EVALUATION EFFICIENCY OF SOME BIOLOGICAL AGENTS AND CHEMICAL FUNGICIDES AGAINST *Fusarium oxysporum* f.sp. *cucumerinum* IN CUCUMBER PLANTS

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ABSTRACT

The study was conducted to evaluate of the efficiency of some biological agents and chemical fungicides to reduce disease incidence and disease severity of Fusarium wilt disease in cucumber, which caused by *Fusarium oxysporum* f.sp. *cucumerinum* under plastic house conditions. Results showed superiority treatments of biological agents *Trichoderma viride*, *Trichoderma harzianum*, bio-preparation Biohealth and bacterial preparation Fluramel (separately or in mixture). Treatments reduced disease incidence and disease severity, to **0** % , followed by Biohealth with Uniform in presence of pathogen 10 and 8.67% respectively ,and then the interaction between *T. harzianum* with Uniform in presence of pathogen 16.67 and 10.67% respectively, compared to control treatment (only pathogen) where 86.67 and 68% respectively. In addition to the results showed biological agents superiority to improve plant growth and yield. Where superior treatment of Biohealth (alone) on the other treatments in the increase plant height, wet and dry weight and yield, where amounted to 394 cm/plant, 930.7 gm/Plant, 145.3 gm/ Plant and 4.333 kg/plant respectively, followed by treatment of Fluramel (alone) was record 385 cm/plant, 926.0 gm/Plant, 138.6 gm/ Plant and 4.067 kg/plant respectively, while control treatment (only pathogen) where amounted to 231 cm/plant, 474 gm/Plant, 67.67 gm/Plant and 1.167 kg /plant respectively.

Keyword : Cucumber, *Fusarium oxysporum* f.sp. *cucumerinum*, Biological Agents, Chemical Fungicides.
***Part of Ph.D. dissertation of the first author**

1- INTRODUCTION

Cucumber (*Cucumis sativus* L.) is considered as one of the major economically summer crops, which belongs to family cucurbitaceae, the crop is widely grown in different season throughout the year, in open field or under protected plastic houses (Nayar and More , 1998). In Iraq spread crop cultivation through winter and autumn seasons under plastic houses conditions. Cucumber is consider important economical crop at many farmers in Iraq (Matloob et al. ,1989). The crop is attacked by several pathogens settlement in the soil during different growth stages causing significant losses and reduce the yield. The agent *F. oxysporum* f.sp. *cucumerinum* causal Fusarium wilt disease in cucumber (Martinez et al. ,2003; Rur, 2016). *F. oxysporum* f.sp. *cucumerinum* is considered highly specific in cucumber infection (Michail et al.,1989). Due to the importance of the spread of Fusarium wilt disease in the cucumber plants in Iraq, and cause of losses heavy, been proposed study which aimed to use some biological agents *T. viride*, *T. harzianum*, bio-preparation (Biohealth) and preparation of bacterial (Fluramel) for induced systemic resistance (ISR) in cucumber plants. Treatments reduced disease percentage and disease severity against *F. oxysporum* f.sp. *cucumerinum*, and improve growth and yield of cucumber plants under plastic house conditions.

2-MATERIALS AND METHODS

2-1: Isolation of *F. oxysporum* f.sp. *cucumerinum* from roots of cucumber

Samples of infected roots of cucumber were brought to the laboratory and but in a beaker 250 ml in continuous water to remove suspended soil of roots. Then samples were cut to small pieces 1cm length. sterilized with

sodium hypochlorite (1% free chlorine) for 3 minutes, washed in distill and sterile water for 2 minutes, dried on filter papers, sample pieces were put in petri dishes 9 cm diameter contain Potato Dextrose Agar (PDA) media, and chloramphenicol 100 mg/L to prevent bacteria growth, petri dishes incubated in temperature 25±2 C^0 .

2-2: Diagnosis isolates of *F. oxysporum* f.sp. *cucumerinum*

The isolates of *F. oxysporum* f.sp. *cucumerinum* was identified morphologically to genus and species after purification by single spore depending on shape of conidiospores (Microconidia, Macroconidia and Chlamydospores), conidiophores and forming structures using classification key Nelson et al. 1981.

2-3: Test pathogenicity for isolates *F. oxysporum* f.sp. *cucumerinum*

Seeds of cucumber were surface sterilized for cultivar Beit Alpha using 1% sodium hypochlorite for 1 minute. Then seeds washed with distill and sterile water, seeds dried on filter papers, 10 seeds planted in plastic pots contain each pot 1 Kg sterile soil (sterilization in autoclave 121 C^0 , 1.5 Kg /cm^2), sterilization for one hour repeated for three days. Fungal inoculum was added at rate 1% (10 gm for each pot). Then transferred to plastic house.

Percentage of seed germination was calculated : -

$$\% \text{ seeds germination} = \frac{\text{Number of germinated seeds in treatment}}{\text{Number of germinated seeds in control}} \times 100$$

Percentage of infection was calculated after 60 days of planting : -

$$\% \text{ infection} = \frac{\text{Number of infected plant}}{\text{Number of examined plants}} \times 100$$

Disease severity on root was calculated after 60 days of planting , according to the disease index :-

0= Healthy plant **1**= rot of secondary root **2**=Rot of secondary and part of the main root. **3**=Rot of main root (no rot of stem base) **4**= Rot of main root and stem base

5= Plant death.

