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Suppression of Fusarium Wilt of Watermelon by Biological and Chemical Control

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Abstract: Watermelon (*Citrullus lanatus* Thunb.) Matsum. & Nakai is one of the most extensively planted summer fruit crops in Egypt. *Fusarium* wilt caused by the soilborn fungus *Fusarium oxysporum* Schlechtend: Fr .f. sp. *niveum* (E. F. Sm.) W. C. Snyder & H. N. Hans., is one of the most serious diseases of watermelon and in many areas is a factor that limits production. Fusarium wilt (*F. oxysporum f.* sp. *niveum*) is a limiting factor to watermelon production in Egypt. Effective control of this disease generally has been achieved only through the use of disease resistant cultivars. Isolation trials from watermelon plants showing root-rot and stem rot or wilt symptoms yielded several fungi belonging to six genera. The most frequent fungi isolated were *Fusarium* spp. (69.6%). The possible interaction between the pathogens tested showed that a reduction in infection percentages were obtained by inoculum combinations, which refers to a relationship between causal pathogens looks like antagonism. El-Beheira isolate was the highest virulence one on watermelon plants (reached 44.2% Pre-emergence and 77.4% Post-emergence respectively). Meanwhile, Ismailia isolates was the lowest one on watermelon in this concern (38.8% and 67.2% respectively). Watermelon only found to be infected in field with *F. oxysporum*, other family plants were not observed to be infected under greenhouse conditions. *Fusarium* wilt fungus strain is very host specific, however, making it possible to rotate tomatoes with watermelons. Data also reveal that among the four tested antagonistic microorganisms, *Pseudomans fluorescens* (B4) gave the highest reduction to the wilt incidence after 4, 8 weeks of application when compared with the control treatment. The efficacy of certain fungicides, biocides as seed dressing or soil drench on watermelon wilt was evaluated under greenhouse condition. Data reveal that, all the tested treatments have significantly reduced the percentage of watermelon wilt incidence. Soil treatments showed higher significant reduction on disease incidence than the seed treatment. Topsin-M as seed dressing or soil drench showed superior effect on disease incidence.

Key words: *Fusarium oxysporum f.sp. niveum*, Fusarium wilt, Watermelon (*Citrullus lanatus* (Thunb.) Suppression and Biological control

INTRODUCTION

Arndt *et al.*[2] Found that, three preselected *fluorescent Pseudomonas* isolates controlling damping-off of cucumber seedlings caused by *Pythium ultimum* and *Rhizoctonia solani* in laboratory experiments were tested as biological control agents in greenhouse trials by soil drenches with cell suspensions (50 or 100 ml, 2x109 colony forming units/ml) of 3 Pseudomonas strains (WB1, WB15 and WB52). Larkin and Fravel[17] tested the numerous fungi and bacteria, against soil-borne fungal pathogens, for their efficacy in controlling *Fusarium* wilt diseases. Specific non-pathogenic strains of *Fusarium* spp., isolated from wilt-suppressive soils, were the most effective antagonists for the reduction of Fusarium wilt diseases of tomatoes, watermelons and muskmelons, providing consistent and significant disease control (50-80% reduction) in several repeated tests. Other organisms, including isolates of *Gliocladium virens* and *Trichoderma hamatum* also significantly reduced wilt compared with controls (30-60% reduction). Singh *et al.*[25] Mentioned that two chitinolytic bacterial strains, Paenibacillus sp. 300 and *Streptomyces* sp. 385, suppressed *Fusarium* wilt of cucumber (*Cucumis sativus*) caused by *Fusarium oxysporum f.sp.* cucumerinum in nonsterile, soilless potting medium. A mixture of the two strains in a ratio of 1:1 or 4:1 gave significantly ($P<0.05$) better control

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of the disease than each of the strains used individually or than mixtures in other ratios.

