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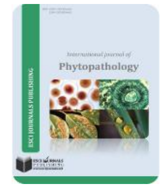

FULVIC ACID: A TOOL FOR CONTROLLING POWDERY AND DOWNY MILDEWS IN CUCUMBER PLANTS

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ABSTRACT

A greenhouse experiment was carried out on cucumber cultivar DP-164 during the seasons 2012-2013 to evaluate the efficacy of three concentrations of fulvic acid (FA) to control downy and powdery mildew diseases compared with the recommended fungicides and their effects on plant growth, chlorophyll content and yield of cucumber plants. Results revealed that all FA concentrations significantly reduced disease severity of both diseases. The highest reduction in disease severity of downy mildew was recorded using 75 ppm of FA, which was more effective than the recommended fungicide. The significant effect of inhibition of powdery mildew diseases increased gradually with increased FA concentration. At the same time, the reduction of diseases severity was greater than or equal to the recommended fungicides. Foliar application of FA concentrations and Ridomil fungicide increased significantly all morphological characters. Sumi-8 fungicide increased the morphological characters except plant height, dry weight/ plant and leaves number/ plant. The yield components (weight of fruits and mean of fruit weight /plant) recorded the same values with all FA concentrations. Fulvic acid had direct effect on soil microbiology by increasing plant exudates, which enhanced the activity of microorganism. Fulvic acid at 150ppm showed the highest increase in dehydrogenase and nitrogenase activity. This study revealed that the foliar application of FA improved plant growth and yield quantity and quality as well as controlling powdery and downy mildews of cucumber plants. Additionally, FA has the advantage as effective and environmental friendly agent.

Keywords: Control, humic substance*, Podosphaera fusca, Pseudoperonospora cubensis*.

INTRODUCTION

Powdery and downy mildew diseases on cucumber (*Cucumis sativus* L.) caused by *Podosphaera fusca* (syn. *Sphaerotheca fuliginea)* and *Pseudoperonospora cubensis*, respectively, are considered the most economically important and widespread diseases occurring throughout the world in both greenhouse and open fields (Sitterly, 1978; Brunelli and Davi, 1987). Although, chemical synthetic fungicides succeed to control several plant diseases, they increase production costs, incidence of health problems and pollution of environment. Therefore, a need has arisen for alternative strategies for controlling plant diseases.

Natural compounds obtained from microorganisms and organic fertilizers have the advantage of being less harmful to the ecosystem, and of being biodegraded *in situ* by the microflora (Sanchez Rodriguez *et al.,* 2002). Fulvic acid extracted from the rachis of waste plants contains high concentrations of potassium and tends to induce resistance to some diseases (Alvarez *et al.,* 2002). Studies conducted by Weltzein (1992), Yohalem *et al.* (1994) and cited by Alvarez *et al.* (2002), indicated that these lixiviates have been used for many years as foliar sprays to control fungal plant diseases. Also, studies reported by Alvarez *et al.* (2002) stated that the application of 5% fulvic acid extracted from banana lixiviate succeeded to reduce the severity of powdery mildew in roses.

The aim of the current research was to evaluate the use of fulvic acid extracted from waste rachis as an effective and low cost alternative controlling powdery and downy mildew diseases in cucumber, beside improving quantity and quality of yield and enhancing soil microbial activity.

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MATERIALS AND METHODS

Source of Fulvic Acid: Biogas manure sample was provided from Recycling of Agric. Residue Training Center Moshtohor, El- Kaliobiya Governorate, Egypt. Biogas manure was manufactured by drying the anaerobic cow dung effluent. Fresh cow dung was fermented in a biogas digester at retention time of 45-50 days. Table (1) shows the physical, chemical and biological properties of biogas manure. Physical parameters including moisture content and dry matter were determined according to the methods described by Black *et al.* (1965). Chemical parameters of electrical conductivity (EC), pH, organic matter (OM), organic carbon (OC), C/N ratio, total phosphorus and total potassium were determined according to standard methods of APHA, (1989), while total nitrogen (TN), ammoniacal nitrogen (NH_4^+-N), nitrate (NO3) were determined for fresh materials using the recommended methods published in Chapman and Pratt (1961). The plate count using the suitable serial dilutions and specific media was applied for estimation of the examined microbial groups. Total and fecal coliform and Salmonella and Shigella were counted according to APHA (1989).

