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Effect of Humic Acid on the Efficacy of some Biocontrol Agents in Controlling Damping-off of Cotton Seedlings Caused by *Fusarium oxysporum*

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A mixture of four isolates of *Fusarium oxysporum*, the causal agent of cotton damping-off, five biocontrol agents (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Streptomyces griseus*, *Trichoderma harzianum* and *Trichoderma viride*), and fungicide Monceren were evaluated on cotton cultivar Giza 90 (*Gossypium barbadense* L.) in the presence and absence of humic acid (potassium humates) which was applied as soil drench. The disease incidence was evaluated as seedling survival, plant height, and dry weight. Activities of some oxidative enzymes (peroxidase, polyphenoloxidase and catalase) were also evaluated. The results showed that humic acid caused deleterious effects on seedling stand, plant height and dry weight of cotton cultivar Giza 90. The application of some biocontrol agents negated these deleterious effects. Therefore, from practical stand point, it is not desirable to use humic acid if the soil is infested with *Fusarium oxysporum*. If it is necessary to use humic acid; this should be accompanied by the application of suitable biocontrol agent.

Keywords: Cotton (*Gossypium barbadense* L.), *Fusarium oxysporum* Humic Acid (potassium humates) and Oxidative enzymes.

Cotton (*Gossypium barbadense* L.) is an important crop in Egypt and has an excellent reputation all over the world. Seedling disease in cotton is a worldwide problem; causing serious stand loss when it is not controlled (Blasingame and Patel 2013). In this disease, root and stem tissue become discoloured and turn brown internally then rotted (Watkins, 1981). The disease is caused by a complex of seed or soil borne fungi including *Fusarium* species (*F. solani* Mart., *F. moniliforme* J. Sheld. *F. oxysporum* Schlecht). *F. oxysporum* and *F. moniliforme* are important pathogens in the aetiology of cotton damping-off in Egypt (Abd-ELSalam *et al.*, 2006). Seed treatments by fungicides are used to control diseases that cause seed rot and damping-off before and after emergence. Treating cotton seeds with fungicides reduced damping-off incidence under field conditions. Treating cotton seeds with fungicide *i.e.*, monceren before planting in infested soil in the greenhouse gave the highest seedling emergence and the highest percentage of surviving seedlings (Aly *et al.*, 2001).

Humic acid is a mixture of dark-brown or organic substances, which can be extracted from soil with diluted alkali and precipitated by acidification to pH 1-2. Humic acid as suspension (potassium humates) can be applied successfully in many areas as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases and pests (Senn, 1991). Hafez (2003) reported that humic acid applications led to a significant increase in soil organic matter, which improves plant growth and crop production. The role of humic acid in plant diseases may be due to the correlation between carboxylic acids and plant health (Scheuerell and Mahaffee, 2004), stimulation of plant growth through increased cell division as well as optimized uptake of water and nutrients especially nitrogen, potassium, and phosphorus, which are necessary for plant growth and increases in cell permeability and soil physical conditions, enzyme activation and/or inhibition, changes in membrane permeability, protein synthesis and finally the activation of biomass production (Prakash *et al.*, 2010). The application of humic acid (potassium humates) as soil amendment resulted in significant increases in plant growth and crop yields in reclaimed saline soils probably due to improvement of hydro-physical properties and nutrient availability of these soils (Osman and Rady, 2012).

Biological control of different plant diseases is applied by using bacteria or fungi. *Bacillus subtilis* Cohn., *Pseudomonas fluorescens* Flügge., *Trichoderma harzianum* Rifai., *Trichoderma viride* Harz., and *Streptomyces griseus* Waksman. are considered the most important genera of antagonistic microorganisms for controlling fungal diseases (Abdel-Kader *et al.,* 2012). Biological control agents inhibit plant pathogens through one or more of the following mechanisms: mycoparasitism, competition for key nutrients and colonization sites, production of antibiotics, or stimulation of plant defense mechanisms (Cook and Baker, 1983).

