Effect of some biological agents and chemical fungicides to induce systemic resistance in cucumber plants against *Fusarium oxysporum* f.sp. *cucumerinum*

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Abstract:
The aim of the study was to evaluate the efficiency of some biological agents and chemical fungicides to reduce disease percentage and disease severity of Fusarium wilt disease in cucumber, which caused by *Fusarium oxysporum* f.sp. *cucumerinum* under plastic house conditions, and detection of the effectiveness of factors induction through the investigation for activity peroxidase enzyme , the amount of phenols as well as the amount of total chlorophyll in cucumber plants leaves . Results showed superiority treatments of biological agents *Trichoderma viride* , *Trichoderma harzianum* , bio-preparation Biohealth and preparation of bacterial Fluramel (alone or in mixture). Treatments reduced disease percentage and disease severity to 0 and 0 % respectively, compared to control treatment (pathogen) was 86.67 and 68% respectively. Results showed that after 7and 14 days of the addition pathogenic fungus to cucumber plants , was superior treatment of interaction between Biohealth with chemical fungicide (Uniform) in the presence of pathogen in the increase activity of peroxidase enzyme where amounted to average absorption 7.17 and 5.89 min/gfw respectively . Also the same treatment superior in recording highest phenols in leaves cucumber plants after 7and 14 days of the addition treatment pathogenic fungus to cucumber plants to 7.77 and 7.38 mg/gfw respectively, while where amounted to treatment of pathogen 2.63 and 2.48 mg/gfw respectively , and superior treatment of Biohealth on the other treatments in increase total chlorophyll after 7and 14 days of treatment with pathogenic fungus to cucumber plants to 38.3 and 36.5 Spad respectively, while in treatment of pathogenic fungus recorded 27.7 and 24.4 Spad respectively.

Keyword : Cucumber , *Fusarium oxysporum* f.sp. *cucumerinum* , Biological Agents , Induce Systemic Resistance.

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1-Introduction

Cucumber (*Cucumis sativus* L.) is considered one of the major economically crops , which belongs to family cucurbitaceae , the crop is widely grown in different seasons throughout the year, in open field or under protected plastic condition (Nayar and More , 1998). In Iraq crop cultivation through winter and autumn seasons under plastic conditions, cucumber is considered important economical crop at many farmers in Iraq(Matloob,1989). The crop is attacked by several pathogens settle in the soil during different growth stages causing significant losses and reduce the yield .The agent *Fusarium oxysporum* f.sp. *cucumerinum* causal Fusarium wilt disease in

cucumber (Martinez et al.2003;Rur,2016). *Fusarium oxysporum* f.sp. *cucumerinum* is considered of fungus highly specific in crop infection (Michail et al.,1989). Due to the importance of the spread of Fusarium wilt disease in the cucumber plants in Iraq , and causes heavy losses. Some biological agents *Trichoderma viride* , *Trichoderma harzianum* , bio-preparation (Biohealth) and bacterial preparation (Fluramel) induced systemic resistance (ISR) in cucumber plants and detect of the effectiveness of factors induction through the investigation for activity peroxidase enzyme , the amount of phenols as well as the amount of total chlorophyll in cucumber plants leaves.

2- Materials and methods

2-1: Isolation *Fusarium oxysporum* f.sp. *cucumerinum*

Samples of infected roots of cucumber were brought to the laboratory and put in a beaker 250ml in continuous water to remove suspended soil from roots . Then samples were cut to small pieces 1cm length . sterilized with sodium hypochlorite (1% free chlorine) for three minutes , washed in distill and sterile water for two minutes , dried on filter papers , sample pieces were put in petri dishes 9 cm diameter contain Potato Dextrose Agar (PDA), and chloramphenicol 100 mg/L to prevent bacteria growth , petri dishes incubated in temperature 25+2 C^0 .

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

Seeds of cucumber verity Beit Alpha were surface sterilized for using 1%sodium hypochlorite for one minute . Then seeds washed with distill and sterile water ,seeds dried on filter papers , 10 seeds planted in plastic pots contain each pot one Kg. sterile soil (sterilization in autoclave 121 C^0 , 1.5 Kg /cm2) , sterilization for one hr. for three times .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 estimated after 60 days from 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 for stem base **4**= Rot main root and stem base **5**= Plant death.

Disease severity was calculated according to Mickeny , 1925 .