Table 1. Physical, chemical and biological analyses of biogas samples.

S. No.	Content	Ratio
1	Density kg/ m^3	400
2	Moisture content %	17.7
3	Dry matter %	82.3
4	pH (1:10)	7.51
5	EC dS/m (1:10)	3.75
6	Ammonia ppm	51.7
7	Nitrate ppm	277.3
8	Total Nitrogen %	1.36
9	Organic matter %	54.80
10	Organic carbon %	31.78
11	Ash %	45.2
12	Carbon/ nitrogen ratio	23:1
13	Total Phosphorus %	0.69
14	Total Potassium %	0.58
15	Total coliform *cfu* /g x10^2	nd
16	Faecal coliformx *cfu*/g 10^2	nd
17	*Salmonella* and *Shigella* *cfu*/g x 10^1	nd
18	Parasites	nd

nd: not detected

Extraction and Purification of Fulvic Acid: Extraction of fulvic acid was done from biogas manure according to the method described by Sanchez *et al.,* (2002). Purification of fulvic acid was done as described by Kononova (1966). The chemical properties of fulvic acid were determined as total phosphorus reluctance (Murphy and Riley, 1962) and total potassium by using a flame photometer (Chapman and Pratt, 1961). Elemental analysis (C, H, N, S and O_2) of the purified fulvic acid was performed by gas microanalyser (Vario elementor C, H, N, S Germany 2004) as described by Goh and Stevenson (1971). Total acidity of fulvic acid was determined following the method described by Dragunova (1958). Carboxyl groups were determined as described by Schnitzer and Gupta (1965), while phenolic groups as described by Kononova (1966).

Greenhouse Experiment: An experiment was carried out under plastic greenhouse at Sakha Agricultural Research Station, Agricultural Research Center, during the seasons 2012-2013 using cucumber cultivar DP-164. Cucumber seeds were planted for 25 days. Seedlings were transplanted on two sides of the ridge at spacing of 50 cm between plants and 30 cm within the row. The treatments were accomplished before appearance of downy and powdery mildews. Each greenhouse was divided to equal experimental units (replicates), each unit contained 12 plants. These plants were distributed in 3 rows; each row was 0.7 m wide and 2 m length. Plants were irrigated whenever needed and fertilized with calculated doses of the mineral elements as recommended. Fulvic acid (added as three concentrations 50, 75 and 150 ppm) [based on the previous experience of conducting the selection of optimal concentrations] was mixed with an adhesive surfactant (bio-film 1265, registered by Ministry of Agric., Egypt) at rate 30 ml /100L and hand homogenized before fine spraying onto the upper and lower leaf surface of plants (21 days old). Another set of plants were sprayed by using water which mixed with the adhesive surfactant served as control treatment. Two synthetic fungicides applications [(1, 2, ethanediylbis (carbamodithioato)) (2-) manganese mixture with (1,2, ethanediylbis (carbamodithioato)) (2-) zinc](Ridomil Gold MZ 68 WG Syngenta) at the concentration 250 g/100 L for downy mildew and [(E)–(R)–1-(2,4-Dichlorophenyl)-4,4-Dimethyl-2-(1H-1,2,4-triazol-1-yl)-1-penten-3-ol)](Sumi-8 5%) obtained from Somotomo Chemical Ltd, Japan at the concentration 35 cm3/100 L for powdery mildew were conducted for comparison. Spraying was repeated weekly. All treatments were applied as protective agents (before appearance of symptoms).