Bacillus subtilis produces more than 66 antifungal and antibiotic substances, which play a role in suppression and inhibition of pathogen, consequently lead to reduction in disease incidence (Singh *et al*., 2008).

Pseudomonas fluorescens is considered as an important group of the antagonistic bacteria, which is effective against several soil borne pathogens in field and greenhouse trails (Karunanithi *et al.,* 2000). Moreover, *Pseudomonas* spp. received great attention as bio-control agents because of their catabolic versatility, excellent root-colonizing abilities and production of broad range antifungal metabolites (Raaijmakers *et al.*, 2002).

Abd-El-Kareem (2007) stated that *Trichoderma* spp. are effective biocontrol agents for a number of soil borne plant pathogens inducing systemic resistance mechanism in plants. *Trichoderma* species are strongly antagonistic to other phytopathogenic fungi which produce hydrolytic enzymes (Abd-El-Moity and Shatla 1981). Abd-El-Moity (1985) stated that *Trichoderma harzianum* produces antifungal substance (Gliotoxin), which can inhibit growth of pathogenic fungi, and also produces organic acids, such as gluconic, citric or fumaric acids, that decrease soil pH and permit the solubilisation of phosphates, micronutrients and mineral cations like iron, manganese and magnesium, which are useful for plant metabolism (Benitez *et al.*, 2004).

Soil actinomycetes particularly *Streptomyces* spp. enhance soil fertility and has antagonistic activity against a wide range of soil-borne plant pathogens (Hallmann *et al.*, 1997). Attempts have been made to develop *Streptomyces* species for controlling root disease agents, since *Streptomyces* spp. are capable of producing a remarkably wide spectrum of antibiotics as secondary metabolites (Franklin *et al.*, 1989).

Bradley *et al.* (1992) reported that increasing peroxidase (PO) activity has been correlated with resistance in many plants including cotton and this enzyme is involved in the polymerization of proteins and lignin or suberin precursor into plant cell wall, thus constructing a physical barrier that could prevent pathogen penetration of cell walls or movement. Polyphenoloxidase (PPO) usually accumulates upon wounding in plants. Biochemical approaches to understand PPO function and regulation are difficult because the quinonoid reaction products of PPO covalently modify and cross link the enzyme (Misaghi, 1982). Catalase activity reduces the level of hydrogen peroxide, which may accumulate up to toxic levels in diseased tissues and turns it into water and free oxygen that possesses biocidal activity (Misaghi, 1982).

The objective of the present study was to evaluate some biocontrol agents for controlling cotton damping-off caused by *Fusarium oxysporum* in the presence or absence of humic acid. Seedling survival, plant height, dry weight and oxidative enzymes were used as criteria for evaluating the efficacy of treatments.

Materials and Methods

1. Source of Fusarium oxysporum isolates and cotton seeds:

Four isolates of *Fusarium oxysporum* Schlecht. used in this study were obtained from the fungal collection of Cotton and Fiber Crops Dis. Res. Dept., Plant Pathology Res. Inst. Agric. Res. Center, Giza (ARC) which were isolated originally from cotton seedlings infected with damping-off. Cotton seeds (Cultivar Giza 90) were obtained from Cotton Research Inst., Agric., Res., Centre.

2. Preparation of bioagents:

Bacillus subtilis, Pseudomonas fluorescens, Streptomyces griseus,Trichoderma harzianum and *Trichoderma viride* were obtained from Central Lab of Organic Agriculture, ARC, Giza. *T. harzianum* and *T. viride* were grown on liquid gliotoxin fermentation medium (GFM) developed by Brain and Hemming (1945) for 11 days under complete darkness conditions at 25ºC. *B. subtilis* isolate was grown in liquid nutrient glucose medium (NGM) developed by Dowson (1957) for 2 days, at 25ºC. *P. fluorescens* isolate was grown in King's medium (King *et al.*, 1954) for 2 days, at 25ºC and *S. griseus* was grown in Starch Nitrate Agar media (StNAM) (Waksman, 1959). Different bio-agents were prepared as suspensions at concentration of 30 x 10^6 cfu/ml for *B. subtilis*, *P. fluorescens* and *Streptomyces griseus* 2 x 10^6 cfu/ml for *Trichoderma harzianum* and 5x 10^6 cfu/ml *Trichoderma viride*.