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

Then repeated isolation of *Fusarium oxysporum* f.sp. *cucumerinum* from infected plants as in 2-1 (Soliman et al. 1988 , modified by Awad , 2004). And choose the most aggressive isolate .

2-3: Diagnosis isolate of *Fusarium oxysporum* f.sp. *cucumerinum*

The isolate of *Fusarium oxysporum* f.sp. *cucumerinum* was identified morphologically to genus and species after purification by single spore depending on shape of conidial spore , conidiophore and forming structures using classification key Nelson et al. 1981.

2-4: Biocontrol agents

Trichoderma viride and *Trichoderma harzianum* were obtained from the Biological laboratory of The General Board of Plant Protection- Ministry of Agriculture .

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

Studying the effect of one isolate of *T. viride* and one isolate of *T. harzianum* , plugs of 5 mm diameter taken from *Fusarium oxysporum* f.sp. *cucumerinum* isolate Foc3 and *T. viride* and *T. harzianum* (7 days old) were transferred on to PDA in petri dish of 9 cm diameter and incubated at 25$\pm$ 2 C^o for 7 days (Maurhofer et al. , 1995) . 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 *Fusarium 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* was placed in the center of plates with PDA and Fluramel growth was 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 *Fusarium oxysporum* f.sp. *cucumerinum*

Biohealth consists of humic acid , seaweed extracts , *Trichoderma* spp. and *Bacillus* spp. was tested against *Fusarium oxysporum* f.sp. *cucumerinum* growth on PDA medium . Five days cultures of the pathogen on PDA medium used 100 mL flasks , five concentrations (1, 2, 3 , 4 and 5 %) were prepared by mixing the Biohealth with PDA in 100 mL flask, then poured in disposable plastic plates . Each treatment was inoculated with pathogen at center of plate and four replicates for each concentration were prepared then incubated at 25$\pm$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) . Four replications of each concentration were used.

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

Activity of Fanate , Milor , Trymax , Fanate and Uniform were tested against the pathogenic fungus using six concentrations 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 old) colonies .Four replicates were made for each concentration and control (no added Fungicide) then incubated at 25± 2 C^o and calculated inhibition percentage as in the above formula (2-5).

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

After preparation soil in plastic house, two cucumbers seeds of cultivar (Beit Alpha) were planted , two seeds each hole and average 20 holes 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 applied as well on average of 20ml/ hole with concentration of (34×10^7 CFU) during planting (Al-Esawy, 2010) . While pathogen added as a crack at depth 10-15 cm beneath the plant . After that millet seeds were add , on average flask with volume 250 ml which contain 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 at rate 2.5 gm/L ,used each 10ml / hole . Added half the amount of Fluramel , Biohealth , *T. harzianum* and *T. viride* in treatments of mixing with chemical fungicide , used the method of drip irrigation in the watering the plants.

Samples were taken 7 and 14 days after application of pathogen inoculum to analyses of peroxidase enzyme by crush **(1** gram) of leaves of cucumber plans with (**2** ml) from sodium phosphate solution SPB (M 0.01 و PH 6.5) on 4 C^0 . Filtered through four layers - cloth (plain cloth) , then its was put in centrifuge for 6000 Cycle/minute for 20 minutes on 4 C^0 . Then the filtrate was used to asses enzyme activity and neglected sediment. Activity peroxidase enzyme was determined according to (Hammerschidt et al. ,1982) through using 100 ml of extract of enzyme with 1.5 ml of M0.05 Pyrogallo in tube of spectrophotometer device , interaction to be 100 ml of hydrogen peroxide was added at rate of 1% volume :volume and absorption was recorded at wave length 420 nanometer by spectrophotometer device each 30 seconds for tenth readings , and ratio of absorption of differentiation was recorded down to following equation :-

$$\text{Change in absorption} = \frac{\Delta A / \Delta t}{\text{Wet weight gm.}}$$

ΔA = change of device reading Δt = change of time /minute

Amount of phenol in leaves of cucumber plants was calculated 7 and 14 days after addition of pathogen inoculum , by crush 2gm from cucumber s'plant leaves with 5 ml methanol 80% concentration, with continues shaking for 15 minutes at $70C^0$, 1 ml of filtrate was mixed with 5 ml distilled and sterile water and 250µL from (Foline detector) in sterile class tube , and solution was incubate at $25C^0$ for 30 minutes , then absorption was determined by spectrophotometer device , at 725 nanometer wave length , and amount of phenol calculated on the bases of take one gram of phenol for each gram of wet plant tissue , and (Catechol) substance was used as standard material (Meena ,2008) . Amount of total chlorophyll was measured by (Spad device) on average three leaves for each plant with different heights (third , fourth and fifth leaf) near of the growing region. At the end of experiment disease percentage and disease severity was calculated as in the above formula (2-2).

