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Effect of Biofertilizers and Humic Acid on control of Dry Root Rot Disease and Improvement Yield Quality of Mandarin (*Citrus reticulate* Blanco)

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Abstract: The effect of soil drenching with humic acid and commercial biofertilizers *i.e*., phosphorien , microbien and/or cearalien were evaluated for controlling dry root rot disease of mandarin ,fruit quality and total yield during 2005 and 2006seasons . *In vitro* , humic acid at 15.0%(v/v) reduced significantly the radical growth and spore germination of *Fusarium solani* the causal agent of dry root rot . Drenching artificially infested soil with humic acid +Phosphorien or miccrobien biofertilizers as twice applications resulted in decrease both disease infection and severity on mandarin seedlings as well as reduce colonization of *F. solani* in seedlings roots . In Field trials , humic acid +phosphorien or miccrobien biofertilizers treatments were significantly increase the number of recovering diseased trees , decrease the disease severity on the others , inhibiting the activity of the pathogen in rhizospher soil of treed trees . Moreover, these treatments improving the yield and fruit quality of treed trees . Therefore, the usage of combination between humic acid and biofertilizers could be suggested as easily bio-treatment for controlling dry root rot of citrus especially under organic system .

Key words: citrus, dry root rot, humic acid, biofertilizers

INTRODUCTION

Mandarin (*Citrus reticulate* Blanco) is one of the most important fruit crops in many countries including Egypt. Dry root rot (Fusarium root rot) disease of citrus caused by *Fusarium solani* (Mart.) Snyd & Hans was reported to attack most citrus varieties [33,23,38,11,14,9]. *Fusarium solani* induce two syndrome of root rot on citrus. First, the dry root rot is confined to the crown and scaffold roots, and the second, feeder and fibrous root rots as associated with gradual decline of the canopy, leaf curl (wilting), defoliation, dieback, fibrous roots turn soft and appears water soaked, slough their cortex easily by hand.

Control of root rot diseases on citrus depends mainly on Fungicides application [14,48]. Meanwhile, fungicides always undesirable due to high coast, probability of development of resistant strains and potential hazards to the environment.

Using biofertilizers and/or organic substances such humic acid instead of the chemical forms of fungicides or mineral fertilizers could be the way to control soil borne plant pathogens and produce the natural clear fruits free from minerals residues[35]. In this respect, application of biofertilizer for controlling soil borne pathogenic fungi has been applied to several plants[8,50,36,52]. Organic and/or biofertilizers improved vegetative growth, nutritional status and reduced the residuals of nitrate and nitrite in banana and grape fruits [2,19]. Farag[18]and Saleh *et al*[37] they noted that organic fertilizers and humic acid significantly decreased nitrogen nitrate and nitrite content and improved yield and fruit quality of treated vines if compared with control(untreated)

Humic acid (HA) is a heterogeneous mixture of many compounds with generally similar chemical properties it performs various functions in the soil and on plant growth. Humic substances are suitable candidates for use as liquid biofertilizers, moreover they have been shown to have appositive effect against plant pathogens[51,22], Pascual *et al*.[46,47] found that incorporation of humic acid into drip irrigation systems is highly effective for controlling *Pythium ultimum* of pea.. Moliszewska and Pisarek[30] found that humic acid could inhibit the mycelia growth of *F. culmorum* and *Alternaria allernata* on PDA medium. Loffredo *et al*[25] noted that humic substances reduced radial growth and spore germination of *Fusarium oxysporum f.sp melonis* and *F. oxysporum* f.sp. *lycopersici* on PDA medium. Many commercial products containing humic acid (HA) have been promoted for use on various crops. Liquid fertilizer containing humic acid increased apple fruit weight, yield and soluble solids content[26]

The use of biofertilizers and humic substances would permit a reduction in the use of agrochemicals such fungicides and mineral fertilizers. So, this

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investigation was done to evaluate effectiveness of ome commercial biofertilizers and humic acid treatments on control of dry root rot disease, soil microflora in rhizospher soil of mandarin trees as well as leaf mineral content, yield and fruit quality of mandarin.

MATERIALS AND METHODS

Fungal Isolate: *Fusarium solani* (Mart. Appal & Wr. Emeed Snyd & Hans) was previously isolated from naturally infected roots of citrus trees affected by root rot disease. This isolate was recorded to cause root rot on different citrus rootstocks in previous studies[14,15]

Bio-Fertilizers: Three commercial bio-fertilizers were used i.e, phosphorein (phosphate dissolving bacteria (*Bacillus megatherium* var *Phosphatircum*), microbien (*Azotobacter* spp) and Cerealien (nitrogen-fixing Cyanobactria) The biofertilizers were produced by General organization for Agriculture Equalization Fund (G.O.A.E.F.), Ministry of Agric. Egypt.