Larkin and Fravel[18] found that, three biocontrol isolates (CS-1, CS-20, and Fo47), demonstrated some degree of induced systemic resistance in tomato (*Lycopersicon esculentum*) and watermelon (*Citrullus lanatus*) plants, as determined by split-root tests, but varied in their relative abilities to reduce disease. Isolate CS-20 provided the most effective control (39 to 53% disease reduction), while Fo47 provided the least effective control (23 to 25% reduction) in split-root tests.

Ozaktan and Bora[24] isolated 126 fluorescent pseudomonad (FP; *Pseudomonas fluorescens*) isolates. Freeman *et al*.[13] found that, a nonpathogenic mutant (isolate 4/4) of *F. oxysporum f.* sp. *melonis* was isolated following UV-mutagenesis and was able to reduce mortality of watermelon seedlings (cv. Odem and Malali) caused by *F. oxysporum f.* sp. *niveum* race 2. Freeman *et al*.[14] Reported that, two non-pathogenic mutant strains 4/4 and 15/15 of *Fusarium oxysporum f.* sp. *melonis* (race 1, 2) were isolated by a continuous dip-inoculation technique following UV mutagenesis of the virulent wild type isolate FOM1.2. No disease symptoms or detrimental effects were observed following inoculation of musk melon [melons] seedlings by strain 4/4. In contrast, strain 15/15 caused mortality of susceptible cultivars although to a lesser extent than the wild type isolate.

Ji-MingShan *et al*.[16] reported that *Fusarium oxysporum* f.sp. *niveum* is the causal organism of wilt disease of some cucurbitaceae plants, *e.g.* watermelon and melon. Thy found that strains, *Trichoderma viride* TR-8 and *Bacillus* sp. B67 were the most effective against watermelon wilt disease under greenhouse conditions. El-Sayed[12] isolated that, *Fusarium avenaceum* [*Gibberella avenacea*] and *F. oxysporum* from watermelon seedlings showing root rot and wilt symptoms and was evaluated in watermelon cv. Balady. FW2 was the most pathogenic (66% disease intensity or DI), whereas FW1 was the least pathogenic (27% DI). And studied effects of Roundup [glyphosate] at 25, 50, 100 and 200 ppm, singly or in combination with fungal antagonists (*Bacillus subtilis, B. megaterium* and *Trichoderma harzianum*), on the growth and sporulation of *F. avenaceum* (FW2) and *F. oxysporum* (FW4), he found that, least fungal growth (5.6-7.3 mm) was obtained with 200 ppm Roundup + *T. harzianum*. The application of Roundup (0.0, 2.5, 5.0 and 10.0 µg/g soil) to infected soil before sowing also reduced wilt disease, especially with increasing concentration. The soil application of Roundup coupled with seed treatment with antagonists was also effective, and up to 75% protection of watermelon was obtained with 10µ/g soil combined with each of the antagonists (against F. avenaceum), or with either *B. subtilis* or *T. harzianum* (against *F. oxysporum*).

Zhuang-Jing Hua *et al*.[34] found that, treatments with conidiospores and chlamydospores of *T. viride* T23 on cucumber seedlings reduced the disease index of *Fusarium* wilt from 33.69 to 13.12 and 10.28, respectively. Bonjar *et al*.[3] found that in greenhouse cucurbits of Kerman Province, Iran, *Fusarium oxysporum f.*sp. *melonis* causes root rot and *Fusarium* wilt. To investigate for new biofungicides, antagonistic activity of soil Actinomycetes isolates were assayed against the pathogen from which *Streptomyces olivaceus* strain 115 showed anti-fusarium activity both *in vitro* and *in vivo* experiments. It is clear that usage of *S. olivaceus* strain 115 as a biofungistatic natural product applied as an amendment in greenhouse soil mix will lead to inhibition or reduction of the pathogen effects. The antifungal activity of the crude axenic filtrate of an endophytic *B. subtilis* strain (BS211) isolated from watermelon and grown in 5 types of liquid media was evaluated against plant pathogens. The antagonistic activity and heat stability of the antagonistic substance obtained from the BS211 filtrate treated with (NH4)2SO4 was evaluated. The inhibitory effect of BS211 on Fusarium wilt [*Fusarium oxysporum f.*sp. *niveum*] was observed in a pot experiment. BS211 exhibited a high level of stable antagonistic effect on 12 plant pathogens. The antagonistic activities of the (NH4)2SO4 sediment and (NH4)2SO4 supernatant from the BS211 axenic filtrate markedly varied, suggesting that the antagonistic substance produced by BS211 was a compound. The antagonistic activity of the BS211 extract treated with (NH4)2SO4 against *Ralstonia solanacearum* in tomato remained stable even during exposure to 121 degrees C for 30 minutes[21].

