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The activity of some Actinomycetes isolates in control of cucumber damping off disease caused by *Rhizoctiona solani* and *Pythium* sp.

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Abstract: A 28 Actinomycetes isolates which collected from different environmental sources in Basra province were described as Gram positive and are characterized by producing branching hyphae. Two isolates were identified by molecular analysis of 16 S r RNA gene. Molecular identification confirmed that two isolates of Actinomycetes from soil had a similarity of 99% with *Streptomyces griseus*. The sequence has been deposited at NCBI with Gen bank accession number (NBRC 14886, AB 184627. 1). While the isolates of date palm roots was analogous to *Brevibacterium celere* and the sequence of this strain deposited at NCBI with Gen bank accession number (DQ164,K414744601). The dual culture technique showed that Actinomyces isolates 44 had high antagonistic activity against *Rhizoctonia solani* as inhibition zone reached 1.7 cm, in contrast to Actinomyces $_{24}$ and *S. griseus* which revealed a high antagonistic activity against *Pythium* sp. with inhibition zone reached 1.2 cm for both isolates. Pots experiment showed all Actinomyces isolates were significantly reduced cucumber seedling damping off caused by *R. solani* and *Pythium* sp. the disease incidence for *R. solani* damping off were reduced to 1.0% in actinomycetes $_{44}$, Actinomycetes $_{24}$ and *B .celere* treatment compared to 11.37 % in control treatment. Disease incidence at *Pythium* sp. damping off was reduced to 1.0% in Actinomycetes isolates compared with that in control treatment (4.33%). According to this study there is possibility for isolating Actinomycetes isolates which isolated from different environments sources have the ability for reducing cucumber damping off disease caused by *R. solani* and *Pythium* sp.

Keywords: *Streptomyces griseus, Brevibacterium celere,* palm roots.

Introduction

Cucumber (*Cucumis sativus* L.) is one of the most important vegetable corps in Iraq and other countries (Al-Bayati *et al*., 2012). Which planted in green houses and open fields all over the country. During the growth season, cucumber plants are attacked by different diseases such as bacterial, fungal diseases, and nematodes in addition to the insect pests. Damping off of seedling caused by *Pythium* sp. which is one of the most

important disease on this crop (Al-Ani *et al*., 1989) and caused by *Rhizoctonia solani*causing root rot diseases as well for many corps including cucumbers plants (Muriungi *et al*., 2014).

Several methods have been used to control seedling damping off disease. The farmer and producers used to spry fungicides to control and decrease the disease, but the chemical control cause problems to the human health and environment (Qin *et al*., 2011). The biological control is an applicable method that involves the microorganisms having an antifungal and antibacterial activity. Some biocontrol agent can be developed into field applicable products called bio pesticide which are more effective and less poisonous and harmful to environment (Nawari, 2011). More than any other microorganisms, Actinomycetes, especially *Streptomyces* genus, which occur widely in the *Rhizosphere* and interior tissues of roots, are characterized by producing antibiotics and volatile organic compounds (VOC) that control the growth of plant pathogenic fungi and bacteria . Moreover, they also play a significant role in the supporting of the plant growth (Viaene *et al*., 2016). The goal of this study is to evaluate the efficiency of different isolates of Actinomyces in controlling the pathogenesis damping off disease.

Materials and Methods

Isolation of Actinomycetes

To obtain Actinomyces isolates, different samples were collected from various sources including soil, animal manure, peat moss, water as well as roots of some plants such as date palms, cucumbers, eggplants, and tomatoes. Soil samples were collected randomly from different agricultural zones in Basra. Samples were taken from the rhizosphere zone at (20 cm depth). 1g of CaCo3 was added per 10g of soil and the mixture was placed in the incubator at 37ºC for four days (Abdulhameed, 2013). Glycerol-Yeast extract Agar medium (GYEA), glycerol 1 ml, yeast extract 0.4 g, K_2HPO_4 0.02g, peptone 5g, agar 3g, nystatin 50mg, distilled H_2O 200ml was used to isolate Actinomycetes from different samples. The petri dishes (9 mm diameter) incubated at 28 ºC for (4-7) days. After the incubation period, part of each colony was transferred to Petri dishes containing the culture medium (GYEA). The dishes then placed in the incubator at 28 ºC (Oskay *et al*., 2004).