Laboratory Studies:

Effect of Humic Acid on *F. solani in Vitro*:

A- Effect on Linear Growth: Serial quantities of humic acid were added to conical flasks containing melted PDA medium to obtain final concentrations of 0.0, 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0% and mixed gently and then dispensed in sterilized Petri dishes (10 cm diameter). Plates were individually inoculated at the centre with equal disks (5 mm diameter) of 10 day old culture of *F. solani* and incubated at 25°C. The overage linear growth of fungi was calculated after 7 days. Each treatment was represented by 5 replicates.

B- Effect on Spore Germination: The spores of *Fusarium solani* obtained from the 10-days-old cultures on PDA medium were collected, suspended in distilled sterile water, and mixed with appropriate aliquots of stock aqueous suspension/solutions of humic acid to obtain a density of 5 x 10^5 spores/ml, and the concentrations of humic acid of 0.0, 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0% of humic acid. Three drops (about 50ml) of each treatment were then placed on microscopic slide , kept at 20°C in moisten filter paper placed in Petri dishes for 28 h. .The germination percentages of conidia (50 conidia for each treatment) were measured using an Olympus Cx40 microscope. The conidium's was considered germinated when the germ-tube length was at least equal to the conidial diameter. The experiment was replicated four times.

Green House Experiments: The experiments were carried out to evaluate the efficacy of biofertilizers *i.e.* phosphorien, microbien and cerealien as well as humic acid individually or in combination treatments for controlling dry root rot disease of mandarin seedlings and the inoculum density of *F. solani* in rhizospher soil of seedlings.

Plastic pots (20 cm diam) containing sand Loam soil (2:1 v:v) infested by *F. solani* according to El-Mohamedy[14] were used. Mandarin (Baladi cv.) seedlings (3 years old) grafted on sour orange rootstock were inoculated by the same pathogen and replanting in infested soil according to Strauss and Labuschagen[38] Five seedlings(pots) were used as replicates for each treatment. The following treatments were applied : Control (non treated infested soil).- Humic acid at the rate of 10% (v/v) of water.- Phosphorien at the rate of 50 g/pot.- Microbien at the rate of 50 g/pot - Cerealien at the rate of 50 g/pot. - Humic acid 5% + phosphorien 25g. - Humic acid 5% + Microbien 25 g. - Humic acid 5% + cerealien 25 g. Mandarin seedlings were replanting after 15 days of soil infestation. Biofertilizer and humic acid treatments were applied twice, the first at replanting time of seedlings as one application, and 30 days from replanting as second application.

Disease Assessment: After 90 days from seedlings replanting time the number of infected seedlings was recorded and the development of disease severity of Fusarium root rot (dry root rot/on seedling for each treatment were estimated following the scale rated from 0 to 4 degree (0=healthy plant 4=died plant) according to Morgan and Timmer[31] and El-Mohamedy[14]. Percentages of disease infection and disease severity of each treatment as well as control (non treated infested soil) were calculated.

Root Colonization and Count of Propagules of *F. Solani* in Rhizospheric Soil of Mandarin Seedlings: At the end of the experiment (90 days), all plants were gently pulled off. Roots were then washed under running water, 100 segments (5mm) of feeder and major roots were cute from seedling roots, washed in sterile water and sterilized by 5% NaOCL for 2 min. Ten root segments were placed on the surface of PCNB medium (Nash and Snyder[32] in Petri dishes. Ten Petri dishes were used for each treatment, and then all dishes were incubated at 27°C for 8 days. Root pieces yielded *Fusarium* spp. were counted and total pieces were expressed as percentage of pieces yielding *Fusarium* spp.

The plate count technique according to Allen[4] was followed for determination the total count of *Fusarium* spp. in soil. Peptone PCNB agar medium was used for counting *Fusarium* spp. in the dilution of 10^{-4}. Four plates were used as replicates. Plates were incubated at

27°C and *Fusarium* spp colonies were counted after 6-8 days, and the number of propagules per gram dry weight rhizosphere soils was calculated.

Field Experiments: This study was carried out during two successive seasons (2005 and 2006) using eight years old of mandarin trees cv. Baladi grafted on sour orange rootstock, with the history of root rot disease infection . Planted on sandy soil under drip irrigation system in a private farm located at Emam Malek village, Nobaria, Beheria governorate, Egypt. Sixty diseased mandarin trees showing typical symptoms of dry root rot disease were selected, 15 diseased trees with different rates of disease severity (1,2 and 3 degree, according to El-Mahamedy[14] were used as replicate for each treatment, as well as control treatment. The following treatments per tree were used:

Control (non – treated healthy trees). - Control (non – treated diseased trees).
Phosphorien 100g + humic a acid 10% (v/v) water-
Microbien 100g + humic acid 10%. (v/v) water -
Cerealien 100g + humic acid 10% (v/v) water .