Suarez-Estrella *et al*.[28] investigated that in vitro and in vivo inhibition and suppression of antagonistic bacteria and fungi from several compost classes toward *Fusarium oxysporum f.*sp. *melonis* (FOM). Microbial strains (493) were isolated from compost samples at different maturation phases (245 bacteria, 73 actinomycetes and 175 fungi). Initial soil dilution plate screening detected 179 isolates inhibiting FOM growth. Cell-free extracts of the 179 selected strains were prepared, concentrated and then assayed for their effects on FOM growth from which 10 fungi were antagonistic. The effect of selected fungi on *Fusarium* wilt of melon was assayed under greenhouse conditions. Results showed that optimal aeration during the composting process was favourable for the isolation

of biocontrol agents. Strains with the highest biological control activity were isolated from mature compost samples and were mostly identified as *Aspergillus* spp. These were consequently considered as potential biocontrol agents against FOM.

The aim of this work to investigate *in vitro* and *in vivo* suppression of *Fusarium* wilt of watermelon by biological and chemical control.

MATERIALS AND METHODS

Microorganisms: The biocontrol agents *Bacillus subtilis* (A), *Bacillus subtilis* (B), *Pseudomonas fluorescence, Pseudomonas putida, Pseudomonas cepacia* were obtained from Water and Environment Research Institute, Agricultural Research Centre (ARC).

Isolation, Purification and Identification of the Causal Pathogen: The fungus isolated from roots of wilted and rotted watermelon cucumber, squash and melon plants which showed typical symptoms of wilt were mostly identified as *Fusarium oxysporum var. niveum* were collected from different locations, *i.e.* Giza (El-Ayyat), Ismailia (El-Salhyia) and Beheira (Nubaryia).

Infected roots parts were cut into small fragments, washed thoroughly with tap water, then sterilized with sodium hypochlorite solution of about 1% chlorine (20 ml. Commercial Clorax in 80 ml. Water) for 1-2 minutes, rinsed several times in sterilized water, and then dried between two sterilized filter papers. Fragments were then placed on potato dextrose agar medium in Petri dishes and incubated at 27ºC for 7 days after incubation. Developed fungal colonies were purified using either hyphal-tip or single-spore technique. The isolated fungi were picked from the edges of growing colonies or germinating spores on water agar plates, using the methods suggested by Nelson *et al.*[23] and Booth[4] and the purified colonies were then transferred on PDA slants. All the obtained isolates were microscopically identified according to the morphological features using the description of Waterhouse[31]. Identification of the selected isolates was confirmed at the fungal Taxonomy Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. The cultures of identified purified fungi were kept at low temperatures (5-10ºC) for further studies.

Laboratory Experiments:

Effect of Antagonistic Microorganisms on Mycelial Growth: All bioagents candidates were tested for their efficacy against the *F. oxysporum var. niveum* isolate *in vitro*. *F. oxysporum var. niveum* isolate were grown on Cazapek's agar media for 10 days at 28ºC. Inocula a disk (5mm in diameter) of the pathogenic fungus was inoculated at one side, whereas the opposite side was inoculated by streak for antagonistic bacteria 6 cm apart (the antagonistic microorganisms were inoculated before and or after 24 hours from inoculation of the pathogenic fungus). Plates only inoculated by *F. oxysporum* var. *niveum* isolate were kept as control. Five Cazapek's plates were used for each treatment and incubated at 28ºC. When mycelial growth covers the entire medium surface in control treatment, plates were then examined and linear growth was determined.