3-Results and discussion:

3-1: Isolation of pathogenic fungus .

Results of diagnosis Table (1) showed that the pathogen *Fusarium 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 *Fusarium 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	Hi 1	55	13	Sh 1	15
2	Hi 2	50	14	Sh 2	10
3	Hi 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		
sequence Average *F. oxysporum* in Sample			% 31.25		

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

3-2 : Test pathogenicity

Results indicated that there was difference in pathogenicity among isolates of *Fusarium oxysporum* f.sp. *cucumerinum* in seeds germination ,disease percentage

and disease severity . Percentage of seeds germination was 100% in isolate Foc22 followed by the isolates Foc18 , Foc16 and Foc14 seed germination were 96.6 , 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: Cucumber seed germination , disease percentage and disease severity of *Fusarium 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.

3-3: Diagnosis isolates of *Fusarium 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 and conidiophores .

3-4: Effect of antagonistic *T. viride* and *T. harzianum* against *Fusarium oxysporum* f.sp. *cucumerinum* on PDA

Study of antagonism showed good activity of *Trichoderma harzianum* and *Trichoderma viride* against *F. oxysporum* f.sp. *cucumerinum* on culture media PDA and antagonism degree was **1** which means highly antagonistic relationship (Figure **1**and **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* on growth of *Fusarium oxysporum* f.sp. *cucumerinum* on culture media PDA.

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

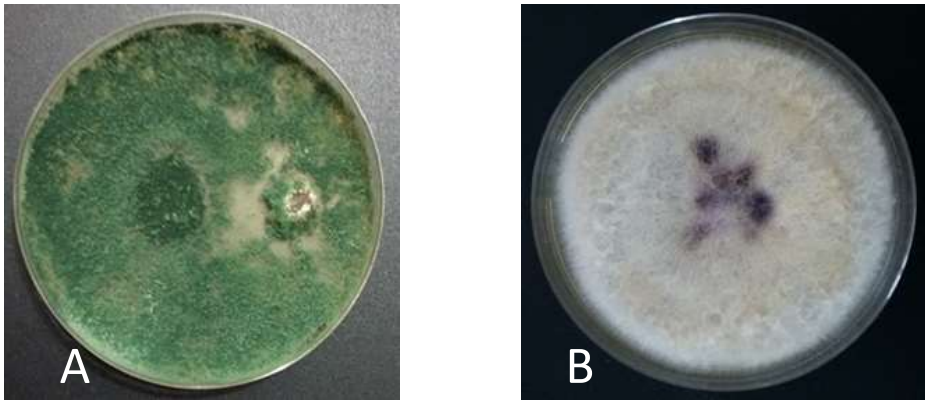

Figure 2: Effect of fungus *T.viride* on growth of *Fusarium oxysporum* f.sp. *cucumerinum* on culture media PDA.

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

3- 5: Effect of Fluramel on growth of *Fusarium 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 *Fusarium 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 , 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 competitive with pathogen on nutrient , minerals , and prevent germination of conidiospores as well as inhibit growth mycelium of pathogen , 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 Fluramel in inhibition growth of *Fusarium 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 *Fusarium oxysporum* f.sp. *cucumerinum* in laboratory

Results showed the ability of inhibitor of bio-preparation (Biohealth) in inhibited mycelium of *Fusarium oxysporum* f.sp. *cucumerinum* on culture media PDA , and 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% while growth reached 1.62 and 1.87 cm respectively , inhibition percentage was 81.94 and 79.16% respectively (Table 4). This can be explained to the role of Biohealth to *Bacillus* spp. , *Trichoderma* spp. , Seaweed and Humic acid in inhibition of *Fusarium oxysporum* f.sp. *cucumerinum* . *Trichoderma* spp. has the ability of mycoparasitism , competition on nutrients and space , produce of enzymes that damage the cell wall of fungi such as Cellulases , Hemicellulases , B- 1,3- glucanase beside 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 ,reproduction and its competing on nutrient (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) .