All treatments were applied to soil around the main stem of the tree as twice application per season, first at the first of March, the second at the first of April. After 60 and 120 days from applying treatments, number of recovered trees and the development of disease severity were calculated as mentioned before.

Rhizospheric Microflora: Rhizospheric soil samples from treated and non-treated (diseased trees) were taken at 0 (before applied treatments), 60 and 120 days after applied all treatments. Soil dilution method was applied according to Louw and Webley[28]. A sample of rhizospheric soil of each treatment was dried at 105°C for 24 hours to its constant weight. Plate count technique[4], using Peptone PCNB agar medium[32] was used to determine total count of Fusarium spp. Total actinomycetes were counted on starch nitrate agar medium[49] and total fungal count were counted on Martin's agar medium (Allen, 1961) and the total count of bacteria were counted on soil extract yeast agar medium (Mohmoud *et al*., 1964).

Effect of Biofertilizers and Humic Acid on Leaf Minerals Content, Yield and Fruit Quality of Mandarin:

A- Leaf Minerals Content: In late August of each season, about fifty leaves of six months old from lagged non. Fruiting and non-flushing terminals of spring growth cycle were collected[21]. Leaf samples were washed with tap water and dried at 70°C until constant weight, finally ground and digested using the method of Piper[44]. The digested solution was used to determine N,P and K content using the methods described by Pregi[45], Troug and Meyer[42] and Brown and Lilleland[7] respectively, N,P and K were calculated as percentage per dry weight bases.

B-yield and Fruit Quality of Mandarin: Yield and fruit quality of mandarin were determined at the end of first and second season 2005 and 2006.

At harvesting time under the experimental conditions (4^{th} week of December) yield expressed in weight (kg) and number of fruits per tree was recorded. For fruit quality, 15 fruits were picked at random from each replicate in order to evaluated fruit weight (gm), fruit volume (cm^3), fruit height (cm), fruit diameter (cm), Juice weight, percentage total soluble solid (TSS), acidity and TSS/acid ratio according to A.O.A.C[5].

Statistical Analysis: The data were subjected to analysis of variance and Duncan's multiple range test was used to differentiate means[13].

RESULTS AND DISCUSSION

Effect of Humic Acid on Linear Growth and Spore Germination of *F. Solani*: Seven concentrations of humic acid were tested *in vitro* against linear growth and spore germination of *F. solani*. Results in Table (1) show that increasing humic acid concentrations caused decrease on both linear growth and spore germination percentage. The linear growth and spore germination of *F. solani* were not affected by humic acid up to 5% in PDA medium. Meanwhile, at 10.0, 12.5 and 15.0% concentrations reduction in linear growth and spore germination as 38.9, 46.7, 53.3 and 30.0, 42.0, 60.0% respectively. These results are in accordance with Moliszewsha and Pisarek[30], and Loffredo *et al*.,[25]. They noted that humic acid could inhibit the growth and spore germination of many plant pathogenic fungi, they attributed this inhibition effect to the presence of some toxic compounds and functional properties especially COOH group content and elemental composition. Also, Loffredo *et al*.,[25] 2007 found that humic acid substances reduced significantly the redial growth and spore germination of *Fusarium oxysporum f sp . melonis* and *Fusarium oxysporum f sp .lycopersici* .

Effect of Biofertilizers and Humic Acid on Dry Root Rot Incidence in Greenhouse: The effect of different biofertilizers and humic acid treatments as one or twice soil application on the incidence of dry root rot (Fusarium root rot) of mandarin seedlings, Fusarium density in rhizosphere soil and root colonization by *F. solani* was studied.

Table 1: Linear growth (mm) and spore germination (%) of *F. solani* affected by different concentrations of humic acid (HA) on PDA medium

Humic acid concentration %	Linear growth (mm)	Reduction (%)	Spore germination(%)	Reduction (%)
0	90a	0	100a	0
2.5	90a	0	100a	0
5	90a	0	92.0ab	8
7.5	72b	20	87.0b	13
10	55c	38.9	70.0c	30
12.5	48c	46.7	58.0d	42
15	42c	53.3	40	60

Values in a column followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range tests (Duncan 1955).

Effect on Dry Root Rot Incidence: Data in Table (2) show that all combined treatments of biofertilizers and humic acid were significantly reduced the infection and disease severity of dry root rot on mandarin seedlings compared with control (non-treated seedlings). Combination between humic acid and phosphorien, microbien and/or cearalien biofertilizers reduced percentage of disease infection from 100% of control to reach 62.5, 75.0 and 87.5% in one application and 50.0, 62.0 and 62.0% in twice soil application. The same treatments resulted in a reduction of disease severity estimated by 53.6, 50.0 and 35.7% & 71.4, 67.8 and 53.6% in one and twice soil application respectively. Meanwhile, the highest percentage of disease infection and the least reduction of disease severity were recorded when humic acid and biofertilizers were applied to the soil as individually treatment. The most effective treatment in reducing dry root rot incidence was humic acid + phosphorien followed by humic acid + microbien, but humic acid + cearalien show considerable effect. These results are similar to these obtained by others authors using different organic materials ,e.g., humic acid ,humic acid fractions and biofertilizers to reduce the effect of many soil borne plant pathogens on many crops[22,6,52,8,50,36,25]. Pascual *et al*[446,47] noted that the incorporation of humic fractions from MSW compost into drip irrigation systems not only will benefit the chemical and biological characteristics of the soil but will improve soil health by enhancing the suppression of plant pathogens.