The inhibition percent in mycelial growth of *Fusarium oxysporum* var. *niveum*

was calculated using the formula as follow:

$$I = \frac{C - T}{C} X\,100$$

Where:

I = Percent of inhibition of fungal growth.
C = Fungal growth of control.
T = Fungal growth of treatment.

***In vitro* Effect of the Fungicide on Mycelial Fungal Growth of *Fusarium* Spp.:** Topsin M-70 was evaluated in laboratory following the method described by Horsfall[15], where 7 different concentrations of the fungicide tested were prepared (5, 10, 15, 20, 25, 50 and 100 ppm, according to active ingredient). Cazapek's medium was sterilized and lifted for cooling to about 45-55C and then the fungicide was added to the medium in flasks under aseptic conditions, just before pouring. Four replicates were used for each treatment as well as other dishes without treatment served as check were inoculated with 4 mm mycelial disks taken from periphery of 10 days-old culture then incubated at 27ºC for 7 days. Data were recorded after 7 days as mentioned before.

Determination of Siderofores and Hydrogen Cyanide: Bacterial strains were tested for their capabilities to produce siderophores[1] and hydrogen cyanide on Tryptone Soya Agar Medium (TSA)[8].

Greenhouse Experiments:

Pathogenicity Test: The fungal inoculum was prepared by growing-each fungus in 500 ml. sterilized bottles containing 100g Oat medium and was incubated for

one month at the 25ºC. The sandy clay soil (1:1) by weight was autoclaved at 20 Lbs/in^2 for 2 hours. The soil was packed in 15 cm formaldehyde sterilized pots. Each pot was inoculated with 2% oat cultures. Eight seeds of cucumber, squash, watermelon and melon were planted in each pot. Three replicates were made for each fungus. Three pots filled with sterile soil were used as a check. The hosts were planted 4 days after soil inoculation. Relative plant infections caused by the fungus were measured after month from cultivation as a percentage of post emergence damping-off.

Host Rang: The aim of this study is to determine the susceptibility of different plant species to infect with the wilt pathogen *Fusarium oxysporum* var. *niveum*). A number of geniuses belonging the Cucurbitiaceae (cucumber, squash, watermelon, melon, Leguminaceae (trifolium and Bean), Rosaceae (strawberry) and cruciferae (cabbage).

Pots (30 cm in diam.) containing sterilized sandy-clay soil (1: 1 w/w) each pot was inoculated with 2% oat cultures and was sown with 3 seed and/or seedling with 4 replicates of each mentioned hosts. Plants were inoculated with water only and served as control. Plants were incubated under greenhouse condition. Disease symptoms and the reaction of these hosts were recorded two weeks after inoculation.

Evaluation of Biological and Chemical Treatments for Controlling Wilt Disease: In greenhouse experiment, evaluation of biological and chemical treatments for controlling wilt disease of watermelon were carried out using seeds sown in autoclaved soil. Seeds watermelon immersed in either bioagents {*Pseudomans ceparcia* (B1); *Bacillus polymyxa* (B2); *Bacillus subtilis* (B3) and *Pseudomans fluorescens* (B4) suspension (2.5×10^8)} or Topsin M-70 suspension (2g./L) for 3 hrs, another sowing of seeds was immersed in sterilized water and used as comparison treatment. Treated and untreated watermelon seeds were plated in pots (25 cm diameter) containing sand-clay soil artificially infested with Beheira isolate, the most aggressive isolate tested of the pathogen *F. oxysporum* var. *niveum* at the rate of 5% (w:w) as described before. A set of watermelon seeds applied with biocontrol agent were sprayed (after soil coverage with plastic sheet to prevent the fungicide from reaching the soil) with Topsin-M (2g/l) as additional treatment after two weeks of the sowing date. Four pots, each containing three watermelon seeds, were used as replicates for each particular treatment. Percentage of wilted plants was calculated two, four and eight weeks after sowing.

Statistical Analysis: Obtained results were statistically analyzed, whenever needed, according to Steel and Torrie[27].