Table 4: Effect of Biohealth on growth of *Fusarium oxysporum* f.sp. *cucumerinum* on culture media PDA

No. Item	% Concentration	Mean of Colon 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

3-7: Effect of Fungicides on radial growth of *Fusarium 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 *Fusarium oxysporum* f.sp. *cucumerinum* , Uniform fungicide at rate 1 and 0.5 ml/L inhibited growth of *Fusarium 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 effect on growth of colony 0 and 0.5 cm respectively, and inhibition percentage was 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 5:Effect of chemical fungicides on growth of *Fusarium oxysporum* f.sp. *cucumerinum* on culture media PDA

No. Item	Name of Fungicide	Concentration(mL. L^{-1})	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**

3-8: Efficiency of some biological agents and chemical fungicides on *Fusarium oxysporum* f.sp. *cucumerinum* and induce systemic resistance in cucumber plants

Results indicated in Table (6) showed that all treatments reduced disease percentage 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 disease percentage and disease severity reached to 10 and 8.67% respectively, with high significant differences compared to treatment of pathogenic fungus only, amounted to 86.68 and 68 % respectively. This can be attributed 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 reduced disease percentage and disease severity because of its contents 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) , polyphenoloxidase (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 the rhizosphere therefore provides protection of plant against pathogen through the produce chitinase , B-1,3 glucanase ,siderophores , Indol -3 acetic acid , HCN which are inhibit 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 6: Evaluation of the efficiency some biological agents and chemical fungicides against *Fusarium oxysporum* f.sp. *cucumerinum*

No. item	Treatments	% Infection	% Disease Severity
1	Tv	00.00	00.00
2	Th	00.00	00.00
3	Bio	00.00	00.00
4	Flu	00.00	00.00
5	Tv+Fu	46.67	23.33
6	Th+Fu	33.33	20.67

7	Bio+Fu	23.33	16.67
8	Flu+Fu	40.00	20.00
9	Uni+Fu	20.00	13.33
10	Mil+ Fu	40.00	20.00
11	Fan+ Fu	43.33	23.33
12	Uni+Fu+Tv	33.33	18.00
13	Mil+ Fu+Tv	46.67	24.00
14	Fan+ Fu+Tv	46.67	24.67
15	Uni+Fu+Th	16.67	10.67
16	Mil+ Fu+Th	26.67	16.67
17	Fan+ Fu+Th	30.00	18.00
18	Uni+Fu+Bio	10.00	8.67
19	Mil+ Fu+Bio	20.00	12.67
20	Fan+ Fu+Bio	30.00	16.67
21	Uni+Fu+Flu	26.67	15.33
22	Mil+ Fu+Flu	43.33	22.67
23	Fan+ Fu+Flu	26.67	13.33
24	Fu	86.67	68.00
25	Control	0.00	0.00
L.S.D 0.05		**9.58**	**4.19**

*****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

Results in figure (**3**) explain role of biological agents increased activity of peroxidase enzyme in cucumber plats with highly significant differences compared to treatment of pathogenic fungus only , 7and 14 days after addition of pathogenic fungus to cucumber plants , treatment of interaction between Biohealth with chemical fungicide Uniform in the presence of pathogen were superior in increasing activity of peroxidase enzyme, while absorption average was 7.17 and 5.89 min/gfw respectively, followed by treatment of interaction between Biohealth with chemical fungicide Milor in the presence of pathogen, reached 5.92 and 5.71 min/gfw respectively, while treatment of pathogenic fungus only was 2.37 and 2.32 min/gfw respectively. Results were showed superior Biohealth in all treatments whether it use alone or with chemical fungicides or with pathogenic fungus , This could be attributed to Biohealth contains a *Bacillus* spp. and *Trichoderma* spp. that help to activate the defence enzymes in cucumber plants such as peroxidase , it also contains humic acid and seaweed which are considered rich source of amino acids such as Cysteine , Serine, Lysine, Glycine, Alanine ,Valine and Methionine and some vitamins such as B1, B2, B12 and C , and some minerals such as Fe, N ,Ca ,I, Mn and Zn. In addition , it contains some growth tonics such as Mannitol ,Fucoidan، Methyl pentosan and Oligo-

saccharide which were very active in induce systemic resistance in the plants (Jayaraman et al., 2011: Singh et al., 2014 : Namukose et al.,2016).