Effect on Fusarium Population Density in Rhizosphere Soil: Data in table (3) clearly show that the most reduction in propagules counts of *Fusarium* spp as well as the incidence and colonization of the pathogen in root of mandarin seedlings were recorded at twice soil application with humic acid combined with phosphorien, or microbien or cearalien biofertilizer. These treatment reduced Fusarium propagules counts from 4.6 x 10^4 cfu/g soil in non treated soil (control) to 1.8 x 10^4, 2.0 x 10^4 and 2.3 x 10^4, cfu/g soil, respectively. Meanwhile, these values were 2.6x10^4, 2.8 x $10^4$and 3.0 x 10^4 in rhizospheric soil of seedling received one application of the same treatments. All individual treatments of biofertilizers or humic acid show less effect in decreasing Fusarium density and the incidence of the pathogen in roots of seedling in one or two soil application. Twice soil application of humic acid and biofertilizers combined treatments were highly effective compared with one application in controlling of *Fusarium solani* to colonize roots of mandarin seedlings, as the percentages of root pieces yield *Fusarium* spp 32, 38 and 46% compared with 100% of control treatment. These results are coincidence with those obtained by Pascual *et al*[47]; Ziedan[52] ;Loffredo *et al*.[25] They used humic acid or their fractions and/or biofertilizers to suppress plant pathogens. Pascual *et al*[47] showed that soil amended with humic acid (HA) or its fractions decreased significantly the population of *Pythium ultimum* and the damage this pathogen caused to pea plants. Moliszewska and Pisarek[30] demonstrated tha the germination process of *F. culmorum* and *Alternaria alternate* spores under laboratory condition can be inhibited by humic acid . Meanwhile, Ziedan[52] noted that population density of *Aspergillus niger* and *Fusarium oxysporum* the causal agents of root and pod rot diseases of peanut were lower in rhizospher soil of plants treated with biofertilizers i.e., cerealien and rhizobactrien.

Control of Dry Root Rot Disease on Mandarin Trees: The experiments were carried out on a number of diseased mandarin trees (Baldi cv. padded on sour orange rootstock), 60 trees showing typical symptoms of dry root rot disease were selected. The effect of combination between humic acid and/or phosphorien or microbien or cearalien biofertilizers treatments on the development of dry root rot, total soil micro flora counts in mandarin rhizosphere soil, as well as the effect on the fruit yield, physical and chemical properties of fruit yield was studied during 2005 and 2006 two successive seasons.

Table 2: Effect of different biofertilizers and humic acid(HA) treatments on Fusarium root rot incidence on mandarin seedlings planting in artificially infected soil in greenhouse.

Soil treatment	No of application	Fusarium root rot incidence			
		Disease infection %	Reduction %	DiseaseSeverity (DS)	Reduction (DS)%
Control	Once	100a	0.0	2.8a	0
Phosphorien		75.0cb	25.0	1.9c	32.1
Microbien		75.0cb	25.0	2.0cb	28.6
Cearilien		87.5ab	12.5	2.3b	17.9
Humic acid (HA)		100a	0.0	2.5b	10.7
Phosphorien + HA		62.5c	37.5	1.3d	53.6
Microbien + HA		75.0cb	25.0	1.4d	50
Cearilien + HA		87.5ab	12.5	1.8cd	35.7
Phosphorien	Twice	75.0cb	25.0	1.5c	46.4
Microbien		62.5cb	37.5	1.6cd	42.8
Cerealien		75.0c	25.0	1.9c	32.1
Humic acid (HA)		87.5ab	12.5	2.3b	17.8
Phosphorien + HA		50.0c	50.0	0.8	71.4
Microbien + HA		62.0c	37.5	0.9	67.8
Cerealien + HA		62.0c	37.5	1.3d	53.6

Values in a column followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range tests (Duncan 1955).