3. Humic acid:

Humic Acid (Potassium humates, pH 9) was obtained from Central Lab of Organic Agriculture, A.R.C, Giza, where this salt is manufactured. Rate of application was 10 ml /kg clay soil as a suspension.

4. The fungicide:

The fungicide Monceren (Pencycuron) 25% WP was used as seed dressing fungicide at rate 3g/kg seeds.

5. Greenhouse experiments:

The greenhouse experiment was conducted in Cotton and Fiber Crops Dis. Res. Dept., Plant Pathology Res. Ins. ARC, Giza, Egypt where the temperature ranged from 25-35ºC. Inocula of *Fusarium oxysporum* isolates were prepared by growing each isolate in sterilized 500- ml glass bottles containing 50 gm of sorghum grains and 40ml of water. The sterilized bottles were inoculated with fungal growth of each isolate separately taken from one-week culture grown on PDA plates. The inoculated bottles were incubated for two weeks at 26±2ºC. During that period the inoculated bottles were shaked for 5 min. every three days to ensure uniform distribution of the fungal growth. The growing cultures on sorghum were air dried under greenhouse conditions. The air dried cultures were triturated to a powder by a blender (Aly, 1988). The powdered inoculum of each isolate was stored in polyethylene bags at 5ºC until use.

Treatments were divided into two groups: the first group included sterilized clay soil dispended in pots as non-infested control, infested clay soil with a mixture of *Fusarium oxysporum* isolates at equal rates by weight (1:1:1:1) at the rate of 30g/kg clay soil dispended in pots as infested control. The five bio-agents were added separately as soil drench to infested clay soil to evaluate their potential in controlling damping-off. Monceren was used as seed dressing to evaluate its efficiency in controlling the disease. Five pots/treatment with ten cotton seed/pot were used. The second group included the same previously mentioned treatments but with the addition of humic acid to all treatments. After forty five days the disease parameters were recorded as plant survival, plant height, and dry weight.

All pots were distributed in a randomized complete block design on a greenhouse bench. Random samples of cotton seedlings were used for further biochemical studies.

6. Preparation of enzyme extracts and the assay methods:

One gram of leaves samples from each cotton treatment, healthy or infected was crushed well in 2 ml sodium phosphate buffer 0.1M at pH 7.1. The homogenate was filtrated through Whatman No.1 filter paper. The suspension was centrifuged at 6000 rpm at 4^{o}C for 20 min and stored at 18^{o}C until use.

6.1 Phosphate buffer preparation:

Eight grams of di-sodium hydrogen phosphate were dissolved in 250 m. distilled water in a volumetric flask (solution A) and 3.9g. Sodium hydrogen phosphate was dissolved in 250 ml. distilled water in another flask (Solution B). Solution A was added to solution B to have pH (7.1).

6.2 Peroxidase activity:

One tenth extracted sample was added to 0.5 ml sodium phosphate buffer 0.1M at pH 7.1and 0.1ml H_2O_2 1% finally 0.3 ml pyrogallol 0.05 µl. Enzyme activity was calculated as mg/gm fresh weight. The mixture was completed to 3 ml using distilled water and colour density was read in absorbance spectrophotometer Miltonroy spectronic 601 at 425 nm every 30 second for 10 reads (Kochba *et al*. 1977).

6.3 Polyphenoloxidase activity:

One tenth extracted sample was added to 0.5 ml sodium phosphate buffer 0.1M at pH 7and 0.5 ml. catechol 0.001 N. The mixture was completed to 3M using distilled water and colour density was read in spectrophotometer Miltonroy spectronic 601 at 495 nm. every 30 second for 10 reads (Esterbauer *et al.* 1977).

