39.7bc	38.7bc	354.3d	38.9b	6.1c
HA (15 ml/l)	296.6 a	42.9 ab	41.0 a	362.9ab	33.2 c	5.44 cd	311.6a	43.5a	44.3a	373.2a	40.2a	6.4ab
Topas 100 EC	257.3 c	37.3 cd	31.0 d	348.8 c	36.3 ab	4.75 e	296c	37.9de	35.7e	361.2c	37.4c	5.2d
Ridomil Gold MZ 68 WG	270.0 b	36.2 de	36.0 c	370.5 a	36.7 a	6.06 ab	276.3e	37.1e	39.3b	366.9b	37.8c	6.1bc
Control (Untreated)	248.3d	31.4 f	25.0 e	316.9 d	25.7 d	4.06 f	254.7f	32.1f	27.7f	317.3f	27.2f	4.2e

Values in the same column followed by the same letter (Duncan's test) are not significantly different *P* < *0.05*. HA = Humic acid

Table 7. Correlation between effects of different treatments on some plant growth parameters of treated cucumber plants.

Parameter	Plant height (cm)	Chlorophyll (SPAD unit)	Number of leaves	Leaf area (cm^2)	Fresh weight /plant (g)	Dry weight/ plant
Plant height (cm)	-					
Total chlorophyll	0.744*	-				
Number of leaves	0.753*	0.923**	-			
Leaf area (cm^2)	0.604	0.598	0.720*	-		
Fresh weight /plant	0.719*	0.889**	0.903**	0.770*	-	
Dry weight/plant	0.506	0.873**	0.896**	0.546	0.853**	-

Cell Contents: Pearson correlation P-Value * significant **highly significant

Data in Table (9) revealed that correlation among some yield components may or may not significant. For example, Increase percent of plant product was highly significant correlation (r = 0.966, p = 0.000 and r = 0.997, p = 0.00) with fruit number/plant and product/plant, respectively. At the same time the correlation between loss of shelf live was not significant.

Table 8. Effect of different treatments on some yield components of treated cucumber plants.

Treatment	Season 2016/2017							Season 2017/2018						
					Shelf life							Shelf life		
	Fruit number /plant	Fruit weight (g)/fruit	Product /plant (kg)	*Increase of product %	1ST day	7th day	*Rate of loss	Fruit number /plant	Fruit weight (g) /fruit	product /plant (kg)	*Increase of product %	1ST day	7th day	*Rate of loss
B. subtilis	34.0 b	107.5 c	3.7bc	125.8	89.7 cd	72.5 c	17.2 d	36.3b	106.2bc	3.82b	144.1	85.2e	69.1f	16.1cd
T. asperellum	34.3 b	105.2 d	3.6 bc	124.1	88.9 cd	73.4 c	15.5 e	35.7c	106.5bc	3.72b	140.4	90.1d	73.5e	16.6c
HA (5 ml/l)	32.0 c	107.6 c	3.44 c	118.6	93.2abcd	75.1b	18.1 c	33.3e	105.4c	3.48c	131.3	94.5c	75.3d	19.2b
HA (10 ml/l)	34.3 b	110.8 a	3.8 b	131.0	96.9 a	77.2 a	19.7 b	34.7d	108.5ab	3.76b	141.9	98.3ab	77.4c	20.9a
HA (15 ml/l)	37.7 a	109.5ab	4.11a	141.7	96.1 ab	75.4b	20.7 a	39.3a	110.4a	4.38a	165.3	99.2a	78.1bc	21.1a
Topas 100	32.0 c	108.3 bc	3.46 c	119.3	91.1 bcd	73.2 c	17.9cd	33.7e	104.2c	3.51c	132.4	94.7c	79.3ab	15.4e
Ridomil Gold	33.0 bc	103.2 bc	3.57 c	123.1	93.9 abc	74.6b	19.3 b	35.3c	105.6c	3.73b	140.7	95.8bc	80.5a	15.3e
Control (Untreated)	29.0 d	102.3e	2.9 d	-	85.3 d	65.2d	20.1ab	27.2f	97.4d	2.65d	-	83.6e	68.1f	15.5de

***Increase of product % = (treatment product /control product)* 100**

****Rate of loss= At once harvest - After 7 days. Values in the same column followed by the same letter (Duncan's test) are not significantly different *P* < *0.05*. HA = Humic acid**

Table 9. Correlation between effects of different treatments on some yield parameters of treated cucumber plants.

Parameter	Fruit number /plant	Fruit weight /fruit	Product /plant	Increased percent of product	Shelf life After 1day	Shelf life after 7days	Loss of shelf life
Fruit number/plant	-						
Fruit weight/fruit	0.616	-					
product/plant	0.976**	0.713*	-				
Increased percent of product	0.966**	0.479	0.997**	-			
Shelf life after 1day	0.675	0.699	0.781	0.580	-		
Shelf life after 7days	0.712*	0.712*	0.817*	0.445	0.898**	-	
Loss of shelf life	0.038	0.093	0.060	0.620	0.386	-0.059	-

*** significant **highly significant**

Powdery and downy mildew are a big problem in production of cucumber (*Cucumis sativus* L). They can be found all around the world in both plastic houses and field production, where they are common and can cause serious infections, yield reductions and quality impaired fruits. Today, there is a need to increase cucumber yield and at the same time keep low impacts on the environment using as little resources of chemicals as possible. Pest control needs to be efficient, but environmentally friendly. To create a sustainable pest management system, it is necessary to increase the use of non-chemical methods. Management of irrigation and fertilizers has been reported to be efficiently against rice bakanae disease (Gabr *et al.,* 2016). In some cases, an alternative is to use microbiological control. The microbiological products typically do not leave any residues in harvested crops, which means that no time of restraint is needed (Romero *et al*., 2007).