Water samples were collected from different sources including well water (Area: Khor Al-Zubair) mixed with R.O water, one sample was taken from Al-Dair sub-river and other samples were also collected from tap water after sterilizing the taps (Area: city centre), 1 ml of each sample was transferred to Petri dishes and then the culture medium (GYEA) was added to each sample with three replicates per each sample were performed, then incubated at 28 ºC for (4-7) days.

Actinomyces isolates were inoculated from roots of some plants by taking three pieces (1cm) each from plant roots and transferred onto GYEA, the Petri dishes were incubated at 28 ºC for four days.

Identification of Actinomycetes

The Actinomycetes isolates were identified according to morphological, biochemical and molecular characteristics:

1. Morphological identification includes gram staining, slide culture technique and biochemical tests include hydrolysis of the starch test, hydrolysis of gelatin test and catalase test.
2. Molecular Identification.

Two of the Actinomycetes isolates were identified by the molecular tool, one isolate from soil and the other from date palm roots at Molecular Genetics Lab. College of Agriculture. University of Basrah. DNA was extracted from Actinomycetes isolates using GSYNC^{TM}DNA Extraction Kit supplied by Geneaid Co. according to manufacturer's instructions. The purity and quantity of the extracted DNA were both measured by Nano drop device. The primers Strep B 5-ACAAGCCTGGAAACGGGGT-3 (forward) and Strep F 5 ACGTGTGCAGCCCAAGACA- 3 (reverse) were used to multiply 16s r RNA gene (Igbinosa *et al.*, 2017)

Isolation of *Pythium* sp.

Pythium sp. was isolated by using cucumbers fruits as plant traps. Three wounds with depth (1-2) cm were made in each cucumber fruit. The cucumbers fruit were buried in a wet soil for 3 days. Later, they were removed and washed with distilled water and eternally sterilized with NaOCl. Cucumber fruit cut to small pieces, each 2-3 pieces and transferred into Petri dishes containing PDA medium incubated at 25 ±2^{o}C. Identification of *Pythium* sp. was based on the taxonomic characteristics mentioned in (Domsch *et al*., 1980) while *Rhizoctonia solani* was obtained from Assist Prof. Dr. Diya'a S. Al Wa'ely from Department of Plant Potection. College of Agriculture, University of Basrah. The pathogenicity of both fungi on cabbage seeds was tested according to Bolcan & Butler (1974).

Preparation of *R. solani* and *Pythium* sp. inoculum

The fungal inoculum was prepared by 200g of clean and washed millet (*Ponicum miliaceum* L.) seeds in a 300ml bottle. After that, they were sterilized by the autoclave under 121^{o}C temperature and 15 pounds/ Inch^2 pressure for 15 minutes. They were re-sterilized the next day to ensure the sterilization of seeds thoroughly. The bottles were left to get cooled and later five disks (0.5cm each) were taken from edges of 4 days cultures of *R. solani* and *Pythium* sp. separately and transferred into each bottle. The bottles were incubated at 25 ± 2^{o}C for 14 days with shaking them every (2-3) day to ensure uniformity of colonization on all seeds.

Preparation of Actinomycetes inoculum

Actinomycetes isolates were grown in 100 ml nutrient broth in 250 ml conical flask, for four days at 28±2°C., dilution method was done to determine the colony forming unit in1ml. The number of colony forming unit (CFU) were calculated by the dilution plate method according to the following equation (Kamal & Sharma, 2014):

CFU= No of colonies × dilution factor.

Evaluation of the antagonistic ability of Actinomycetes against *R. solani and Pythium* sp.

The antagonistic ability of actinomycetes isolates was determined by dual culture technique. Five mm mycelium plug from four-day-old PDA culture of *R. solani* and *Pythium* sp. were placed three centimeters from the edge of actinomycetes isolates disk. The dishes were incubated for four days at 25±2°C and the antagonistic activity was calculated according to the following formula:

C = A – B

C= Zone of inhibition

A= The distance between the actinomycetes and the fungal disk

B= The distance growth of pathogenic fungus towards the disk of Actinomycetes.

The Actinomycetes isolates considered to have the weak inhibitory capability if the rate of C equal or less than 0.9 and with medium inhibitory capability if C = 1-1.9cm or with high inhibitory capability if C= 2cm or more (Aghighi *et al*., 2004).

Effect of Actinomycetes isolates on the damping-off disease of cucumber seedlings caused by *R. solani* and *Pythium* sp.