Table 3: Fusarium total count (cfu) and root colonization (%) as affected by different treatments of biofertilizer and Humic acid soil treatments in green house:

Soil treatment	No of application	Fusarium propagules count cfux10^4g dry soil	% root segments yield *Fusarium* spp
Control	Once	4.6[a]	100a
Phosphorien		3.8b	88a
Microbien		4.0b	81ab
Cerealien		3.7b	73b
Humic acid		4.2b	100a
Phosphorien + HA		2.6cd	48d
Microbien + HA		2.8cd	51cd
Cerealien + HA		3.0c	64bbc
Phosphorien	Two	2.9c	60c
Microbien		3.6bc	65bc
Cerealien		3.2c	55cd
Humic acid (HA)		3.8bc	77b
Phosphorien + HA		1.8de	32
Microbien + HA		2.0d	38
Cerealien + HA		2.3cd	46d

Values in a column followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range tests (Duncan 1955).

A- Effect on Dry Root Rot Development on Mandarin Trees: The effect of different combined treatment of humic acid and biofertilizers on recovering naturally infected mandarin trees began to be Cleary observed after 4 months from soil drenching.

Data in Table (4) show that humic acid + biofertilizers treatments reduced the number of infected trees and reduced percentage of disease severity on these trees. Humic acid + phosphorien was the most effective treatments in controlling dry root rot on mandarin trees followed by humic acid + microbien. After 60 days from treated soil with humic acid combined with phosphorien or microbien or cearalien treatments resulted in recovering 6, 6 and 4 trees and reduced percentage of disease severity from 63.3% (control to 33.3, 28.3 and 36.7% during the first season. Also in the second season the same treatments caused recovery of 9, 5 and 4 trees and reduced disease severity from 58.3% (control) to 20.0, 25.0 and 35.0% respectively. Meanwhile, after 120 days, humic acid + phosphorine or microbien or cerealien cause high significant effect in decreasing both the number of infected trees and percentages of disease severity. As, these treatments resulted in recovering 12, 11, 8 trees in the first season and 11, 11, 10 in the second season, and reduced disease severity from 68.2% (control in the first season) to 13.3, 16.7, 26.7% and from 73.3% (control in the second season) to 10.0, 16.7, 26.7 respectively.

Two soil application with humic acid + phosphorin or microbien, however significantly reduced the number of infected trees and also the percentage of disease severity compared with other treatments. The highest number of recovered trees from infection and also the least percentages of disease severity were detected when soil was treated twice with these treatments. These results agree with those of Hoitink and Fahy[22]; Bettiol[6]; Ziedan[52]; Buonassisi[8]; Zaki and Ghaffar[50]; Saleh and Ahmed[36]; Loffredo[25]. In this respect Pascual *et al*[47] noted that the addition into soil of whole composts and/or their humic fractions reduced the number of root lesion and Pythium population and avoiding reduction of plant growth[6]. Craft and Nelson[10] they noted that humic substances and the humic fractions extracted from composts have been shown to have suppressive effect on different soil plant pathogens ,such *Rhizoctonia solani* , *Phytophthora* spp., *Pythium* spp., *F. solani* and F. oxysporum.

B- Effect on Rhizospheric Microflora of Mandarin Trees: The effectiveness of treated soil of infected mandarin with humic acid combined with phosphorien or microbien and/or cerealien on the activity of soil microorganisms in rhizosphere soil of treated mandarin trees such as *Fusarium* spp, total fungi, total bacteria and total actionomycetes counts was assessed using selective media for counting each group during the two successive seasons 2005 and 2006.

Data in table (5) indicate that all tested treatments reduced the number of fusarium propagules in treated soil, while it increased in untreated one (control of diseased trees). Such data are clear commencing 120 days after application. Humic acid + phosphorien or microbien were the most effective treatments in inhibiting fusarium activity in rhizosphere soil followed by humic acid + cerealien. Where as these treatments reduced the total counts from 6.8 x 10^3 propegules in untreated control to 1.6x10^3, 2.0x10^3 and 2.4x10^4 propagules after 20 days from application. The same trend was observed during 2006 season. Whereas , the best treatments exhibit reduction in the count of fusaria compared with control were humic acid + phosphorien or microbien. On the other hand, soil drenching with humic acid mixed with phosphorien or microbien or cerealien did not affecting total counts of bacteria and actinomycetes in rhizosphere soil, but the counts of these groups lead to increase if compared with control (untreated trees) on contrast total fungi counts was less affected by all soil treatments, as these no significant differences between total fungi counts in rhizosphere soil of treated and non treated (control) mandarin trees. It is obvious that twice application of soil with humic acid + phosphorien or microbien obtained the highest effect in reducing Fusarium propagules counts, moderate effect on total fungal counts, but the counts of bacteria and actinomycetes, lead to increase. In this respect many investigators[8,36,52] noted that most biofertilizer treatments highly reduced population density of the causal organism such *Fusarium* spp, *Fusarium oxysporum* and *Phythium* spp. In addition most biofertilizers containing plant promoting rhizobacteria (PGPR) which produce antibiotics that suppress deleterious microbes Moreover biofertilizers and humic acid substance were reported as enhancement of increasing total bacteria and actinomycetes could in rhixosphere soil[52]