Results in figure (**4**) revealed the importance of biological agents in elevating accumulated phenols leaves cucumber plants, as readings were taken 7and 14 days after applying of pathogenic fungus to cucumber plants .However treatment of interaction between Biohealth with fungicide (Uniform) in the presence of pathogenic fungus was the highest in amount of accumulated phenols 7.77 and 7.38 mg/gfw respectively compared to all treatments with high significant differences, followed by treatment of Biohealth in the presence of pathogenic fungus which reached 7.50 and 7.32 mg/gfw respectively, while treatment of pathogenic fungus only was 2.63 and 2.48 mg/gfw respectively. Results showed significance increase when use Biohealth in accumulation phenols , this can be associated with role of *Bacillus* spp. and *Trichoderma* spp. to increase induction and accumulation of phenols in plant tissue nearby to the infection site, also it prevent develop and growth of pathogenic fungus in the plant tissue , at the same time it improves synthesis lignin in cell wall of cucumber plants . Also, the seaweed contains natural phenols and tannins which impact toxin to pathogenic fungus , also contains on Alginic acid which is consider chelates ions such as Ca, Mg, Mn, Zn and Fe ,therefore it is active in synthesis of polysaccharide which play an important role in plant defenses against pathogenic fungi, as well as it contains some composites sugar and amino acids and Betaine material which will activates the systematic resistance in plant through the synthesis the Phytoalexin (Craigie ,2011 :Zhang et al. ,2011).

Figure (**5**) showed efficiency of biological agents to increase total chlorophyll in leaves cucumber plants, that has been obtained 7and 14 days after application of pathogenic fungus to cucumber plants , Biohealth treatment showed superiority to increase in total chlorophyll , to 38.3 and 36.5 Spad respectively, followed by treatment of Fluramel which were 37.8 and 36.5 Spad respectively , then treatment of *T. harzianum* reached 36.5 and 35.3 Spad respectively , while treatment of pathogenic fungus were 27.7 and 24.4 Spad respectively, this may due to role of *Bacillus* spp. , *P.fluorescens*, *Trichoderma* spp. and *T. viride* in boosting the readiness of some nutrients which involve in the synthesis of chlorophyll pigment and prevent its degradation . Humic acid and seaweed are considered a rich source of vitamins and amino acids which may increase chlorophyll pigment of cucumber plants and increase plant ability to absorption of major elements such as Nitrogen , Phosphorus and Potassium which are important to balance growth of plant and increase total chlorophyll even in the cultivated crop in a few lighting places or few solar brightness (Khan et al. ,2009 :Zamani et al., 2013).