Soil containing peat moss (1:3) was sterilized by 1/50 concentration of formalin. After 4 days of sterilization, the soil put into sterilized pots. *R. solani* and *Pythium* sp. inoculum were added to the pots with 1% ratio W/W. The soils were moistened and left for two days. Six isolates of actinomycetes which were showed high antagonistic ability against *R. solani* and *Pythium* sp. in vitro experiment were tested. 100 ml of each isolate (prepared on nutrient broth) were added to each pot. Two days later 10 sterilized cucumber seeds were sown per pot. Control treatment includes soil infected with *R. solani* or *Pythium* sp. alone. After four weeks the germination percentage and germination speed were calculated.

The germination speed is calculated according to the following equation:

$$\text{The germination speed} = \frac{(A)T1+(A+B)T2+(B+C)T3+(C+D)T4+\cdots}{A+B+C+D}$$

A, B, C, D, is the number of germinating seeds

T1, T2, T3, T4, the period occurs between each numeration and the last day of germination (Hamad *et el*., 2013).

Disease incidence was calculated as follow:

$$\text{Disease incidence \%} = \frac{\text{the number of the infected plants}}{\text{the total number of plants}} \times 100$$

Disease severity was measured according to visible symptoms composed of 5 degrees shown in the below table (1).

Table (1): Guide (0-4 degrees) for disease severity of damping off disease caused by *R. solani* and *Pythium* sp. on cucumber plants.

Description	Degree
Intact roots	0
(1-3) roots with brown spots	1
(4-6) roots with brown spots	2
(7-8) roots with brown spots	3
(9-10) roots with brown spots	4

$$\text{D.S. \%} = \frac{\text{sum of the plant for each degree} \times \text{degree}}{\text{total number of plants x maxmum degree}} \times 100$$ as mentioned in Al-Wa'ely, (2004)

The dry and wet weights of the shoot and root systems, as well as the plant height, were calculated.

Statistical Analysis

All experiments were conducted according to Completely Randomized Design (C.R.D). The averages were compared Least Significant Design (L.S.D) based on possibility level 0.01 for lab experiments and 0.05 for pots experiments. The results were statistically analyzed as per Genstat Discovery Edition.

Result and Discussion

Twenty eight actinomycetes isolates were recorded from different geographical areas in Basra (Table 2) suggesting spread of actinomycetes in different environments. Results of this study were consistent with (Zhang, 2011) how stated that actinomycetes can be found in different environments such soil, water, rhizosphere and plant surface.

Table (2): Sources of Actinomycetes isolates.

Source	Area	Isolate No.
	College of Agriculture/ garden	1
	AL MDAINAH	2
Soil	AL ZUBAIR	5
	Al FAO	6
	AL SEEBA	7
	Al FAO	8
	College of Agriculture / greenhouse	10
	SAFWAN	12
	College of Agriculture / garden	13
	AL DAIR	14
	College of Agriculture / garden	15
Soil	College of Agriculture / garden	18
	College of Agriculture / garden	19
	MANAWILIGAM	20
	AL QURNA	24
	Shat AL Arab Area	35
	AL MDAINAH	36
Tap water	AL BASRAH	25
Sheep dung		30
Sheep dung	AL BASRAH	40
Cow dung		50
Peat moss		60
Date palm roots		44
Date palm roots		55
Date palm roots	AL BASRAH	66
Date palm roots		77
Eggplant roots		88
Eggplant roots		99

All the isolated actinomycetes showed waxy and leathery appearance on GYEA medium, some of them are cream colored while others have white to yellowish colored. Under light microscope they appeared to have branching hyphae (Fig., 1-2).

Biochemical test (Table 3) indicated that all the Actinomycetes isolates were hydrolysis of starch and produce catalase enzyme. These results revealed capability to produce amylase enzyme and such enzyme may have role in supporting plant growth and disease control (Mansur *et al*., 2015; Sousa *et al*., 2008). Moreover 23 Actinomycetes isolates have a positive gelatin hydrolysis. While the other 5 isolates have negative gelatin hydrolysis. Results of this study consistent with Mamatha *et al.* (2014), who stated that some actinomycetes isolates gave a positive catalase and starch hydrolysis but a negative gelatin hydrolysis.

A B C D

Fig. (1): Shapes of colonies of different Actinomycetes (A, B, C& D) on GYEA medium.

A: Actinomycetes isolate isolated from FAO soil.

B: Actinomycetes isolate isolated from QURNA soil.