6.4 Catalase activity:

The activity of catalase was determined as described by Aebi (1974). Enzyme extract (0.1ml) was added to 2.9ml of a reaction mixture containing 0.3M. H_2O_2 5% and 0.5M. sodium phosphate buffer (pH 7.6). The activity of catalase was measured by monitoring the reduction in the absorbance at 240 nm as a result of H_2O_2 consumption. Catalase activity was expressed as unit's $min^{-1}mg^{-1}$protein. One unit of enzyme activity was defined as the decomposition of 1µmol of H_2O_2 per min.

7. Statistical analysis:

All experiments were set up in a randomized complete block design. Data were subjected to statistical analysis procedure according Steel and Torrie (1960). The means differences were compared by the least significant difference test (L.S.D.) at 5% level of significance.

Results and Discussion

ANOVA in Table 1 show highly significant (p=0.00) effects for humic acid (H) and treatments (T) on seedling survival. On the other hand, the interaction between humic acid and treatments was not significant (p=0.21) source of variation.

Due to the non-significant interaction, general means were used to compare between treatments (Table 2). These comparisons showed that Fusarium mixture was pathogenic as it significantly ($p \leq 0.05$) reduced survived seedlings from 85% (uninfested control) to 61.67% (infested soil). All treatments, regardless the presence or absence of humic acid significantly increased seedling survival except *Pseudomonas fluorescens*, which significantly reduced seedling survival (Table 2). The deleterious effect of some Pseudomonas strains on seedling stand is well documented in literature (Aly *et al*., 2017 and Schippers *et al*., 1987).

Table 1. Analysis of variance ANOVA of the effects of humic acid (H), treatments (T) and their interaction on seedling disease variables caused by *F. oxysporum* on cotton (Cultivar Giza 90)

Variable and source of variation	d.f.	M.S.	F. value	P>F
(1) Survival				
Replication	2	27.083	0.367	0.696
Humic acid (H)	1	27000.0	36.610	0.000
Treatments (T)	7	363.905	8.636	0.000
H x T	7	109.524	1.485	0.210
Error	30	73.750	-	-
(2) Plant height				
Replication	2	5.453	2.018	0.151
Humic acid (H)	1	20.672	7.648	0.010
Treatments (T)	7	34.713	12.843	0.000
H x T	7	26.709	9.882	0.000
Error	30	2.703	-	-
(3) Dry weight				
Replication	2	0.005	0.465	0.633
Humic acid (H)	1	0.918	16.787	0.000
Treatments (T)	7	0.240	20.402	0.000
H x T	7	0.163	13.841	0.000
Error	30	0.012	-	-

Table 2. Effects of humic acid (H), treatments (T) and their interaction on seedling survival (%) of cotton (Cultivar Giza 90)

Treatments	Humic Acid		Mean
	Absent (H-)	Present (H+)	
Control 1 (autoclaved soil)	86.67[a]	83.33	85.00
Control 2 (infested soil)	76.67	46.67	61.67
Bacillus subtilis	76.67	70.00	73.34
Pseudomonas fluorescens	66.67	46.67	56.67
Streptomyces griseus	76.67	56.67	66.67
Trichoderma harzianum	90.00	76.67	83.34
Trichoderma viride	83.33	73.33	78.33
Moncern	86.67	70.00	78.34
Mean	80.42	65.42	

[a] mean of three replicates, L.S.D. 0.05 for: Humic acid (H)= 1.012, Treatments (T) = 4.048, H x T = n.s.

The application of humic acid caused significant reduction in survival of seedlings regardless of treatments (Table 2). Yigit and Dikilitas (2008) found a positive relationship between the increasing concentrations of humic acid and appearance of the disease caused by soil pathogen *Fusarium oxysporum*.