Disease severity was calculated according to Mickenny, 1925.

$$\% \text{ Disease severity} = \frac{\text{(number of plants in grade 0×0) + (number of plants in grade 1× 1) +…… in grade 5×5)}}{\text{number of examined plants} \times 5} \times 100$$

Then repeated isolation of *F. oxysporum* f.sp. *cucumerinum* from infected as in (2-1), and choose the most aggressive isolate (Soliman et al. ,1988).

2-4: The biocontrol agents

T. viride and *T. harzianum* were obtained from The Biological laboratory of The General Board of Plant Protection / Ministry of Agriculture.

2-5: Antagonistic of *T. viride* and *T. harzianum* against *F. oxysporum* f.sp. *cucumerinum* in culture media PDA

Studying the effect of one isolate of *T. viride* and one isolate of *T. harzianum* , 5 mm diameter of *F. oxysporum* f.sp. *cucumerinum* isolate Foc3 and *T. viride* and *T. harzianum* (7 days old) were transferred to PDA in petri dish of 9 cm diameter, incubated at 25± 2 C^o for 7 days. The radial growth of isolate Foc3 was recoded and the inhibition percentage was calculated by the formula I= C-T/C × 100, where I= percent growth inhibition, C = radial growth of pathogen without antagonistic agent, and

T= radial growth of pathogen with antagonistic agent. Four replicates were used for each fungal isolate.

2- 6: Effect of Fluramel on growth of *F. oxysporum* f.sp. *cucumerinum* on culture media PDA

Fluramel was provided by College of Science / Karbala university. To assess Fluramel antagonism against *F. oxysporum* f.sp. *cucumerinum*, 5 mm agar discs from (7

days old) mycelium of *F. oxysporum* f.sp. *cucumerinum* were placed in the center of plates with PDA and Fluramel growth were placed equidistant site 1 cm from plate periphery as spots around the center**,** used the dilutions from 10^{-1} to 10^{-10} . After 7 days of incubation , the percentage of growth inhibition was calculated as before. The mean of two crossed diameters of fungus colonies was calculated to estimate inhibition percentage as in the above formula (2-5). Four replications of each dilution were used.

2-7: Effect of Biohealth against *F. oxysporum* f.sp. *cucumerinum* growth in laboratory

Biohealth is consisting of humic acid, seaweed extracts, *Trichoderma* spp. and *Bacillus* spp. was tested against *F. oxysporum* f.sp. *cucumerinum* growth on PDA medium. Five days cultures of the pathogen growth on PDA medium were used 100 mL flasks, five concentrations (1, 2, 3 , 4 and 5 %) were prepared by mixing the right amount of Biohealth with PDA medium in 100 mL flasks, then poured in disposable plastic plates. Each treatment was inoculated with pathogen at the plate center and four replicates for each concentration were prepared then incubated at 25 ± 2 C^0 . The mean of two crossed diameters of fungus colonies was calculated to estimate inhibition percentage as in the above formula (2-5).

2-8: Effect of Fungicides on radial growth percentage of *F. oxysporum* f.sp. *cucumerinum*

Activity of Fanate, Milor, Trymax, Fanate and Uniform against the pathogenic fungus was tested using six concentration 1, 0.50, 0.25, 0.15, 0.01 and 0.001 ml /L.

Each Fungicides homogenized with the PDA medium and poured in to 9 cm disposable petri dish. The center of each Fungicides treatment was inoculated with a piece of mycelium sliced from the edge of (7 days) colonies. Four replicates were made for each concentration and control (no added Fungicide) then incubate at 25 ± 2 C^o inhibition percentage as in the above formula (2-5).

2-9: Efficiency of some biological agents and chemical fungicides against *F. oxysporum* f.sp. *cucumerinum*

Cucumber seeds cultivar Beit Alpha were planted, after preparation soil in plastic house, two seeds planted each hole and average 20 hole each treatment. *T. harzianum* and *T. viride* were grown on Millet seeds and added before 5 days from culture of seeds cucumber and mixed well with soil culture, bacterial preparation (Fluramel) also was apply as well on average of 20ml/ hole with concentration of 34×10^7 during planting (Al-Esawy, 2010) . While pathogen added a crack with depth of 10-15 cm beneath the plant. After its was grown on Millet seeds as well, on average flask with volume 250 ml which contain on 75 gm from pathogen (Dewan,1989 ; Fayadh,1997). Furthermore the chemical fungicides (Uniform , Milor and Fanate) were applied at rate 1 ml/L after One day of the addition pathogen on average 20ml/hole (Al-Guboory, 2002 ; Hasson , 2005).While bio- preparation Biohealth used after planting in rate 2.5 gm/L

(Recommendations of the manufacture), used each 10ml / hole. Added half the amount of Fluramel, Biohealth, *T. harzianum* and *T. viride* in treatments of added with chemical fungicide , used the method of drip irrigation in the watering the plants. At the end of experiment calculated disease percentage and disease severity as in the above formula (2-3), and calculated height of plant, wet and dry weight and yield at the end of experience.