C: Actinomycetes isolate isolated from date palm roots

D: Actinomycetes isolate isolated from eggplants root.

Fig. (2): Microscopic appearance of *Streptomyces griseus* by using slide culture technique.

Table (3): Enzymatic activity of Actinomycetes isolates.((+) positive result, (-) negative result.

Enzymatic activity of Actinomycetes isolates							
No. of Isolate	Gelatinase	Catalase	Amylase	No. of Isolate	Gelatinase	Catalase	Amylase
1	+	+	+	24	+	+	+
2	-	+	+	25	+	+	+
5	+	+	+	30	+	+	+
6	-	+	+	35	-	+	+
7	+	+	+	36	+	+	+
8	+	+	+	40	+	+	+
10	+	+	+	44	+	+	+
12	+	+	+	50	+	+	+
13	+	+	+	55	-	+	+
14	+	+	+	60	-	+	+
15	+	+	+	66	+	+	+
18	+	+	+	77	+	+	+
19	+	+	+	88	+	+	+
20	+	+	+	99	+	+	+

Molecular identification

Only two isolates of Actinomycetes were identified by DNA sequencing, isolate No.6 (from the soil) and isolate No.66(from the date palm roots). The molecular analysis showed that the isolate No. 6 and LKA2 for isolate No.66. have similar nucleotide sequence with *Streptomyces griseus* (99%) and isolate No.66 have similar nucleotide sequence with *Brevibacterium celere* (99%). The nucleotide sequence has been deposited at NCBI with gene bank accession number NBRC 14886 AB 184627.1 for isolate No.6 (Table 2) and number DQ164 AB 184627.1.

For isolate No.66 .from previous studies experience, it seems how it is valuable to use molecular technology to exam the genetic material in microorganisms as well as how it difficult to identify it by using morphological characteristics (Sonya *et al*., 2015; Trabelsi *et al*., 2016; Amin *et al*., 2017).

Pathogenicity test for *R. solani* and *Pythium* sp.

Pathogenicity test results conducted on cabbage seeds showed that both *R. solani* and *Pythium* sp. had high pathogenic effects on examined seeds, no germination at both pathogens was observed compared with 100% at control one.

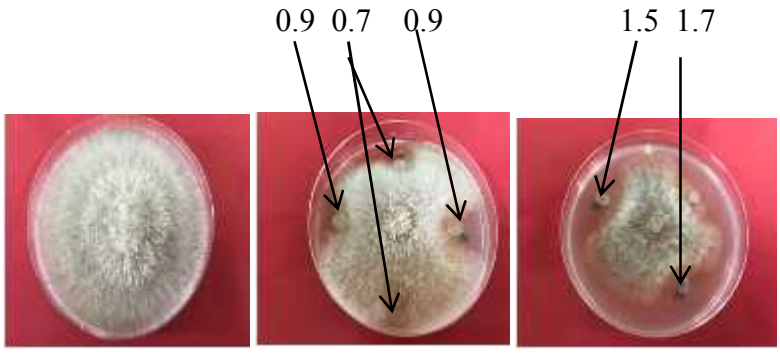
Antagonistic ability of Actinomycetes against *R. solani* and *Pythium* sp.

Dual culture technique showed that actinomycetes isolate had a high antagonist ability against *R. solani* and *Pythium* sp. (Table 4 and Figure3). The most effective isolates were Actinomycetes No. 44 and *B. celere*. The ability of Actinomycetes isolates to inhibit the growth of many pathogenic fungi has been mentioned in many previous studies (Kang & Sowndhararajan, 2012; Goudgjal *et al.*, 2014; Shrivastava *et al.*, 2017).

The antagonistic capability of actinomycetes against pathogenic fungi may be attributed to its ability to produce anti-fungal compounds and the nutrient competition by the production of Siderophores (Goudgjal *et al*., 2014) or to produce cell wall degrading enzymes such as Chitinase (Sharivastava *et al*., 2017; Ohike *et al*., 2018).

Table (4): Actinomycetes capability against *Pythium* sp. and *R. solani*. (Abbr. (-) no inhibition (+) weak inhibitory capability (++) medium inhibitory capability; Each number mentioned in the table represents an average of three replicate.