Humic acid, treatments and their interaction were all highly significant (P=0.00) sources of variation in plant height (Table 1). Due to the significant interaction, an interaction LSD was used to compare treatments in the presence and absence of humic acid (Table 3). These comparisons showed that Fusarium mixture caused non-significant increase in seedling height in the absence of humic acid. On the contrary, the Fusarium mixture caused significant decrease in the plant height in the presence of humic acid. In the absence of humic acid, the comparisons between treatments and infested soil showed that each of *P. fluorescens* and *S. griseus* caused significant decrease in plant height, while the other treatments caused non-significant effects on plant height. In the presence of humic acid, all the tested treatments caused significant increases in plant height compared with the infested control, except *P. fluorescens* which caused non-significant increase in plant height (Table 3).

Table 3. Effects of humic acid (H), treatments (T) and their interaction on plant height (cm/plant) of cotton (Cultivar Giza)

Treatment	Humic Acid		Mean
	Absent (H-)	Present (H+)	
Control 1 (autoclaved soil	16.73[a]	19.87	18.30
Control 2 (infested soil)	18.07	10.63	14.35
Bacillus subtilis	17.23	20.60	18.92
Pseudomonas fluorescens	14.77	11.97	13.37
Streptomyces griseus	14.03	21.70	17.87
Trichoderma harzianum	18.97	21.07	20.02
Trichoderma viride	19.27	21.43	20.35
Moncern	16.73	18.97	17.85
Mean	16.98	18.28	

[a] mean of three replicates L.S.D. 0.05 for: Humic acid (H) = 0.19
Treatments (T) = 0.78 H x T = 1.55

In case of dry weight, all sources of variation were highly significant (P=0.00). Due to the significant interaction, a L.S.D. was used to compare between treatments in the presence or absence of humic acid (Table 4). Dry weight of seedlings was not affected by Fusarium mixture in the absence of humic acid, while it was significantly reduced from 0.95 mg to 0.22 mg in the presence of humic acid (Table 4). In the absence of humic acid, *B. subtilis*, *P. fluorescens* and *T. viride* caused significant (P =0.05) increases in dry weight compared with the infested control.

The other treatments showed no effects on dry weight. In the presence of humic acid, all treatments significantly increased dry weight compared with infested control.

Table 4. Effects of humic acid (H), treatments (T) and their interaction on dry weight (mg/plant) of cotton (Cultivar Giza 90)

Treatment	Humic Acid		Mean
	Absent (H-)	Present (H+)	
Control 1 (autoclaved soil)	0.81[a]	0.95	0.880
Control 2 (infested soil)	0.80	0.22	0.510
Bacillus subtilis	0.93	1.12	1.025
Pseudomonas fluorescens	1.02	1.20	1.110
Streptomyces griseus	0.87	1.46	1.165
Trichoderma harzianum	0.85	1.09	0.970
Trichoderma viride	1.01	1.04	1.025
Moncern	0.83	1.06	0.945
Mean	0.89	1.018	

[a] mean of three replicates L.S.D. 0.05 for: Humic acid (H) = 0.013, Treatments (T) = 0.052 and H x T = 0.103

Analysis of variance (ANOVA) of the effect of humic acid, treatments and their interaction on activities of some oxidative enzymes (peroxidase, polyphenoloxidase and catalase) in cotton seedlings is shown in Table (5). Data show that there was highly significant interaction (p=0.00) between humic acid (H) and treatments (T) of all tested enzymes. Due to the significant H x T interaction, treatments were compared when humic acid was present or absent.

Data in Table 6 show that infestation of soil with *Fusarium oxysporum* caused significant increase in peroxidase activity in the absence of humic acid while the activity was decreased in the presence of humic acid. Ulukan (2008) reported that biochemical effects of humic substances may include inhibition of enzymes activity. Addition of biocontrol agents to infested soil caused decrease in peroxidase activity in the seedlings when humic acid was absent especially in the case of *S. griseus* and *T. viride* where it decreased from 0.888 to 0.192 and to 0.252 respectively. In the presence of humic acid (potassium humates), peroxidase activity was significantly increased by all treatments in comparison with seedlings grown in the infested control except *Bacillus subtilis* where the increase in peroxidase activity was not significant. Many investigators stated that there is positive relationship between peroxidase enzyme and resistance developed in plants (Mohammadi and *Kazemi*, 2002 and Chen *et al.*, 2000).