3-RESULTS AND DISCUSSION

3-1: Isolation of pathogenic fungus

Results of diagnosis Table (1) showed that the pathogen *F. oxysporum* f.sp. *cucumerinum* was present in all samples of infected plants of cucumber with sequence 10-60 %.

Table 1. percentage of presence and sequence of isolates *F. oxysporum* f.sp. *cucumerinum* isolated from cucumber roots

No. of Sample	Sample Symbol	% Sequence *F. oxysporum* in Sample	No. of Sample	Sample Symbol	% Sequence *F. oxysporum* in Sample
1	Qi 1	55	13	Sh 1	15
2	Qi 2	50	14	Sh 2	10
3	Qi 3	60	15	Sh 3	25
4	Ma 1	35	16	Ta 1	25
5	Ma 2	30	17	Ta 2	30
6	Ma 3	35	18	Ta 3	15
7	Ha 1	60	19	Ab 1	20
8	Ha 2	40	20	Ab 2	30
9	Ha 3	55	21	Ab 3	25
10	Ka 1	25	22	Mu 1	20
11	Ka 2	20	23	Mu 2	10
12	Ka 3	35	24	Mu 3	25
Highest percentage to sequence *F. oxysporum* in Sample			60 %		
Average sequence *F. oxysporum* in Sample			31.25 %		

*Average of **Four** replications, **Five** plant pieces for each petri dish

Qi = Qifel **Ma**=Madhatiya **Ha**= Hashimiya **Ka**= Kasem

Sh = Shomaly **Ta**= Taleaa **Ab**=Abi- Ghaeaq **Mu**=Muhawel

3-2: Diagnosis isolates of *F. oxysporum* f.sp. *cucumerinum* .

Morphological diagnosis showed that there was difference in color of isolates on culture media PDA. Also diagnosis depend on an shape of conidiospores (Microconidia , Macroconidia and Chlamydospores) and conidiophores .

3-3 : Test pathogenicity

Results indicated that there was difference in pathogenicity among isolates of *F. oxysporum* f.sp. *cucumerinum* in seeds germination ,disease incidence and disease severity. Percentage of seeds germination was 100% in isolate Foc22 followed by the isolates Foc18, Foc16 and Foc14 seed germination were 96.67, 93.33 and 90% respectively. while Foc3 reduced seeds germination to 50% , also Foc3 caused highest infection 93.33%, followed by isolate Foc20 reduced seeds germination to 90%.

Regarding disease severity the highest was 74.67% for Foc3 and the lowest was 3.33% for isolate Foc22. In control treatments 100% seed germination and no disease percentage or disease severity (Table 2).

Table 2. Seed germination, disease incidence and disease severity of isolates of *F. oxysporum* f.sp. *cucumerinum*

No. item	Isolates	% Seed Germination	% Infection	% Disease Severity
1	Foc 1	70.00	80.00	38.00
2	Foc 2	66.67	80.00	52.00
3	Foc 3	50.00	93.33	74.67
4	Foc 4	66.67	73.33	54.00
5	Foc 5	63.33	76.67	51.33
6	Foc 6	76.67	66.67	35.33
7	Foc 7	60.00	66.67	36.67
8	Foc 8	73.33	63.33	48.00
9	Foc 9	83.33	56.67	28.67
10	Foc 10	86.67	46.67	20.00
11	Foc 11	56.67	66.67	52.67
12	Foc 12	53.33	80.00	56.00
13	Foc 13	83.33	53.33	23.33
14	Foc 14	90.00	26.67	14.67
15	Foc 15	53.33	76.67	52.67
16	Foc 16	93.33	23.33	12.67
17	Foc 17	63.33	73.33	44.00
18	Foc 18	96.67	20.00	6.67
19	Foc 19	76.67	73.33	36.67
20	Foc 20	56.67	90.00	55.33
21	Foc 21	63.33	80.00	50.00
22	Foc 22	100	6.67	3.33
23	Foc 23	70.00	76.67	42.67
24	Foc 24	73.33	76.67	43.33
Control		100	0.00	0.00
L.S.D 0.05		12.27	12.70	7.77

*Each number average of **three** replicates

3-4: Studying the effect of antagonistic *T. viride* and *T. harzianum* against *F. oxysporum* f.sp. *cucumerinum* on culture media PDA

Study of antagonism showed good activity of *T. harzianum* and *T. viride* against *F. oxysporum* f.sp. *cucumerinum* on culture media PDA and antagonism degree was **1** which means highly antagonistic relationship (Figure 1and 2). Could be attributed to the mechanisms used by *Trichoderma* spp. including mycoparasitism, produce of antibiosis and competition on nutrients and space, as well as disable the effectiveness of pathogen enzymes (Verma et al. ,2007).