RESULTS AND DISCUSSION

Identification of the twelve purified fungal cultures isolated from watermelon cucumber, squash and melon plants samples showing symptoms of wilt diseases revealed that all isolates are belonging to the fungus *F. oxysporum*.

Pathogenicity Test: Twelve *F. oxysporum* isolates, collected from different localities, were tested for their pathogenicity on growing watermelon under greenhouse conditions.

Data presented in Table (1) and Fig. (1) indicate that all isolates were able to attack watermelon plants causing wilt symptoms. The tested isolates significantly varied in their ability to cause wilt infection under greenhouse conditions. El- Beheira isolate was the highest virulence one on watermelon plants (reached 44.2% Pre-emergence and 77.4% Post-emergence respectively). Meanwhile, Ismailia isolates was the lowest one on watermelon in this concern (38.8% and 67.2% respectively). These result are in agreement with those reported by Ozaktan and Bora[24]; Martyn[19]; Zhou and Everts[33]; Martyn[20]; Egel and Martyn[10] and Yang-XiaoHe; *et al.*[29].

Host Rang: This experiment was designed to study the host rang of *F. oxysporum* fungus under greenhouse conditions with some plants species *i.e.* cucumber, squash, watermelon, melon (Family Cucurbitaceae), (trifolium and Bean (Family Leguminaceae), strawberry (Family Rosaceae) and cabbage (Family Cruciferceae). Watermelon only found to be infected in field with *F. oxysporum*, other family plants were not observed to be infected under greenhouse conditions. Data presented in Table (2) indicate that, watermelon plants were the most susceptible host to infect with the causal fungus of the wilt disease. Other tested plant from anther families not infected during this study. There was some significant difference between the tested plants. The obtained results are in according with those

reported by Martyn[19]; Martyn[20] and Egel and Martyn[10]. They reported that, Fusarium wilt can be caused by several different strains of *Fusarium oxysporum*. Its many hosts include rice, peanut, tomato, sorghum, watermelon and summer squash. *F. oxysporum* can survive in the soil for 10 to 12 years, so any ratio in must be long-term. Watermelons, for example, are sometimes grown on a 7-year rotation. Each Fusarium wilt fungus strain is very host specific, however, making it possible to rotate tomatoes with watermelons.

Table 1: Pathogeniciy of twelve *F. oxysporum* isolates to induce wilt disease on watermelon under greenhouse conditions

F. oxysporum isolate		Watermelon wilt incidence (%) *	
Governorate	Plant	Pre-emergence stage	Post-emergence stage
Beheira	watermelon	44.2	77.4
	cucumber	8.8	10.2
	squash	4.4	5.0
	melon	12.4	9.6
Mean	17.45	25.55	
Giza	watermelon	40.2	70.0
	cucumber	8.0	14.4
	squash	4.0	11.8
	melon	11.8	11.2
Mean	16	26.85	
Ismailia	watermelon	38.8	67.2
	cucumber	7.8	11.2
	squash	4.2	6.0
	melon	10.4	6.6
Mean	15.3	22.75	
Check **	0.0	0.0	
Mean	16.25	25.05	
L.S.D at 5% for:	Isolates (I) = 1.2 Time (T) = 0.74 I x T = 2.1		

** Watermelon seeds sown in pots inoculated with water only and served as control.

Table 2: Symptoms of wilt disease caused by *F. oxysporum* on different hosts under greenhouse conditions.

Tested host		wilt incidence (%)
Family	Species	
Cucurbitaceae	*Citullus vulgaris* (Watermelon)	80.8
	Cucurbita pepo (Squash)	0.0
	Cucumis sativus (Cucumber)	0.0
	Cucumis milo Var. *aogyotlacuo* (Melon)	0.0
Leguminaceae	*trifolium*	0.0
	Vicia faba (Bean)	0.0
Rosaceae	*Fragaria ananassa* (Strawberry)	0.0
Cruciferae	*Brassica ole.* var. *capitata* (Cabbage)	0.0

* Data were recorded three weeks after seeded of tested plants with Beheare isolate of *F. oxysporum*.