Table 5. Analysis of variance ANOVA of the effects of humic acid (H), treatments (T) and their interaction on activities of some oxidative enzymes in cotton seedlings (Cultivar Giza 90)

Variable and source of variation	d.f.	M.S.	F. value	P>F
(1) Peroxidase				
Replication	2	0.114	27.504	0.000
Humic acid (H)	1	0.362	86.958	0.000
Treatments (T)	7	0.046	11.014	0.000
H x T	7	0.149	35.757	0.000
Error	30	0.004	-	-
(2) Polyphenol oxidase				
Replication	2	6.77E-006	1.433	0.255
Humic acid (H)	1	0.004	868.951	0.000
Treatments (T)	7	0.004	950.194	0.000
H x T	7	0.004	849.173	0.000
Error	30	4.73E-006	-	-
(3) Catalase				
Replication	2	0.357	8.690	0.001
Humic acid (H)	1	9.063	220.651	0.000
Treatments (T)	7	1.796	43.722	0.000
H x T	7	1.614	39.296	0.000
Error	30	0.41	-	-

Table 6. Effects of humic acid (H), treatments (T) and their interaction on peroxidase activity in cotton seedlings

Treatment	Humic Acid		Mean
	Absent (H-)	Present (H+)	
Control 1 (autoclaved soil)	0.484	0.421	0.453
Control 2 (infested soil)	0.888	0.159	0.524
Bacillus subtilis	0.700	0.242	0.471
Pseudomonas fluorescens	0.550	0.290	0.420
Streptomyces griseus	0.192	0.297	0.245
Trichoderma harzianum	0.539	0.274	0.407
Trichoderma viride	0.252	0.417	0.335
Moncern	0.302	0.419	0.361
Mean	0.448	0.315	

L.S.D. 0.05 for: Humic acid (H) = 0.036
Treatments (T) = 0.072 H x T = 0.102

In plants grown in the infested soil with *Fusarium oxysporum* isolates the polyphenoloxidase activity was increased compared with those grown in non-infested soil in the absence of humic acid, while there was no significant change in polyphenoloxidase activity in the presence of humic acid (Table 7). In the absence of humic acid, the biocontrol agents caused significant decrease in enzyme activity in the plants raised from seeds planted in infested soil with Fusarium except *B. subtilis* treatment where the enzyme activity was increased from 0.072 to 0.173.

In the presence of humic acid, biocontrol agents caused increase in enzyme activity except *S. griseus.*

Increase of polyphenoloxidase activity in host tissues in response to infection by the pathogen has been reported (Chen *et al.*, 2000 and Mayer 1987).

Table 7. Effects of humic acid (H), treatments (T) and their interaction on polyphenoloxidase activity in cotton seedlings

Treatment	Humic Acid		Mean
	Absent (H-)	Present (H+)	
Control 1 (autoclaved soil)	0.039	0.028	0.034
Control 2 (infested soil)	0.072	0.026	0.039
Bacillus subtilis	0.173	0.037	0.105
Pseudomonas fluorescens	0.037	0.035	0.036
Streptomyces griseus	0.024	0.025	0.025
Trichoderma harzianum	0.024	0.045	0.035
Trichoderma viride	0.017	0.030	0.024
Moncern	0.016	0.027	0.022
Mean	0.050	0.032	

L.S.D. 0.05 for: Humic acid (H) = 0.001,Treatments (T) = 0.002, H x T = 0.004

Regarding Table 8 catalase activity was significantly increased in the cotton seedlings grown in the infested soil compared with seedlings grown in non-infested soil in the absence of humic acid while, in the presence of humic acid there was no significant decrease in catalase activity. Effect of treatments on plant grown in infested soil with biocontrol agents in the absence of humic acid can be divided into three groups. Group (1) included: *T. viride* which caused significant increase in catalase activity from 1.981 to 2.483. Group (2) included; *P. fluorescens*, *T. harzianum* and fungicide Moncern which caused significant decrease in catalase activity. Group (3) included: *B. subtilis* and *S. griseus* which did not cause significant effect on catalase activity.