Generally, application of humic acid concentrations, bio-agents and fungicides reduced significantly disease severity of powdery and downy mildew diseases. Antioxidant enzymes such as peroxidase and polyphenol oxidase are scavenging enzymes which regulate hydrogen peroxide (H_2O_2) content, thus inhibiting the production of $OH^.$ radicals which produced from photorespiration and β-oxidation of fatty acids in organelles during metabolic processes (Bhutta, 2011). Down regulation of antioxidants produced by plants could lead to susceptibility process because no enough amount produced to scavenge the free radicals (Ketta, 2015). Damaging of membranes fatty acids could produce small hydrocarbon fragments including MDA which is an important sign of membrane system injury (Moussa, 2006). Results obtained by Moussa, (2006) and Berbara and García, (2014) reported that humic acid was the responsible element for the production of oxygen free radical $O_2^{\bullet-}$ during regulation system of xanthine/xanthine oxidase. Xanthine/xanthine oxidase system is the main factor for increasing the antioxidant compounds during the defense mechanism processes. In addition to participate in defense mechanisms, using of humic acid by different concentrations, bio-agents and fungicides influenced cucumber growth, yield and quality parameters significantly. The significant increases were found with the chlorophyll content, leaf area, stem elongation and yield components.

Humic acid could be applied also as stimulant for plant growth because it based on potassium humate and other elements enhancing the plant development or applied for enhancing the resistance of host plants against invasion of plant pathogens and pests which is very connected with the yield improvement and increasing (Scheuerell and Mahaffee, 2006). Some micronutrients activate the auxin oxidase system in treated plants (Marschner *et al*., 1997). Activating the auxin oxidase system in the plants is responsible for increasing the calcium content and total phenols which are very active materials against plant pathogens (Chowdhury, 2003). Sun *et al*., (2004) reported that enhancing and increasing the antioxidant enzymes (superoxide dismutase) and non-enzymatic antioxidants such as carotene, tocopherols and ascorpic acid, happened in studied plants when applied by humic acid. Enzymatic and non-enzymatic antioxidants play an essential role during the processes of defense mechanisms against plant pathogens (Ziadi *et al*., 2001).

CONCLUSION

Obtained results cleared that, application of humic acid as foliar sprays every week under concentration of 15ml/l played a pivotal role either for controlling powdery (*Sphaerotheca fuliginea* Schlecht., Pollacci) and downy (*Pseudoperonospora cubensis* Berk. and Curt.) mildews of cucumber through enzymes induction or advantage of enhancing the plant growth, chlorophyll content, fresh and dry weights as well as yield components and fresh fruit shelf life.

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الدور المحوري لحمض الهيومك ضد مرضي البياض الدقيقي والزغبي في الخيار تحت ظروف الصوب البلاستيكية

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تم تقييم تأثير حمض الهيومك في مكافحة مرضي البياض الدقيقي (*Sphaerotheca fuliginea* Schlecht., Pollacci) والزغبي (*Pseudoperonospora cubensis* Berk. and Curt.) علي محصول الخيار ودراسة تأثيره علي بعض القياسات الخضرية والمحصولية تحت ظروف الصوب البلاستيكية في موسمي ٢٠١٧/٢٠١٦ و ٢٠١٨/٢٠١٧. تم استخدام ثلاث تركيزات من حمض الهيومك وهي ٥، ١٠ و ١٥ مل/لتر ماء وتم الرش خمس مرات متتالية بفارق ٧ ايام بين الرشة والأخرى ضد مرضي البياض الدقيقي والزغبي مقارنة بكائنات التضاد الحيوية *Bacillus subtilis* and *Trichoderma asperellum* ومبيدين فطريين للمقارنة هما ريدوميل جولد ٦٨ لمكافحة البياض الزغبي ومبيد توبلس ١٠٠ للبياض الدقيقي. سجل التركيز ١٥ مل/لتر ماء من حمض الهيومك انخفاض ملحوظ في الشدة المرضية لكل من البياض الدقيقي (٨٥.٢٣ و ٨٠.٨٨) والبياض الزغبي (٧٠.٣٦ و ٨١.١٤) خلال موسمي الدراسة على التوالي مقارنة بباقي التركيزات. كما ساهمت كل المعاملات سابقة الذكر في زيادة نشاط انزيمي البيروكسيديز والبولي فينول اوكسيديز. تم الحصول علي اعلي نشاط من انزيم البيروكسيديز والبولي فينول اوكسيديز عند المعاملة بال *Trichoderma asperellum* تبعها المعاملة بحمض الهيومك بتركيز ١٥ مل/لتر ماء خلال الموسم الاول وتشابهت النتائج المتحصل عليها خلال الموسم الثاني. ادى استخدام حمض الهيومك بتركيزاته المختلفة رشا علي المجموع الخضري الي زيادة معنوية في طول النبات ومحتوي الكلوروفيل والوزن الرطب والجاف وايضا في القياسات المحصولية ومده بقاء الثمار طازجة. وتبين هذه النتائج ان لحمض الهيومك دورا فعالا ومحوريا في مكافحة مرضي البياض الدقيقي والزغبي في نبات الخيار من خلال استحثاث النبات للمقاومة بالإضافة الي اهميته في تحسين النمو والمكونات المحصولية.