Disease Assessment: Severity of powdery and downy mildews was assessed based on the percentage of affected leaf area. Percentage of protection was expressed as (Check-treatment/Check) x 100. It was worthy to calculate the disease inhibition (DI %) to evaluate the real comparable efficacy of treatments.

Plant Parameters Measurements: Plant growth parameters (plant height, leaves number/plant, leaf area, fresh and dry weight) and yield components (fruit number/plant, fruit weight, yield/plant) were estimated at harvesting stage, which extended for 90 days. Additionally, total chlorophyll content was determined by using SPAD-501 portable leaf chlorophyll meter (Minolta Corp) (Yadava, 1986). Shoot fresh and dry weight of plants (80^{o}C for 36 hours) were measured. Average number and weight of fruits/plant were estimated by harvesting fruits at marketable size. Harvesting was repeated day after day and extended for 90 days, and the accumulated yield was expressed as number and weight of fruits / plant.

Statistical Analysis: The randomized complete block design was used. The data were subjected to analysis of variance (ANOVA) using XLSTAT PRO statistical analysis software (Addinsoft). The experiments were repeated at least three times, and treatment means were separated using a Fisher's least significant difference (LSD) test. All analyses were conducted at a significance value of $P \leq 0.05$.

RESULTS

Characteristics of Biogas Manure: Data presented in Table (1) show the chemical and physical properties in biogas manure. The density of manure was 400 kg/m^3. It is suitable for extraction of fulvic acid (FA), because the density of manure should not exceed 700 kg/m^3. Biogas manure is more mature when ammonia contents are lower meanwhile, nitrate content is high (Zucconi and de Bertoldi, 1987). It was worthy to notice that the organic matter in biogas sample recorded up to 54%.

The C: N ratio of the sample was 23:1 resembling the optimum less than 25: 1 for the mature manure as stated by TMECC (2002). The total phosphorus and total potassium in biogas manure were 0.69 and 0.58, respectively. The biogas sample recorded no existence of pathogenic bacteria and parasites. Data in Table (2) show the main characteristic of FA extracted from biogas manure. Phenolic groups were higher than both total acidity and carboxylic groups. Moreover, fulvic acid contained NPK and high percentages of carbon, hydrogen and sulfur.

Table 2. Characteristic of the fulvic acid (FA) extracted from biogas manure.

Treatment		C%	N%	H%	S%	O_2%	P ppm	K ppm	Total acidity (mmol / 100g)	CooH group (mmol/100g)	Phenolic group (mmol / 100g)
KOH 1.0N.	FA Bio. m.	48.6	2.8	4.3	2.3	42.0	0.016	0.240	600	300	650

Effect of FA on Downy Mildew Disease: Data in Table (3) show that all concentrations of FA (50, 75 and 150 ppm) and the fungicide Ridomil significantly decreased disease severity compared with control treatment. Fulvic acid at concentration 75 ppm achieved the best result for disease inhibition (78.33%), while the recommended fungicide Ridomil recorded 60.0%. Fulvic acid showed significant antifungal activity against downy mildew of cucumber. However, these results represent the first report of FA against downy mildew of cucumber.

Effect of FA on Powdery Mildew Disease: Data in Table (4) demonstrated that FA at 150 ppm was more efficient for controlling powdery mildew on cucumber plants. The fungicide, Sumi-8, succeeded to decrease disease severity to 5.7%, similar to recorded value by FA at 75ppm.

Effect of FA on Soil Dehydrogenase and Nitrogenase Enzymes: Different concentrations of FA showed good effect on the activity of soil microorganisms. All fulvic acid concentrations increased activity of dehydrogenase and nitrogenase enzymes. The percentage increase at a concentration of 75 ppm in the case of dehydrogenase enzyme (50.3%), while concentration 50 ppm achieved (53.7%) in the case of an nitrogenase enzymes. Sumi-8 treatment had negative effect on soil microorganisms which appeared in low enzyme activities. On the other hand, Ridomil fungicide had positive effect on soil microorganisms, which may be due to its short half age period in plant (Table 5).