Effect of Soil Treatment with Humic Acid and Biofertilizer on Leaf Mineral Content, Yield and Fruit Quality of Mandarin:

A- Effect on Leaf Minerals Content: Data in table (6) show the effect of soil drenching with humic acid combination with phosphorien or microbien or cerealien biofertilizers on leaf N,P and K content of Balady mandarin trees during two seasons 2005 and 2006. Nitrogen content in the leaves was highly affected due to all tested treatments. The percentages of N content ranged between (2.18-2.53%) and (2.12-2.48%) compared with 2.05% and 2.12% N content in leaves of diseased mandarin trees (control) during 2005 and 2006 seasons, respectively.

Table 4: Dry root rot disease development on mandarin trees as affected by biofertilizer and humic acid (HA) treatments under field condition during 2005 and 2006 seasons.

Season	Soil treatment	Dry root rot development after					
		0 month		2 months		4 months	
		Infection%	Severity%	Infection%	Severity%	Infection%	Severity%
2005	Control(diseased trees)	100	50	100	63.3	100	68.3
		15⁺a	30⁺⁺a	15 a	38a	15a	41a
	Phosphorien+HA	100	50	60	23	20	13.3
		15a	30a	9c		3c	8c
	Microbien+HA	100	50	60	28.3	26.7	16.7
		15a	30a	9c	14	4c	10c
	Cerealien+HA	100a	50	73.3	36.7	46.7	26.7
		15a	30a	11b	22	7b	16b
2006	Control(diseased trees)	100	50	100	58.3	100	73.3
		15⁺a	30⁺⁺a	15a	35a	15a	44a
	Phosphorien+HA	100	50	46.7	20.0	26.7	10.0
		15a	30b	7d	12c	4b	6d
	Microbien+HA	100	50	66.7	25.0	26.7	16.7
		15a	30b	10c	15c	4b	10c
	Cerealien +HA	100	50	80	35.0	33.3	26.7b
		15a	30b	12b	21b	5b	16b

Values in a column followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range tests (Duncan 1955).

* Total No. of infected trees HA: humic acid

** Total degrees of disease severity

Table 5: Effect of different biofertilizers and humic acid (HA) treatments on total count of soil microflora in rhizospheric soil of mandarin trees under field conditions during 2005 and 2006 season.

Season	Soil treatment	Total count of Soil microflora											
		*Fusarium s*ppAV. Number of propagules x 10^3/g dry soil			Total fungi AV. Number of propagules x 10^3/g dry soil			Total bacteria AV. Number of propagules x 10^3/g dry soil			Total actinomycetes AV. Number of propagules x10^4xg dry soil		
		0 day	60 day	120 day	0 day	60 day	120 day	0 day	60 day	120 day	0 day	60 day	120 day
2005	Control (diseased trees)	4.8a	6.0a	6.8a	9.6a	14.8a	16.0a	0.25	22.0a	20.0a	3.2a	4.0a	5.5a
	Phosphorien+HA	4.5a	2.4b	1.6b	8.1a	14.6a	15.4a	18.2a	28.2b	23.2b	3.0a	5.4b	6.2b
	Microbeien+HA	4.8a	2.7b	2.0bc	9.1a	14.2a	16.2a	17.8a	25.0b	25.0b	3.4a	5.2b	7.0b
	Cearalien+HA	4.5a	2.8b	2.4bc	8.4a	14.2a	14.8a	18.0a	25.6b	26.0b	3.2a	5.0b	6.4b
2006	Control (diseased trees)	2.8a	5.6a	6.1a	9.2a	14.5a	15.2a	15.0a	20.6a	19.2a	5.0a	5.2a	5.8a
	Phosphorien+HA	2.6a	2.3a	1.8a	9.2a	13.0b	15.0a	15.4a	21.0a	24.0b	4.8a	7.8b	7.0b
	Microbeien+HA	2.9a	2.5b	2.1b	8.8a	13.6b	15.4a	15.2a	22.8b	22.8b	4.8a	7.4b	6.6b
	Cearalien +HA	2.5a	2.9b	2.5b	8.5a	13.0b	15.4a	14.8a	20.4a	24.2b	4.4a	7.0b	6.2b

Values in a column followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range tests (Duncan 1955).

Table 6: Nitrogen, phosphorus and potassium percentages in leaves of Mandarins trees as affected by different Bio-fertilizers and humic aside (HA) soil treatments during 2005 and 2006 seasons.