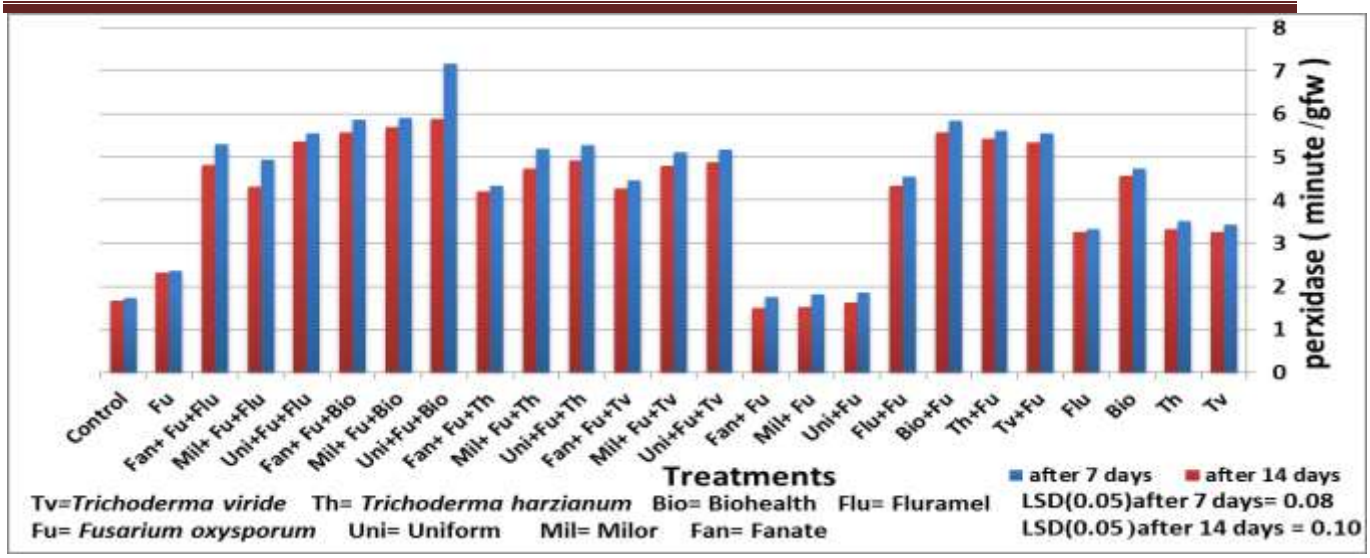

Figure 3: Effect of biological agents in increase peroxidase enzyme activity in leaves cucumber plant

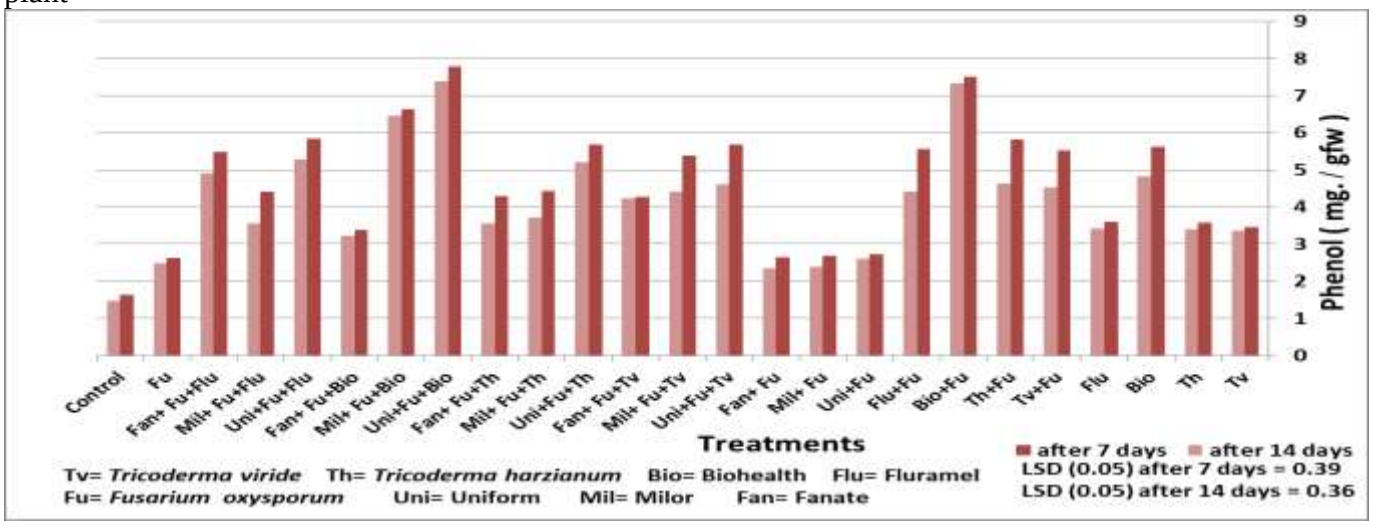

Figure 4: Effect of biological agents phenol accumulation of leaves cucumber plant