Plant Growth Parameters: Botanical measures represented in Table (6) showed that all FA concentrations and Ridomil treatments significantly increased growth parameters with different extent. In this respect, application of FA came in the top of the mentioned treatments in increasing plant height and leave number/plant.

Table 3. Impact of different concentrations of fulvic acid (FA) on cucumber downy mildew.

Treatments	Disease Severity (%)	Disease Inhibition (%)
Fulvic acid (50) ppm	$11^{b} \pm 0.82$	63.33
Fulvic acid (75) ppm	$6.5^{a} \pm 0.41$	78.33
Fulvic acid (150) ppm	$9^{a} \pm 0.82$	70.00
Ridomil	$12^{b} \pm 1.63$	60.00
Control	$30^{c} \pm 1.63$	-

Means followed by the same letter (s) within a column in each block are not significantly different (*P≤ 0.05*).

Table 4. Impact of different concentrations of fulvic acid (FA) on cucumber powdery mildew.

Treatments	Disease Severity (%)	Disease Inhibition (%)
FA (50) ppm	$8^{a} \pm 1.14$	73.33
FA (75) ppm	$5.7^{a} \pm 0.81$	81.00
FA (150 ppm	$5.3^{a} \pm 0.76$	82.33
Sumi-8	$5.7^{a} \pm 0.81$	81.00
Control	$30^{b} \pm 4.28$	-

Means followed by the same letter (s) within a column in each block are not significantly different (*P≤ 0.05*).

Table 5. Effect of different treatments on soil dehydrogenase and nitrogenase enzymes.

Treatment	Periods	Enzymes	
		Dehydrogenase UgTPF/100g	Nitrogenase n.mol C_2H_4/g dry soil/hour
FA (50) ppm	Initial	15.1	0.4269
	Final	16.7	0.6562
	Increase	10.6%	53.7%
FA (75) ppm	Initial	19.5	0.4866
	Final	29.3	0.7121
	Increase	50.3%	46.3%
FA (150) ppm	Initial	39.6	0.8015
	Final	44.1	0.9564
	Increase	11.4%	19.3%
Sumi-8	Initial	13.4	0.342
	Final	12.3	0.1652
	Increase	- 8.2%	- 51.7%
Ridomil	Initial	14.0	0.395
	Final	18.1	0.5418
	Increase	29.3%	37.2%
Control	Initial	12.3	0.239
	Final	12.8	0.3108
	Increase	4.1%	30.0%

Table 6. Influence of different concentrations of fulvic acid (FA) on plant growth parameters of cucumber plants during the season 2012/2013.

Treatments	Plant Growth Parameters					
	Plant Height (cm)	Total chlorophyll (SPAD)	Leave number /plant	Leaf area (cm^2)	Fresh weight/ plant (g)	Dry weight/ plant (g)
FA (50) ppm	$273.4^{a} \pm 3.27$	$40.8^{ab} \pm 0.57$	$35.0^{b} \pm 1.15$	$317.3^{bc} \pm 21.2$	$33.0^{ab} \pm 1.15$	$5.2^{a} \pm 0.56$
FA (75) ppm	$280.0^{a} \pm 5.77$	$42.7^{a} \pm 3.88$	$42.3^{a} \pm 1.85$	$347.6^{ab} \pm 26.46$	$32.0^{c} \pm 1.15$	$5.2^{a} \pm 0.72$
FA (150) ppm	$293.3^{a} \pm 8.81$	$43.0^{a} \pm 1.09$	$42.6^{a} \pm 2.30$	$401.3^{a} \pm 14.2$	$37.1^{a} \pm 1.07$	$6.5^{a} \pm 0.56$
Sumi-8	$193.3^{c} \pm 12.01$	$37.4^{ab} \pm 2.82$	$24.7^{c} \pm 0.33$	$400.7^{a} \pm 35.45$	$36.1^{ab} \pm 1.35$	$4.7^{a} \pm 0.58$
Ridomil	$270.0^{a} \pm 5.77$	$44.9^{a} \pm 2.80$	$36.0^{b} \pm 2.51$	$370.3^{ab} \pm 0.88$	$36.3^{b} \pm 1.12$	$6.0^{a} \pm 0.60$
Control	$245.0^{b} \pm 2.88$	$33.5^{b} \pm 2.25$	$26.3^{c} \pm 2.18$	$276.3^{c} \pm 9.49$	$27.6^{d} \pm 1.18$	$4.7^{a} \pm 0.49$