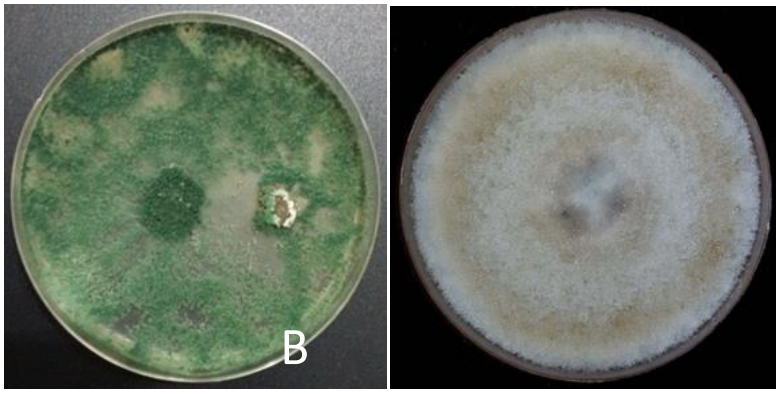

Figure 1. Effect of *T.harzianum* to inhibit growth of *F. oxysporum* f.sp. *cucumerinum* on culture media PDA.

A- Treatment of pathogenic + *T.harzianum* B- Treatment of pathogenic

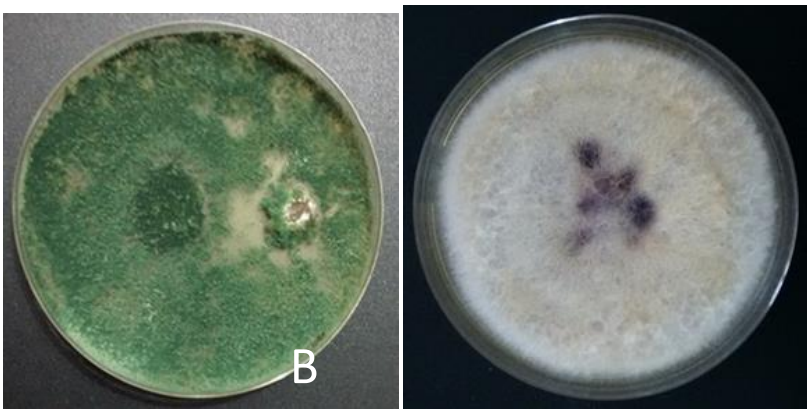

Figure 2. Effect of fungus *T.viride* in inhibit growth of *F. oxysporum* f.sp. *cucumerinum* on culture media PDA.

A- Treatment of pathogenic + *T.viride* B- Treatment of pathogenic

3- 5: Effect of Fluramel on growth of *F. oxysporum* f.sp. *cucumerinum* on culture media PDA

Results in Table (3) showed the ability of bacterial preparation (Fluramel) component of *P. fluorescence* and *B. subtilis* to inhibit the pathogen *F. oxysporum* f.sp. *cucumerinum* on culture media PDA. Dilutions 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} superior on others dilutions to reduce growth colony of pathogen, where amounted to 0cm , and the percentage inhibition was 100% for all dilutions. This can be attributed to the inhibition ability to bacteria *P. fluorescence* and *B. subtilis* its ability to competition with pathogen on nutrient, minerals, and prevent conidiospores germination as well as inhibit growth mycelium of pathogen, its effect on the metabolism process such as protein synthesis, and produce many enzymes that damage cell wall of fungi such as Chitinase and B- 1,3 glucanase, in addition to produce toxic compounds to fungi such as Hydrogen Cyanide, and their ability on produce volatile and non-volatile compounds as secondary metabolism which inhibit for the growth of mycelium (Karimi et al. , 2007).

Table 3. Effect of dilutions (active of the highest dilution) from Fluramel in inhibition growth of *F. oxysporum* f.sp. *cucumerinum* on culture media PDA

No. Item	Dilution	Mean of Colony Growth(cm.)	% Inhibition
1	**10^{-1}**	**0.0**	**100**
2	**10^{-2}**	**0.0**	**100**
3	**10^{-3}**	**0.0**	**100**
4	**10^{-4}**	**0.0**	**100**
5	**10^{-5}**	**0.0**	**100**
6	**10^{-6}**	**2.37**	**73.60**
7	**10^{-7}**	**2.62**	**70.83**
8	**10^{-8}**	**3.87**	**56.94**
9	**10^{-9}**	**4.50**	**49.99**
10	**10^{-10}**	**6.25**	**30.55**
11	**control**	**9.00**	**00.00**
L.S.D 0.05		**0.42**	**4.66**

*****Each number average of **Four** replicates

3-6: Effect of Biohealth against *F. oxysporum* f.sp. *cucumerinum* in laboratory

Results showed the ability of inhibitor of bio-preparation (Biohealth) in inhibition mycelium growth of *F. oxysporum* f.sp. *cucumerinum* on culture media PDA, showed rate of 5% was superior on other rates in reducing growth of pathogen completely, and the percentage of inhibition 100%, followed by the concentration 4.5and 4% where growth reached 1.62 and 1.87 cm respectively, inhibition percentage was 81.94 and 79.16% respectively (Table 4). Could be attributed to the role of Biohealth to *Bacillus* spp. , *Trichoderma* spp. , Seaweed and Humic acid in inhibit of *F. oxysporum* f.sp. *cucumerinum*. Where has *Trichoderma* spp. The ability of mycoparasitism, competition on nutrients and space, produce of enzymes that damage the cell wall of pathogen such as Cellulases, Hemicellulases, B- 1,3- glucanase as well as produce antibiotics and secondary metabolites (Intana and Chamswarng , 2007). The importance of bacteria *Bacillus* spp. in inhibition growth of pathogen may be it has different mechanisms in inhibition as parasitism and its produce antibiotics such as Subtiline, Surfactin, Bacitracin, Bacillin and Bacillomycin, and the enzymes that destructs the cell wall of fungi such as B-1,3- glucanase and endochitinase, as well as of its reproduction and its competing on nutrient and apace within the Petri dishes (Montealegre et al. ,2003 ; Noubuhiro et al. , 2005). While the role of the Seaweed and Humic acid in inhibition growth mycelium in the dishes comes as a results their containment on natural phenols that are bacterial and fungal antibiotics (Nardi et al. , 2002 ; Khan et al. , 2009) .