Soil treatment	Nitrogen %		Phosphorus %		Potassium %	
	2005	2006	2005	2006	2005	2006
Control (Healthy trees)	2.73a	2.78a	0.232a	0.225a	1.78a	1.75a
Control (diseased trees)	2.05b	2.12b	0.171b	0.162b	1.42b	1.48b
Phosphorien + HA	2.53a	2.48a	0.240a	0.251a	1.72a	1.58ab
Microbien + HA	2.26ab	2.12ab	0.212ab	0.210ab	1.70a	1.63a
Cearalien + HA	2.18ab	2.15ab	0.192b	0.183b	1.56b	1.58ab

Values in a column followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range tests (Duncan 1955).

Soil drenching with humic acid + phosphorien improved N percentages in the mandarin leaves rather than those treatments. As, the values of N contents in leaves of treated trees during two seasons lead to reach to a level of N content in leaves of healthy trees (2.73-2.78%). Such results was recorded by Tattini *et al*[40,41]; Olk and Cossman[43]; David *et al*[12]; Adani[3]. Rengrudki and Partida [34] noted that humic acid treated avocado trees increased by 28% in shoot height and 19.2 %in shoot diameter. Leaf analysis showed a high level of nitrogen and a slight increase in potassium in trees treated with humic acid compared with untreated trees.

Concerning phosphorus and potassium content in the leaves, results cleared that all tested treatments slightly raised up both P and K percentage in leaves of treated trees. As, the percentages of P and K content during 2005 and 2006 season were 0.192 - 0.240%, 0.183 - 0.251% and 1.56 - 1.72 % , 1.58- 1.63 % compared with 0.171, 0.162% and 1.42, 1.48% of P and K content in leaves of untreated diseased trees respectively.

From the above mentioned results. It is obvious that the compination between humic acid and biofertilizers had a favorable effect on N, P and K content in the leaves of treated trees in compared with untreated trees. The obtained results are in line with those reported. by Lobartini *et al*.,[24]; Olk and Cassman 1995 they noted tat the addition of humic acid to vermiculited soils reduced K fixation and resulted in grater total extracted K and highly labile K as well as plant K.

Humic acid + phosphorien treatment caused the highest percentage of P and K content (0.240, 0.251% and 1.72 1.58%) compared with 0.171, 0.162% and 1.42, 1.48% of P and K content in leaves of untreated trees (control) during 2005 and 2006 season respectively. During the past several years humic acid and /or biofertilizers have been promoted by many investigators as plant growth amendments to increase plant nutrient content and improving plant growth[43,34,39,19,1,27].

B- Effect on Yield of Mandarin Trees: Results in Table (7) cleared that drenching soil around stem of diseased mandarin trees with humic acid combined with either phosphorien or microbien or cerealin biofertilizers were significantly affected yield and fruit number in both seasons compared with control (untreated diseased trees).

However, humic acid + phosphorien treatment caused the highest number of fruit/tree, highest yield as kg/tree and ton/feddan (413, 53.7, 8.6 and 410, 57.7, 9.1) compared with 290 fruit/tree, 30.0 kg/tree, 4.8 ton/feddan and 300, 31.5, 5.0 ton feddan of control (untreated diseased tree) in 2005 and 2006 seasons, respectively.

Meanwhile, humic acid + microbien or cearalien biofertilizers improved yield fruit number and total yield (ton/feddan). This positive effect was cleared in the two seasons. As, these treatments resulted in increasing total yield of mandarin trees over the control (non treated diseased trees) by 62.5, 47.9% and 64.0, 50.0% during 2005 and 2006 season, respectively. Beneficial effects of biofertilizers and /or humic acid applications on the growth and yield of some field crops or fruit trees were shown by Abd EL-Monem(Eman)[1]; Liu *et al*[27]; El-Shenawey and Fayed[16]; Saleh *et al*[37]; El-Zeiny *et al*[17]; Gomaa and AbdEl-Naby[20].

C- Effect on Fruit Quality of Mandarin Trees: The effect of twice application of humic acid combined with biofertilizers, phosphorien or microbien or cerealin on fruit quality as physical characteristics (Fruit Weight, fruit size, fruit height and fruit diameter) and fruit chemical properties (TSS%, Acidity %, Juice weight % and TSS/ acid ratio was studied during two successive seasons 2005 and 2006.

Data in Table (8) show that all treatments improved the weight, size, height and diameter of fruit in both seasons. The heaviest and largest fruits were harvested from trees treated with humic acid + phosphorien or microbien in the two seasons followed by humic acid + cerealin treatment. The lowest values of such physical characteristics were recorded by those taken from control (non treated diseased trees) in both seasons. The highest values of fruit weight (130.0 and 135.0 gm), fruit size (140.2 and 146.8 cm^3), fruit height (5.5 and 5.6 cm) and fruit diameter (7.2 and 7.1 cm) were determined in the case of humic acid + phosphorien treatments . But these values were 88.6 and 84.0 gm, 121.3 and 116.8 cm^3, 4.9 and 5.0 cm and 6.5, 6.3 cm of the same fruit characters in non treated diseased trees (control) in the same two seasons respectively. These results are agree with those obtained by El-Shenawey and Fayed[16] and Saleh *et al*[37]. In this respect Li et al[26] reported that liquid fertilizer containing humic acid increased apple fruit weight , yield and soluble solids content .