Means followed by the same letter (s) within a column in each block are not significantly different (*P≤ 0.05*).

Both FA at 150 ppm and Sumi-8 fungicide treatments showed the best effects on leaf area (401.3, 400.7 cm^2) respectively. Fulvic acid concentration at 150 ppm achieved the best result in fresh weight compared with control treatment (37.1 g). No significant differences were found between treatments in dry weight. The chlorophyll content in fresh plants was determined as shown in Table (6). The fungicide Ridomil was the best treatment in chlorophyll pigment (44.9 SPAD) followed by FA at both 150 ppm and 75 ppm (43.0 SPAD). No significant differences were found between Ridomil fungicide and FA.

Yield Components: The yield as average number and weight of fruits/plant were significantly increased using different concentrations of FA. The application of fungicides (Sumi-8 and Ridomil) induced the same trend on yield components (Table 7).

Table 7. Influence of different concentrations of fulvic acid on yield components of cucumber.

Treatments	Yield parameters		
	Average Fruit number / plant	Average weight / fruit (g)	Mean yield/plant (kg)
FA (50) ppm	33.7^{bc}±0.88	81.0^{b}±8.66	2.73^{ab} ± 0.16
FA (75) ppm	36.0^{b}±2.0	82.3^{b}±12.8	2.96^{a} ± 0.47
FA (150) ppm	41.3^{a}±1.2	81.0^{b}±4.58	3.35^{a} ± 0.12
Sumi-8	24.0^{c}±1.5	86.0^{a}±11.53	2.06^{bc} ± 0.03
Ridomil	33.0^{bc}±1.73	91.0^{a}±8.71	3.00^{a} ± 0.29
Control	19.0^{d}±1.73	75.0^{c}±6.51	1.43^{c} ± 0.02

Means followed by the same letter (s) within a column in each block are not significantly different (*P≤ 0.05*).

DISCUSSION

The biogas sample suppresses both pathogenic bacteria and parasites (Iannotti *et al.,* 1993). In general, humic substances are a suspension based on potassium humates which can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases and pests (Scheuerell and Mahaffee, 2004, 2006). Furthermore, El-Ghamry *et al.* (2009) indicated that the use of humic substances as foliar application improve growth and mineral content as well as decrease the damage of chocolate spot and rust diseases of faba bean. The role of humic substances in overcoming the harmful effects of diseases in plants may be due to the increase in chitinase activity (Abd-El- Kareem, 2007), moreover, regulating hormone level, improving plant growth and enhancing stress tolerance (Piccolo *et al.,* 1992). Humic substances contain three types of organic acid; humin, humic acid and fulvic acid. They have been defined in humic science in accordance with their solubility characteristics (IHSS, 2004). Fulvic acid is a group of subclasses of an entire large class of humic substances compounds. Fulvic Acid is especially active in dissolving minerals and metals when they are in solution with water. Metallic minerals simply dissolve into the fulvic structure and become bio-chemically reactive and mobile (BioAg Technologies International, 1999). Fulvic acid mainly consists of mixtures of polycyclic phenolic compounds. Phenolic compounds play a major role in plant defense which is much higher than both total acidity and carboxylic groups in diseases management (Hahlbrock and Scheel, 1989). Moreover, FA contains high percentage of carbon, hydrogen and sulfur which are active agent in controlling plant diseases, especially powdery and downy mildews.