3-7: Effect of Fungicides radial growth of *F. oxysporum* f.sp. *cucumerinum*

Results in Table (5) showed good activity of chemical fungicides in inhibition growth of pathogen. Although chemical fungicide Uniform was superior in effect on isolate of *F. oxysporum* f.sp. *cucumerinum*, Uniform fungicide at rate 1 and 0.5 ml/L inhibited growth of *F. oxysporum* f.sp. *cucumerinum* completely, while control treatment (without fungicide)the growth colony diameter 9 cm and inhibition percentage 0% , followed by chemical fungicide Milor at rate 1 and 0.5 ml/L growth of colony 0 and 0.5 cm respectively and inhibition percentage 100 and 94.36% respectively .While treatment of chemical fungicide Fanate growth of pathogen was 0 and 0.8cm respectively.

Results could be attributed to the efficiency of chemical fungicide Uniform in inhibition of growth colony of pathogen impact of systemic fungicides widely against many fungi that live in the soil, including the genus *Fusarium* spp. that cause the Fusarium wilt on many of plants, and it contains active substance as Azoxystrobin and Metalaxyl which inhibit the cellular respiration process in mitochondria also inhibit of protein synthesis process (Al- Adil, 2006).

Table 4 . Effect of different concentrations from Biohealth to inhibit growth of *F. oxysporum* f.sp. *cucumerinum* on culture media PDA

No. Item	% Concentration	Mean of Colony Growth(cm.)	% Inhibition
1	**0.5**	**6.25**	**30.55**
2	**1.0**	**6.12**	**31.94**
3	**1.5**	**5.62**	**37.49**
4	**2.0**	**4.62**	**48.61**
5	**2.5**	**3.37**	**62.49**
6	**3.0**	**3.12**	**65.27**
7	**3.5**	**2.62**	**70.83**
8	**4.0**	**1.87**	**79.16**
9	**4.5**	**1.62**	**81.94**
10	**5.0**	**0.00**	**100.0**
11	**control**	**9.00**	**00.00**
L.S.D 0.05		**0.60**	**6.67**

*****Each number average of **Four** replicates

3-8: Efficiency of some biological agents and chemical fungicides against *F. oxysporum* f.sp. *cucumerinum*

Results in Table (6) showed that all treatments reduced disease incidence and disease severity. Treatments of *T.viride*, *T. harzianum*, Biohealth and Fluramel inhibited growth of pathogen completely, while interaction between Biohealth with fungicide Uniform in the presence of pathogen less rate of infection and disease severity reached 10 and 8.67% respectively, with high significant differences compared to treatment of pathogenic fungus only, were 86.68 and 68 % respectively. This can be explained to the role of biological agents in rate of infection and disease severity to effect *T.viride* and *T. harzianum* through mycoparasitism, competition on nutrients and space and product antibiosis and many enzymes which damage cell wall of pathogen such as cellulases, hemicellulases, B-1,3 glucanase and chitinase in addition to produce defense enzymes such as peroxidase and polyphenol-oxidase as well as role of *T.viride* and *T. harzianum* to induce systemic resistance in plant (Sahi and Khalid, 2007, Dubey et al. 2011, Mahdy et al. 2011). Referred to the importance of Fluramel in reducing disease percentage and disease severity because of its content bacteria *P. fluorescens* which has the ability of antagonistic, formation siderophores of iron, formation the toxic compounds such as hydrogen cyanide (HCN) , produce of many antibiotics, produce pathogenesis- related proteins (PR proteins) and produce defence enzymes in plant such as chitinase, peroxidase (PO), polyphenoloxiase (PPO), superoxide dismutase (SOD) and phenyalanine amonialyase (PAL) that effective in infected plant tissue as well as its role in an increase the process of lignification in the plant cell walls (Al-Whaibi , 2006 ; Ardebili et al. ,2011). Also contains Fluramel and Biohealth in bacteria *B. subtilis* that surround region the rhizosphere therefore its provides protection of plant against pathogen through the produce chitinase, B-1,3 glucanase ,siderophores, Indol

-3 acetic acid, HCN which are inhibits growth of pathogen as well as their role in increase of activity defense enzymes in plant such as peroxidase, polyphenol oxidase and phenylalanine ammonia - lyase (Chen et al. , 2010 ; Cao et al. , 2011)

Table 5 . Effect of chemical fungicides on growth of *F. oxysporum* f.sp. *cucumerinum* on culture media PDA