Effect of Antagonistic Microorganisms on Mycelial Growth: Linear growth of *F. oxysporum* var. *niveum* was determined after 2, 5, 7 and 10 days of growing on Cazapek's agar media. Data persented in Table (3) and Fig. (2) indicate that all bioagents tested were significantly decreased the mycelial growth of *F. oxysporum* var. *niveum* isolate compared with the control, when the bioagents inoculated 24 h either before or after inoculation by *F. oxysporum* var. *niveum* isolate.

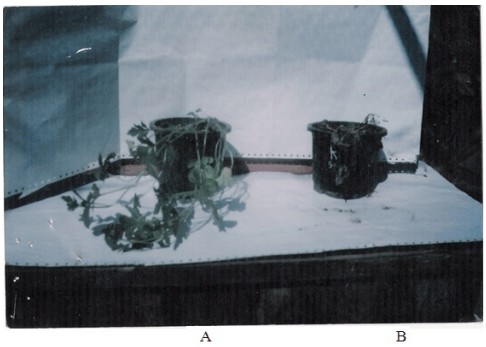

Fig. 1: Symptoms of wilt disease on watermelon plant after artificial infected with Behera isolate. A: Healthy, B: wilted.

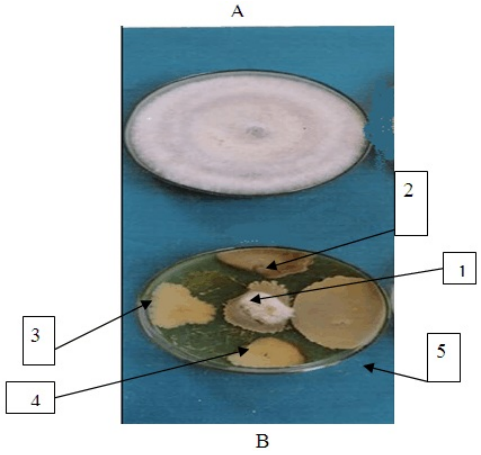

Fig. 2: Effect of antagonistic microorganisms on mycelial growth, A. Control, B. 1. *F. oxysporum* var. *Niveum* 2. *Pseudomans ceparcia* (B1), 3. *Bacillus polymyxa* (B2), 4. *Pseudomans fluorescens* (B4), 5. *Bacillus subtlus* (B3).

Bacillus subtlus (B3) and *Pseudomans fluorescens* (B4) overlapped and the best inhibited the growth of the isolate while *Pseudomans ceparcia* (B1) and *Bacillus polymyxa* (B2) retarted mycelial growth of the fungus at a distance by producing inhibitory zone against the *Fusarium* isolate and reduced the growth In addition, when the bioagents inoculated 24 h after inoculating the *Fusarium* isolate, *Bacillus subtilis* (B3) and *Pseudomans fluorescens* (B4) also reduced the growth of isolate.

Generally, when the bioagents inoculated 24 h after inoculation by *F. oxysporum* var. *niveum* isolate. *Bacillus subtlus Pseudomans fluorescens* were the most effective bioagents (reduction of growth 68.9% and 68.9% respectively) while *Pseudomans ceparcia* (B1) gave less effective (63.3%). The same results were obtained when the bioagents inoculated 24 h before inoculation (71.1% and 68.9% respectively). Similar results concerning the inhibitory effect of tested bioagent on different soilborn plant pathogens are reported by many investigators[16,12,34,3,21].

***In vitro* Effect of the Fungicide Topsin M-70 on Linear Growth of *F. oxysporum* var. niveum:** Effect of Topsin M-70 was tested at 7 different concentrations of 5, 10, 15, 20, 25, 50 and 100 ppm, on linear growth of the pathogens. Data in Table (4) show that linear growth of all fungi tested were reduced gradually as the fungicide concentration was increased and all concentrations significantly reduced the linear growth of the tested fungus, as compared to the check (0.0% conc.). The highest two tested concentrations (50, 100 ppm) showed almost significant complete inhibition to growth of all the tested fungi tested. On the other hand the lowest concentration showed inhibition of more than 50% of the linear growth at 20 ppm. Successfull control of the disease investigated was reported by Cohen *et al.*[6]; Singh *et al.*[26]; El-Habbaa *et al.*[11] and Zhuang-JingHua; *et al.*[34] by using fungicides (Topsin M-70).