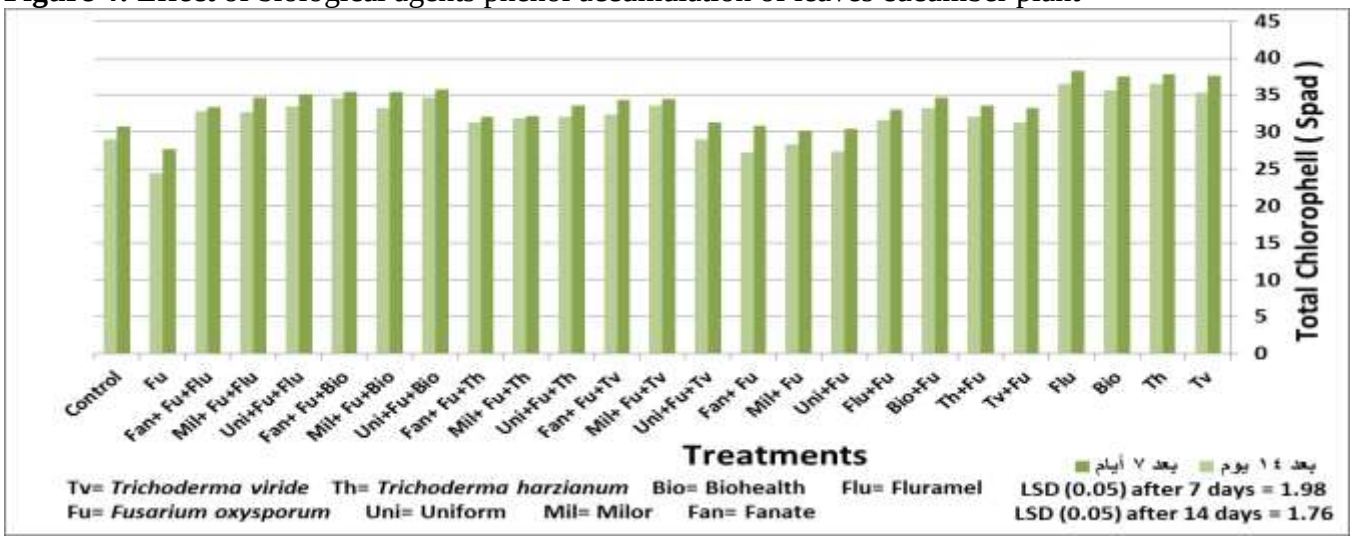

Figure 5: Effect of biological agents in total chlorophyll in leaves cucumber plant

Refrences:

1. **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.
2. **Al- Esawy, J. M. A. F.(2010)** Biological control to damping off disease on egg-plant caused by fungus *Rhizoctonia solani* Kühn . M.Sc. Thesis . 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. **Awad, H.M. (2004)** Studies on root rot diseases of pea. M.Sc. thesis, Faculty of Agriculture, Minufiya University.109 pp.
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. **Craigie, J.S.(2011)** Seaweed extract stimuli in plant science and agriculture .Journal of Applied Phycology .23(3):371-393.
10. **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.
11. **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.
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. **Hammerschidt , R.; F. Nuckles , F. and J. Kuc.(1982)**Association of enhance activity with induced systematic resistance of cucumber to *Colletotricum lagenrium*. Physiol. plant pathol.20:73-82.

14. 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.

15. 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.

16. Jayarman, J. ; J.Norrie and Z.K. Punja .(2011) Commercial extract from the brown seaweed *Ascophyllum nodosum* reduces fungal disease in greenhouse cucumber .Journal of Applied Phycology. 23(3): 353-361.

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. 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.

20. 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.

21. 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.

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. Meena, R. K. ; V. Patni, and D.K.Arora.2008. Study on phenolic and their oxidative enzyme in *Capsicum annuum* L. infected with Geminivirus. Asian J. Exp. Sci. 22:307-310.

24. 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.

25. 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.

26. Namukose , M . ; F. E. Msuya ; S.C.A. Ferse ; M.J. Slater and A. Kunzmann. (2016) Growth performance of the sea cucumber *Holothuria scabra* and the seaweed *Eucheuma denticulatum* , integrated mariculture and effects on sediment organic characteristics .Aquaculture Environment Interactions .8: 179-189.

27. 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.

28. Nayar, N.M. and More, T.A. (1998) Cucurbits . Science Publishers , INC . Enfield , New Hampshire , USA . 340 PP.

29. 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.

30. 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.

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. Singh, B.N. ; A. Singh ; B.R. Singh and H.B. Singh (2014) *Trichoderma harzianum* elicits induced resistance in sunflower challenged by *Rhizoctonia solani* . Journal of Applied Microbiology . 116(3): 654-666.

35. 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.

36. 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.

37. Zamani ,S. ; S. Khorasaninejad and B. kashefi.(2013) The importance role of seaweeds of some characters of plant. International Journal of Agriculture and Crop Sciences. 5 (16): 1789-1793.

38. Zhang, N. ; K. Wu ; X. He ; S. Li ; Z. Zhang ; B. Shen ; X. Yang ; R. Zhang ; Q. Huang and Q. Shen. (2011) A new bioorganic fertilizer can effectively control banana wilt by strong colonization with *Bacillus subtilis* N11.Plant and Soil. 344(1-2): 87-97.