No. Item	Name of Fungicide	Concentration (mL/ L)	Mean of colony Growth (cm)	% Inhibition
1-	Uniform	1.00	0.0	100.00
		0.50	0.0	100.00
		0.25	0.8	91.66
		0.15	1.4	84.72
		0.01	2.1	77.76
		0.001	4.5	48.69
		control	9.0	00.00
2-	Milor	1.00	0.0	100.0
		0.50	0.5	94.36
		0.25	1.1	87.33
		0.15	2.2	74.67
		0.01	3.1	64.78
		0.001	6.5	26.63
		control	8.8	00.00
3-	Fanate	1.00	0.0	100.00
		0.50	0.8	91.41
		0.25	1.2	85.70
		0.15	2.2	74.26
		0.01	3.5	59.96
		0.001	6.8	22.71
		control	8.7	00.00
4-	Swift	1.00	0.0	100.00
		0.50	0.9	90.11
		0.25	1.8	80.30
		0.15	2.9	67.64
		0.01	3.8	56.45
		0.001	7.5	15.52
		control	8.8	00.00
5-	Trymax	1.00	0.0	100.0
		0.50	1.1	87.33
		0.25	1.9	78.84
		0.15	3.2	63.39
		0.01	3.9	54.41
		0.001	8.0	100.0
		control	8.8	00.00
L.S.D to Fungicide			0.15	1.721
L.S.D to Concentration			0.18	2.036
L.S.D to Interference			0.40	4.553

Results Showed in Table (6) role of biological agents and fungicides chemical in improve growth and yield compared to treatment of pathogen. Treatment of Biohealth superior on other treatments for height , wet and dry weight and yield of plant, were 394 cm/plant, 930.7 gm /plant, 145.3 gm /plant and 4.333 Kg/plant respectively. Followed by of Fluramel 385 cm/plant, 926 gm /plant, 138.6 gm /plant and 4.067 Kg/plant respectively and then treatment of *T.harzianum* which was recorded 383 cm/plant , 916.7 gm /plant, 135 gm /plant and 3.833 Kg/plant respectively and treatment of *T.viride* where amounted to 375 cm/plant, 911.3 gm /plant, 134.6 gm /plant and 3.767 Kg/plant respectively, while in treatment of pathogen were 231 cm/plant, 474 gm /plant, 67.67 gm /plant and 1.167 Kg/plant respectively.

Table 6. Efficiency some biological agents and chemical fungicides Against *F. oxysporum* f.sp. *cucumerinum*

No. item	Treatments	% Infection	% Disease Severity	plant height (cm)	wet weight (gm)	dry weight (gm)	Yield (plant) (kg)
1	Tv	00.00	00.00	375	911.3	134.6	3.767
2	Th	00.00	00.00	383	916.7	135.0	3.733
3	Bio	00.00	00.00	394	930.7	145.3	4.333
4	Flu	00.00	00.00	385	926.0	138.6	4.067
5	Tv+Fu	46.67	23.33	314	737.3	113.6	3.067
6	Th+Fu	33.33	20.67	324	756.0	116.3	3.167
7	Bio+Fu	23.33	16.67	347	814.7	119.0	3.300
8	Flu+Fu	40.00	20.00	330	719.7	103.6	3.167
9	Uni+Fu	20.00	13.33	315	733.7	108.6	3.133
10	Mil+ Fu	40.00	20.00	312	727.0	103.6	3.100
11	Fan+ Fu	43.33	23.33	315	725.3	102.6	3.067
12	Uni+Fu+Tv	33.33	18.00	333	854.7	126.0	3.367
13	Mil+ Fu+Tv	46.67	24.00	329	834.0	122.6	3.233
14	Fan+ Fu+Tv	46.67	24.67	332	837.7	124.6	3.133
15	Uni+Fu+Th	16.67	10.67	354	858.0	129.3	3.633
16	Mil+ Fu+Th	26.67	16.67	352	845.7	124.3	3.467
17	Fan+ Fu+Th	30.00	18.00	344	820.0	121.3	3.400
18	Uni+Fu+Bio	10.00	8.67	373	873.0	131.3	3.767
19	Mil+ Fu+Bio	20.00	12.67	368	862.7	130.6	3.733
20	Fan+ Fu+Bio	30.00	16.67	364	764.3	118.0	3.667
21	Uni+Fu+Flu	26.67	15.33	362	825.7	123.6	3.700
22	Mil+ Fu+Flu	43.33	22.67	336	816.3	120.3	3.633
23	Fan+ Fu+Flu	26.67	13.33	359	824.0	122.6	3.667
24	Fu	86.67	68.00	231	474.0	67.67	1.167
25	Control	0.00	0.00	311	725.0	100.6	3.067
L.S.D 0.05		**9.58**	**4.19**	**17.32**	**14.40**	**5.74**	**0.149**

*****Each number average of **three** replicates

Tv = *Trichoderma viride* **Th** = *Trichoderma harzianum* **Bio** =Biohealth **Flu** =Fluramel
Fu= *Fusarium oxysporum* **Uni** =Uniform **Mil** = Milor **Fan**= Fanate