Actinomycetes	Zone of Inhibition(cm) *R. solani*	Inhibitory capability	Zone of Inhibition(cm) *Pythium* sp.	Inhibitory capability
Control	0	--	0	--
S.griseus	0.7	+	1.2	++
Actinomycetes 10	0.7	+	1.1	++
Actinomycetes 14	0.9	+	0.7	+
Actinomycetes 24	0.9	+	1.2	++
Actinomycetes 44	1.7	++	0.0	--
B. celere	1.5	++	0.9	+
L.S.D 0.01	0.44		0.67	



A **B** **C**

Fig. (3): Antagonistic capability of Actinomyces.
A = Control *R. solani*
B = Actinomycetes$_{24}$ + Actinomycetes$_{14}$ + Actinomycetes$_{10}$ + *S. grisues* + *R. solani*
C = Actinomycetes44 + *B. celer* + *R. solani*

Inhibitory effect of Actinomyces isolates against the causal agents of cucumber seedlings damping off disease (*Pythium* sp. and *R. solani*).

The results of this experiments (Table 6) showed that *Pythium* sp. and *R. solani* had no significant decrease in the germination percentage of cucumber seeds that such percentage recorded 80% in the soil infested with *R. solani*, whereas it was recorded 90% in the non-infested soil but it was recoded 86.7% in the soil infested with *Pythium* sp. and 83.3% in the non-infested soil,

The seedlings in the soil infested with *R. solani* and *Pythium* sp. were subjected to stunting and got weak, this may be attributed to the occurrence of cankers in the root hairs. Yet, the germination percentage of the treatments of adding Actinomycetes isolates is better than the treatments of pathogenic fungus that Actinomycetes $_{24}$ isolated from soil recorded the highest average of seed germination percentage which reached to 93.3%, when it was compared to the control treatment, the isolates Actinomycetes $_{24}$, Actinomycetes $_{14}$ and Actinomycetes $_{10}$ isolated from soil recorded increase in the seed germination percentage in their treatments which reached 93.3% compared with control treatment (soil infested with *Pythium* sp.)

The results showed that the seeds germination speed are varied according to the Actinomycetes isolate the highest seed germination speed was recorded in the treatment of Actinomycetes isolate-10, Actinomycetes isolate-14 and *B . celere* isolated from the roots of date palms with an average 8.4, 8.2 and 8.0 respectively compared with that in control treatment (6.1)**.** Seedlings damping off caused by *R. solani* or *Pythium* sp. is negatively related with the seedling germination speed and positively related with the pathogen growth speed. Therefore, increasing the speed of seedlings growth, could increase the opportunity for disease escape. This results agreed with some previous studies which state that Actinomycetes have their own effect on the seed germination speed and protect them against pathogens (Cao *et al*., 2004; Nawari, 2011; Janaki, 2016). Whether isolated from soil or from the inside of the plant tissues, Actinomycetes can produce Indol Acetic acid (IAA) and Ammonia. IAA is regarded as one of the most important hormones for plants which induces the length ,division and differentiation of cells and thus it helps increase seed germination and speed germination (Soltani *et al*., 2010).

Table (5): Inhibitory effect of some Actinomyces isolates against the causal agents of cucumber seedlings damping off disease (*Pythium* sp. and *R. solani*); Each number mentioned in the table represents three replicates.

Treatments	*Pythium* sp.		*R. solani*	
	Germination speed	Germination percentage	Germination speed	Germination percentage
Control1/free from fungi and bacteria	7.8	83.3	6.1	90.0
Control2/ pathogen	7.7	86.7	6.2	80.0
S.grisues	7.7	90.0	7.7	90.0
Actinomycetes 10	8.2	93.3	8.4	90.0
Actinomycetes 14	8.1	93.3	8.2	90.0
Actinomycetes 24	5.7	93.3	6.8	93.0
Actinomycetes 44	6.8	90.0	7.0	90.0
B. celere	4.1	90.0	8.0	90.0
L.S.D 0.05	3.25	16.94	2.08	22.35

Effect of actinomycetes isolates on incidence and severity of seedling damping-off

R. solani and *Pythium* sp. which caused damping off cucumber seedlings induced disease incidence reached 11.37 and 4.33 for *R. solani* and *Pythium* sp. respectively. It's also found that *R. solani* and *Pythium* sp. caused high disease severity in soil infested with them. But it's clear from the results illustrated in (Table 7) that actinomycetes isolates used in this experiment reduced the inhibitory effects of the two pathogens. Isolates No. 44 and *B. celere* were gave the best results for disease severity caused by *R. solani* and *Pythium* sp..