Determination of Siderofores and Hydrogen Cyanide: All strains produced sidrophores. However, hydrogen cyanide producing capability appears to be cyanide could be detected in cultures of bacteria from the other genera. Mechanisms, other than antibiosis, have been proposed to account for the disease suppressive effect of bioagents. Of these are competition for iron and infection sites, production of secondary metabolites as antibiotics and hydrogen cyanide[5,7]. Therefore, the examined bacteria were screened for production of sidrophores and hydrogen cyanide. Hydrogen cyanide is a general biocide forming stable compounds with divalent ions and inhibiting cytochrome oxidase of many organisms[30]. Production pf HCN by *Pseudomonas* spp. was reported by Egamberdiyeva[9].

Effects of Soaking Seeds of Watermelon in Suspension of Different Biocontrol Agents: This experiment conducted under greenhouse conditions. Data presented in Table (6) show that all of the tested biocontrol agents significantly reduced the disease percentages in comparison with the control treatment.

Table 3: Effect of four bioagent on linear growth of *F. oxysporum* var. *niveum* on Czapek's agar medium in vitro.

Tested bioagent	Linear growth in cm.									
	After inoculation					Before inoculation				
	2	5	7	10	Reduction of growth (%)	2	5	7	10	Reduction of growth (%)
Pseudomans ceparcia(B1)	1.2	2.3	3.0	3.3	63.3	1.0	2.1	2.8	2.9	67.8
Bacillus polymyxa (B2)	1.2	2.6	2.8	3.2	64.4	1.1	2.4	2.6	3.0	66.7
Bacillus subtlus(B3)	1.2	2.4	2.4	2.8	68.9	1.1	2.2	2.2	2.8	68.9
Pseudomans fluorescens (B4)	1.3	2.2	2.5	2.8	68.9	1.2	2.0	2.4	2.6	71.1
Control	1.5	4.0	7.0	9.0	--	1.5	4.0	7.0	9.0	--
Mean	1.28	2.7	3.54	4.22	-	1.18	2.54	3.4	4.06	-
L.S.D. at 5%	Time (T) = 0.3, Bioagent (B) = 0.2 and T x B =0.9									

Table 4: Effect of seven concentrations of tested fungicide Topsin M-70 on linear growth of *F. oxysporum* var. *niveum* on Czapek's agar medium *in vitro*.

Topsin-M concentrations (ppm)	Linear growth in cm.									
	After inoculation					Before inoculation				
	2	5	7	10	Reduction of growth (%)	2	5	7	10	Reduction of growth (%)
5	1.4	3.8	6.3	6.4	28.9	1.4	3.8	6.0	5.8	35.5
10	1.4	3.8	6.0	6.0	33.3	1.4	3.6	5.6	5.3	41.1
15	1.3	3.6	5.6	5.8	35.5	1.3	3.5	5.9	5.0	44.4
20	1.2	3.3	3.6	3.9	56.7	1.1	3.2	3.4	3.8	57.8
25	1.2	2.6	2.8	2.8	68.9	1.2	2.4	2.6	2.5	72.2
50	1.0	2.2	2.0	2.1	76.6	1.0	2.1	1.9	1.6	82.2
100	0.8	1.6	1.7	1.8	80.0	0.8	1.4	1.4	0.9	90.0
Control	1.5	4.0	7.0	9.0	--	1.5	4.0	7.0	9.0	--
Mean	1.23	3.11	4.38	4.72		1.21	3	4.23	4.24	
L.S.D at 5%	Time (T) = 0.4, Concentration (C) = 0.2 and T x C =1.0									

Table 5: *In vitro* production of siderophores and hydrogen cyanide (HCN) by bioagents.