The biological agents *T.harzianum* and *T.viride* in increase of plant height, wet and dry weight and yield, could be attributed to its role in increase absorption of some nutrients which are necessary for plant, increase growth and yield in cucumber of plants (Saravanan and Marimuthu ,2003 ; Mathivanan et al. ,2005 ; Bernal- Vicente et al. ,2009). Could be associated with effect of Fluramel in increase plant height, wet and dry weight and yield of cucumber plant, by *P.fluorescens* that increased necessary nutrients in soil, as well as positive impact in improve growth and shape of the roots, and thus improving nutrients uptake (Ganeshan and Kumar ,2005 ; Ozaktan et al. ,2015 ; Nakkeeran et al. , 2017). While attributed the bioagent *B.subtilis* produces Auxins, Gibberellins, Cytokines similarities and stimulate the plant to increase concentration some growth regular of plant and increase number of root hairs, which increase root absorption of nutrients from soil solution, this is reflected positivity in increase average of growth in plant as well as their role in inhibition of ethylene formation, (Fayyad , 2006 ; El- Meleigi et al. 2007) .

4-REFRENCES

1. **Al- Esawy, J. M. A. F.2010.** Biological control to damping off disease on eggplant caused by fungus *Rhizoctonia solani* Kühn . M.Sc. Thesis . Dept. protection of plant . Coll. of Agriculture . Univ. of Baghdad .75 pp.
2. **Al- Adil, K. M. 2006.** Pesticides, principles and its role in agriculture and public health . Dept. protection of plant . Coll. of Agriculture . Univ. of Baghdad .75 pp.
3. **AL- Guboory, H. H.2002.** Effect of growth retardants Cultar and some of extracts plants on *Vicia faba* L. plant infection by root rot pathogens. M.Sc. Thesis . Dept. protection of plant . Coll. of Agriculture . Univ. of Baghdad .90 pp.
4. **Al-Whaibi, M.H. 2006.** Role of diazotrophic bacteria in some non - leguminous plant . J .Saudi Soc. For Agric. Sci . 5 (2) .
5. **Ardebili, Z. O. ; N. O. Ardebili and S. M. M. Hamdi .2011.** Physiological effects of *Pseudomonas fluorescens* CHA0 on tomato (*Lycopersicon esculentum* Mill.) plants and its possible impact on *Fusarium oxysporum* f. sp. *Lycopersici* . AJCS. 5(12):1631-1638 .
6. **Bernal- Vicente, A. ; M. Ros and J.A. Pascual.2009.**Increased effectiveness of the *Trichoderma harzianum* isolate T-78 against Fusarium wilt on melon plants under nursery conditions .Journal of the Science of Food and Agriculture . 89(5): 827-833.
7. **Cao, Y; Z. Zhang ; N. Ling ;Y.Yuan ; X. Zheng ;B. Shen and Q. Shen .2011.** *Bacillus subtilis* SQR can control Fusarium wilt in cucumber by colonizing plant roots. Biology and Fertility of Soil, 47 (5) : 495-506 pp.
8. **Chen, F.; M. Wang; Y. Zheng; J. luo; X. Yang and X. Wang. 2010.** Quantitative changes of plant defense enzymes and Phytohormons in biocontrol of cucumber fusarium wilt by *Bacillus subtilis* B 579. World J. Microbial Biotechol. 2(2): 121-132.
9. **Dewan, M. M. 1989 .** Identify and frequency of occurrence of fungi in root of wheat and ryegrass and their effect on take - all and host growth. Ph.D. Thesis. Univ. West Australia. 210pp.
10. **Dubey, S.C. ; R. Bhavani and B. Singh.2011**.Integration of soil application and seed treatment formulation of *Trichoderma* species for management of wet root rot of mungbean caused by *Rhizoctonia solani*. Pest Management Science .67(9): 1163-1168.
11. **El-Meleigi, M.A. ; Z.M. Hassan and G.H. Ibrahim.2007.** Biological control of common root rot of spring wheat by coating seeds with *Bacillus* or *Trichoderma* spp. JKAU: Met. Env. and Arid Land Agric. Sci. 18(1): 3-12.
12. **Fayadh, M. A.1997.** Response of different genetic structures of sunflower (*Helianthas annus*) to infection by fungus *Macrophomina phaseolina* and role of some biological methods in the control . Ph.D. Thesis . Dept. protection of plant . Coll. of Agriculture . Univ. of Baghdad .91 pp.