Concerning chemical properties of mandarin fruit Data in Table (9) indicate that all tested treatments affected TSS%, TSS/acid ratio and Juice weight % in fruit of treated trees if compared with non treated diseased trees in two seasons. Meanwhile, acidity percentage in Juice were not affected by all soil treatments in both seasons, as the acidity ranged between 1.02-1.12% and 1.01-1.15% of treated trees, compared with 1.20 and 1.18% of non treated diseased trees in 2005 and 2006 season, respectively. Humic acid + phosphorien treatment resulted in enhancing the parameter of fruit quality such TSS% (13.3 and 13.4%), TSS/acid ratio (13.04 and 13.27) and Juice weight (55.3 and 56.4%), as these values nearly similar to those of healthy trees (control-1). Meanwhile the least values were obtained in the case of non treated diseased trees (control-2). The obtained results are in line with those obtained by many authors[26,1,27,16,37].

Table 7: Yield of Mandarins trees as affected by different by different Bio-fertilizers and humic aside (HA) soil treatments during 2005 and 2006 seasons.

Soil treatments	2005				2006			
	No. of fruits/tree	Yield		% Over Diseased trees	No. of fruits/tree	Yield		% Over Diseased trees
		Kg/tree	Ton/feddan			Kg/tree	Ton/feddan	
Control (healthy tree)	378b	55.1a	8.8a	-	390a	56.2a	9.0a	-
Control (diseased trees)	290d	30.0d	4.8d	-	300d	31.5c	5.0d	-
Phosphorien + HA	413a	53.7a	8.6a	79.2	410a	57.7a	9.1a	82
Microbien + HA	362b	48.9b	7.8b	62.5	378b	51.0d	8.2b	64
Cearalien + HA	320c	44.7bc	7.1c	47.9	350c	47.0b	7.5	50.0c

Values in a column followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range tests (Duncan 1955).

Table 8: Effect of different soil treatments of Bio-fertilizers and humic aside (HA) on some physical characteristics of Mandarins fruits during 2005 and 2006 seasons.

Soil treatments	Fruit weight(gm)		Fruit size(cm^3)		Fruit height(cm)		Fruit diameter(cm)	
	2005	2006	2005	2006	2005	2006	2005	2006
Control (H.T)	145.8a	144.0a	169.0a	166.3a	5.8a	5.7a	7.3a	7.1a
Control (D.T)	88.6d	84.0d	121.3d	116.8d	4.9b	5.00bc	6.5b	6.3c
Phosphorien +HA	130.0b	135.0b	140.2b	146.8b	5.5a	5.6a	7.2a	7.1a
Microbien +HA	124.0b	130.2b	136.3bc	140.3bc	5.4a	5.6a	7.0a	6.8b
Cearalien +HA	121.0c	120.0c	131.2c	130.5c	5.3a	5.2a	7.4a	7.1a

Values in a column followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range tests (Duncan 1955).

Table 9: Effect of different soil treatments of Bio-fertilizers and humic aside (HA) on some chemical characteristics of Mandarin fruits during 2005 and 2006 seasons.

Soil treatment	T.S.S%		Acidity %		TSS/acid ratio		Juice weight %	
	2005	2006	2005	2006	2005	2006	2005	2006
Control (H.T)	13.8a	13.6a	1.00a	1.02a	13.8a	13.3a	62.3a	61.5a
Control (D.T)	10.4c	10.5d	1.20b	1.18a	8.67d	8.90d	42.6d	44.5d
Phosphorien +HA	13.3a	13.4a	1.12a	1.15a	13.04a	13.27a	55.3b	56.4b
Microbien +HA	12.5ab	12.6b	1.08a	1.07a	11.57bc	11.78b	56.5b	55.8bc
Cearalien +HA	11.8b	11.4bc	1.02a	1.01a	10.54c	9.91c	51.8c	50.2c

Values in a column followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range tests (Duncan 1955).

In conclusion drenching artificially or naturally infested soil with Fusarium solani the causal agent of dry root rot of citrus by humic acid +phosphorien or miccrobien biofertilizers as twice applications resulted in decrease both disease infection and severity on mandarin seedlings as well as reduce colonization of *F. solani* in seedlings roots . In Field trials , such treatments cause significantly increase the total number of recovering diseased trees, decrease the disease severity on the others as well as cause inhibiting the activity of the pathogen in rhizospher soil of treed trees . Moreover, these treatments improving the yield and fruit quality of treed trees . Therefore, the usage of combination between humic acid and biofertilizers could be suggested as easily bio-treatment for controlling dry root rot of citrus especially under organic system.

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