Table 8. Effects of humic acid (H), treatments (T) and their interaction on catalase activity in cotton seedlings

Treatment	Humic Acid		Mean
	Absent (H-)	Present (H+)	
Control 1 (autoclaved soil)	1.440	3.919	2.680
Control 2 (infested soil)	1.981	3.660	2.821
Bacillus subtilis	1.823	2.246	2.044
Pseudomonas fluorescens	1.639	2.981	2.310
Streptomyces griseus	2.016	2.387	2.202
Trichoderma harzianum	1.246	2.701	1.934
Trichoderma viride	2.483	1.954	2.219
Moncern	1.157	0.871	1.014
Mean	1.723	2.592	

L.S.D. 0.05 for: Humic acid (H) = 0.116, Treatments (T) = 0.232, H x T = 0.327

Infested soil with *F. oxysporum* was always associated with significant increases in the activity of oxidative enzymes (PO, PPO and catalase) in the tissues of Giza 90 cotton seedlings (Bradley *et al*., 1992). The increase in disease pressure in the presence of humic acid was due to the inhibitory effects of humic acid on the activity of some oxidative enzymes, which led to a comparable increase in the susceptibility of seedlings (Ulukan, 2008 and Yigit and Dikilitas, 2008).

Conclusion

The present study demonstrated the deleterious effects of humic acid on seedling stand, plant height and dry weight of cotton seedlings grown in soil infested with *Fusarium oxysporum*. The application of some biocontrol agents such as *Bacillus subtilis*, *Pseudomonas florescens*, *Streptomyces griseus*, *Trichoderma harzianum* and *Trichoderma viride* negated these deleterious effects. Therefore, from practical stand point, it is not desirable to use humic acid if the soil is infested with *Fusarium oxysporum*. If it is necessary to use humic acid; this should be accompanied by the application of suitable biocontrol agent.

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تأثير حمض الهيومك على فعالية بعض عوامل المقاومة الحيوية فى مقاومة مرض موت بادرات القطن المتسبب عن فطر فيوزاريوم أوكسيسبورم

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تم تقييم خليط من أربع عزلات من فطر فيوزاريوم أوكسيسبورم *Fusarium oxysporum* المسبب لمرض موت بادرات القطن وخمس عزلات من كائنات المقاومة الحيوية *Bacillus subtilis, Pseudomonas fluorescens*, *Streptomyces griseus, Trichoderma harzianum* and *Trichoderma viride* والمبيد الفطرى مونسرين من حيث تأثيرهم على مرض موت البادرات فى صنف القطن جيزة ٩٠ و ذلك فى حالة وجود أو غياب حمض الهيوميك (هيومات البوتاسيوم). تم تقييم حدوث المرض بتقدير البادرات السليمة الباقية على قيد الحياة و أطوال البادرات و الوزن الجاف للبادرات. كما تم تقدير نشاط بعض الأنزيمات المؤكسدة (البيروكسيديز - البوليفينول اوكسيديز- الكتاليز). أظهرت النتائج ان إضافة حمض الهيوميك على شكل سائل الى التربة أدى الى تاثيرات ضارة على جميع مؤشرات حدوث المرض. أدى استعمال بعض كائنات المقاومة الحيوية الى إبطال التأثيرات الضارة لحمض الهيوميك. وعلى ذلك ومن وجهه تطبيقية، فانه من غير المرغوب فيه اضافة حمض الهيومك للتربة الملوثة بفطر *F. oxysporum*. أما اذا اقتضت الضرورة إضافة حمض الهيومك للتربة فان ذلك يجب ان يكون مصحوباً باستعمال عامل مقاومة حيوى مناسب.