13. **Fayyad, N.A. 2006.** In vitro control of *Fusarium oxysporum* by soil isolates of *Bacillus* species. submitted in partial fulfillment of the requirements for the degree of master of biological sciences in microbiology. Islamic University - Gaza, Deanery of Higher Education, Faculty of Science, Biological Science. 92 pp.
14. **Ganeshan, G. and A. M. Kumar.2005.** *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. Journal of Plant Interactions. 1(3): 123-134.
15. **Hasson, I. K. 2005.** Biological and chemical control to Potato stem canker pathogen *Rhizoctonia solani* Kuhn . Ph.D. Thesis . Dept. protection of plant . Coll. of Agriculture . Univ. of Baghdad .113 pp.
16. **Intana, W. and C. Chamswarng .2007.** Control of Chinese-kale damping off caused by *Pythium aphanidermatum* by antifungal metabolites of *Trichoderma virens*. Songklanakarin. J. Sci. Technol. 29(4): 919-927.
17. **Karimi, E. ; Z. D. Rouhani ; G. Khodakaramian and M. Taghinasab.2007.** Biological control of vascular wilt disease of carnation caused by *Fusarium oxysporum* f. sp. *dianthi* by *Bacillus* and *Pseudomonas* strains isolated from rhizosphere of carnation . J. Sci. & Technol. Agric. and Nature. Resource. 11(41).
18. **Khan, W ; U. P. Rayirath ; S.Subramanian ; M.N. Jithesh ; P.Rayorath ; D.M. Hodges ; A.T. Critchley ; J.S. Craigie ; J. Norrie and B. Prithiviraj.2009.**Seaweed extracts as biostimulants of plant growth and development. J. Plant Growth Regul. 28:386-399.
19. **Mahdy, A.M.M. ; A.O. Sagitov and G.A. Ahmed.2011.**Efficacy of *Trichoderma* spp. in controlling Fusarium wilt of cucumber under protected. Annals of Agric. Sci. 49(1): 71-77.
20. **Martinez, R.; M.L. Aguilar ; M.L. Guirado ;A. Alvarez and J. Gomez .2003.** First report of Fusarium wilt of cucumber caused by *Fusarium oxysporum* in Spain., Plant Pathology. 52(3): 410 pp.
21. **Mathivanan, N. ;V.R. Prabavathy and V.R.Vijayanandraj .2005.** Application of Talc formulation of *Pseudomonas fluorescens* Migula and *Trichoderma viride* Pers. ex S.F. gray decrease the sheath blight disease and enhance the plant growth and yield in rice .Journal of Phytopathology.153(11-12): 697-701.
22. **Matloob, A. N. , E. D. S. Mohammed and K. S. Abdool.1989.** Vegetables of production .Press Univ. of Al-Mosul .part 2. 337 pp.
23. **Michail, S.H. ; H.M. Sheir and MR. Rasmy .1989.** Cross protection of watermelon and cucumber plants against wilt by prior inoculation with an irrespective forms speciales of *Fusarium oxysporum* . Acta Phytopathological Entomological Hungarica. 24(3-4):301-309.
24. **Montealagre, J. R. ; R. Reyes ; L.M. Perez ; R. Herrera ; P. silva and X. Besoalin.2003.** Selection of bioantagonistic bacteria to be used in biological control of *Rhizoctonia solani* in tomato. Electronic J. Biotechnol. 6 : 115-127.
25. **Nakkeeran, S. ; W.G.D. Fernando and Z.A. Siddiqui. 2017.**Plant growth promoting rhizobacteria formulations and its scope in commercialization for the management of pests and disease .Biocontrol and Biofertilization .257-296.
26. **Nardi, S.; D.Pizzeghello; A. Muscolo and A. Vianello. 2002.** Physiological effects of humic substances on higher plants. Soil Biology and Biochemistry. 34 : 1527-1536.
27. **Nayar, N.M. and More, T.A. 1998.** Cucurbits . Science Publishers , INC . Enfield , New Hampshire , USA . 340 PP.
28. **Nelson, P.E. ; T.A. Toussoun and R.J. Cook.1981**. *Fusarium* : Disease , Biology and Taxonomy . The Pennsylvania state University press , Univ. Prak. London , 365-390 pp.
29. **Nobuhiro, K. ; O. Takeshi ;U. Hidetoshi ; N. Ken; M. Masahumi and S. Makoto. 2005.** Biological control of damping-off of tomato seedlings and cucumber phomopsis root rot by *Bacillus*

subtilis RB14-C.Agricultural Research Quarterly. 39 : 109-114.

30. **Ozaktan, H ; B. Cakir ; A.Gul ; L. Yolageldi ; A. Akkopru ; D. Fakhraei and M. Akbaba. 2015.** Isolation and evaluation of endophytic bacteria against *Fusarium oxysporum* f.sp. *cucumerinum* infecting cucumber plants . Austin J. Plant Biol. 1 (1) .
31. **Rur, M. 2016.** Developing IPM tools for greenhouse cucumber production in Sweden a participatory action research approach . thesis doctoral., department of plant protection biology, Sweden University of Agriculture Sciences. 102 pp .
32. **Sahi, I.Y. and A.N. Khalid.2007.** In vitro biological control of *Fusarium oxysporum* causing wilt in *Capsicum annuum*. Mycopathology. 5(2): 85-88.
33. **Saravanan, T. and T. Marimuthu. 2003.** Development of integrated approach to manage the fusarial wilt of banana. Crop Protection .22(9): 1117-1123.
34. **Soliman, N.K. ; M.S. Mikhaili ; P.K. Jarb and E.M. Khalil.1988.** Response of broad bean plants infected with Rhizoctonia solani to application of growth regulators and calcium. Egypt. J. Phytopath. 20 (1): 1-11.
35. **Verma, M.; S.K. Brar ; R.Y. Surampalli and J.R. Valero .2007.** Antagonistic fungi, *Trichoderma* spp. : Panoply of biological control . Biochemical Engineering Journal. 37 : 1-20.