Bioagents	Siderophores	HCN
Bacillus subtlus(A)	0	-
Bacillus subtlus(B)	0	-
Pseudomonas fluorescence	0	0
Pseudomonas putida	0	0
Pseudomonas	0	0

Table 6: Effects of soaking seeds of watermelon in suspension of different biocontrol agents on the infection (%) of wilt disease.

Test bioagents	Wilt incidence (%) after weeks			Mean	Reduction in wilt incidence (%)
	Two	Four	Eight		
Pseudomans ceparcia (B1)	0.0	12.7	54.3	30.8	45.7
Bacillus polymyxa (B2)	0.0	10.0	50.0	20.0	64.7
Bacillus subtlus(B3)	0.0	11.7	45.0	26.7	52.9
P. fluorescens (B4)	0.0	12.0	44.0	23.0	59.4
Mixture (B1, B2, B3, B4)	0.0	8.0	36.3	14.8	73.9
B1×B2	0.0	11.0	40.3	17.1	70.0
B1×B3	0.0	10.7	47.3	19.3	66.0
B1×B4	0.0	14.3	43.0	19.1	66.3
B2×B3	0.0	14.0	43.7	19.2	66.1
B2×B4	0.0	13.0	44.8	19.3	66.0
B3×B4	0.0	14.3	47.3	20.5	64.0
Control	30.0	50.0	90.0	56.7	-
L.S.D. at 5%	Treatment (T) = 6.42, Fungi (F)= 3.22 and T x F = 7.9				

Table 7: Evaluation of certain fungicides, biocides for controlling watermelon wilt under greenhouse condition.

Treatment	Soil drench			Seed dressing		
	Dose	Wilt (%)	Reduction (%)	Dose	Wilt (%)	Reduction (%)
Rizolex-T	1.5g/Kg	46.8	48.0	1.5g/Kg	44.0	51.1
Topsin-M	1.5g/Kg	42.6	52.7	1.5g/Kg	40.4	55.1
Rhizo-N	4.0g/l	56.5	37.2	4.0g/l	55.5	38.3
Plant Guard	2.5ml/l	60.0	33.3	2.5ml/l	58.8	34.7
Humix	40ml/l	61.6	31.5	40ml/l	60.2	33.1
Control	90.0	--		90.0	--	
L.S.D at 5%	(F) fungicide = 1.2, (A) application = 0.975, (FxA) = 1.45					

Also, obtained data indicate that the disease percentage was highly increased by increasing the time after inoculation with pathogen.

Meanwhile, mixtures of tested antagonistic microorganisms caused more reduction in the disease incidence than that the individual bioagent. Results also indicate that mixtures (B1, B2, B3 and B4) was the most effective treatment in reducing the disease incidence 73.9%, followed by mixtures of (B1+ B2); (B1+B4) and (B2+ B3) which reduced the disease incidence down to 70.0, 66.3 and 66.1% respectively, followed by (B1+B3); (B2+ B4) and (B3+ B4) which reduced the disease incidence to 66.0, 66.0 and 64.0%, respectively.

Evaluation of Certain Fungicides, Biocides for Controlling Watermelon Wilt under Greenhouse Condition: The efficacy of certain fungicides, biocides as seed dressing or soil drench on watermelon wilt was evaluated in pots experiment using soil artificially infested with the disease agents under greenhouse condition

Data in Table (7) reveal that, all the tested treatments have significantly reduced the percentage of watermelon wilt incidence at either applications seed dressing or soil drench.

Soil treatments showed higher significant reduction on disease incidence than the seed treatment. Topsin-M as seed dressing or soil drench showed superior effect on disease incidence followed by Rizolex-T and Rizo-N respectively. Disease incidence (%) for these treatments, recorded 42.6 & 40.4%; 46.8 &44.0%; and 56.5 &55.5% respectively. Meanwhile, Plant Guard and Humix gave the lowest control potential (disease

incidence was between 60.0 & 58.8% and 61.6 & 60.2% respectively).

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