The foliar application of FA at all concentrations showed visible reduction of downy mildew severity compared with control treatment. The highest reduction was recorded with FA at75 ppm, while, reduction in powdery mildew severity occurred ascending with increased FA concentrations. The percentage of reduction was equal for both of powdery mildew fungicide and FA at 75 ppm. Interestingly, Neil (2012) found that FA has the nature to be an effective antifungal agent. This result is in agreement with the findings of Sherry *et al.* (2011). The efficacy of fulvic acid treatment against powdery mildew fungus may be due to the ability of fulvic acid to induce resistance to some diseases (Alvarez *et al.,* 2002). Foliar application of FA (25% active FA) consistently enhanced antioxidants such as α-tocopherol, α-carotene, superoxide dismutase, and ascorbic acid concentrations in turf grass species (Zhang, 1997). This antioxidant may play a role in the regulation of plant development, flowering and inducing of disease resistance (Ziadi *et al.,* 2001; Dmitrier *et al.,* 2003). Additionally) these results indicated that the fulvic acid treatment is a viable, environmentally friendly alternative for managing fungal diseases (Escobar-Vélez and Castaño-Zapata, 2005; Velez and Zapata, 2005; Elisabetta *et al.,* 2008; Fahril and Murat, 2008; Afifi, 2010; Derbalah et al., 2012).

Application of FA showed significant increases in growth parameters and morphological characteristics of cucumber plants. Increasing of growth parameters enhanced fruits quality and yield which led to reduce the reliance on synthetic fertilizers. Fulvic acid and humic acid are excellent foliar fertilizer carriers and activators. Application of humic acids or FA in combination with trace elements and other plant nutrients, as foliar sprays, can improve the growth of plant foliage, roots and fruits. By increasing plant growth processes within the leaves an increase in carbohydrates content of the leaves and stems occurs. These carbohydrates are then transported down the stems into the roots where they are released as root exudates to provide nutrients for various soil microorganisms on the rhizoplane and the rhizosphere. Then, soil microorganisms release acids and other organic compounds which increase the availability of plant nutrients. Other microorganisms release "hormone-like" compounds which are taken up by plant roots (Achuo *et al.,* 2004). Afifi (2010) found that FA extracted from compost (chicken manure and vegetable wastes) increased the numbers of actinomycetes in soil, while FA extracted from biogas manure increased total count of bacteria, fungi and actinomycetes. Additionally, humic substances stimulate plant growth by the assimilation of major and minor elements, enzyme activation and/or inhibition, changes in membrane permeability, protein synthesis and finally the activation of biomass production (Mackowiak *et al.,* 2001; Ulukon, 2008). Russo and Berlyn (1990) reported that humic substances (granular and liquid forms) can reduce plant stress that involved plant diseases as well as enhance plant nutrient uptake. Humic substances contribute significantly to water retention and metal/solute binding and release, and they are necessary for safe plant nutrition (Mac Carthy *et al.,* 1990; Stevenson, 1994). In addition, FA can be used as a growth regulator by regulating endogenous hormone levels (Frgbenro and Agboola, 1993; Piccolo *et al.,* 1992). This is because FA binds with minerals and other molecules and transform them. Finally, several studies demonstrated that humic and fulvic acids preparations increased the uptake of mineral elements (Maggioni *et al.,* 1987; De Kreij and Basar, 1995), promote the root length (Vaughan and Malcolm, 1979; Canellas *et al.,* 2002) and increase the fresh and dry weight of crop plants (Kauser and Azam, 1985; Chen *et al.,* 2004a, b).

In conclusion, due to the positive effects of fulvic acid on controlling both downy and powdery mildew diseases and increasing cucumber growth and yield, we suggest that FA can be inserted in an integrated management program of these diseases, with a reduced application of synthetic fungicides.

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