This results assured the previous studies concerning the ability of actinomycetes in control soil borne fungi such as *R. solani*, *Pythium* sp. and *Fusarium* sp. (Al-Zahrany & Al-Sharary, 2007; El-Tarabily *et al*, 2008; Kamal & Sharma, 2014).

Table (6): Effect of some actinomycetes isolates on the severity and seedlings damping off percentage caused by *Pythium* sp. and *R. solani*.

Treatments	*R. solani*		*Pythium* sp.	
	% Disease severity	%incidence	% Dieses severity	%incidence
control / free from fungi and bacteria	0	0	0	0
control / pathogen	80.83	11.37	41.54	4.33
S. grisues	21.66	4.70	13.10	5.16
Actinomycetes 10	17.10	1.00	6.10	1.0
Actinomycetes 14	19.04	1.00	15.36	1.0
Actinomycetes 24	24.37	1.00	10.59	5.16
Actinomycetes 44	16.94	5.16	13.59	8.87
B. celere	19.58	1.00	7.36	1.0
L.S.D 0.05	23.01	8.52	9.01	8.31

The Plant height and Wet and Dry Weight of Shoot and Root Systems

The results of Table (7) and Fig. (4) showed that the plant elevation, as well as wet and dry weight of shoot and root systems were reduced from 13.27cm, 3.000g, 0.767g, 0.267g and 0.0667g respectively in the uninfected comparison treatment to 6.90cm, 1.4679g, 0.667g, 0.163g and 0.0533g respectively in the comparison treatment infected with the *R. solani*. Treating the soil infected with *R. solani* via the six Actinomycetes isolates used in the experiment has improved the cucumber growth indicators. The ratios were varied depending on the variation of the used isolates that the plant elevation got increased in all treatments of Actinomycetes isolates with significant differences compared with the comparison treatment free from fungi and bacteria and the experiment treatment infected with *R. solani*. The plant highest elevation average was recorded in the *B. celere* isolate which was 15.97 cm compared with the comparison treatments. Wet weight of shoot system in all treatments of Actinomycetes isolates got increased with significant differences compared with the experiment treatments. The highest average of the wet weight was recorded in Actinomycetes $_{44}$ which weighed to 5.100g compared to the experiment treatment while the dry weight of the root system has got increased in all Actinomycetes isolates but with insignificant differences compared with the comparison treatments. The highest average of root system wet weight was recorded in *S. griseus* isolate which recorded 0.933. Also, the results showed that the dry weight of the shoot system was increased in all Actinomycetes isolates treatments with significant differences compared with experiment treatments. The highest average of dry weight was recorded in Actinomycetes $_{44}$ while the dry weight of root system was increased but with insignificance differences compared with the comparison treatments. The highest average of the dry weight was recoded in *B. celere* isolate.

The results of Table (8) showed that the plant elevation, the wet and dry weight of shoot and root systems weighed 10.0cm, 2.000 cm, 0.300g, 0.2000g and 0.0433g respectively in the uninfected comparison treatment while the growth indicators in the comparison treatment infected with *Pythium* sp. weighed 10.40 cm, 2.200g, 0.433g, 0.200g and 0.0600g respectively. Yet, treating the soil infected with *Pythium* sp. via the six Actinomycetes isolates used in the experiment caused the plant elevation to be increased in all treatments of Actinomycetes isolates with significant differences compared with the comparison treatment infected and uninfected with *Pythium* sp. The highest average of plant elevation was recorded in the treatment of *S. griseus* which reached up to 15.17cm compared with comparison treatments while the highest average of wet weight of shoot system was recorded in Actinomycetes $_{24}$ isolate which weighed 4.933g whereas the highest average of wet weight of root system was recorded in *B. celere* isolate which scored 0.967g. The highest average of dry weight of the shoot system was recorded in the Actinomycetes $_{44}$ isolate which scored 0.467 g and The highest average of dry weight of the root system was recorded in the *B. celere* isolate.

Such results conform with(Al Zahrany & Al Sharary, 2007; El- Tarabily *et al*, 2008; Nawary, 2011). These studies show that Actinomycetes (including *Streptomyces* genus) have a negative impact in controlling seedling damping off disease as well as they help increase the biomass of the shoot and root systems through the increase of seed germination ratio, plant elevation, seed proper growth which lead to strengthen the growth of seedlings and support their resistance against pathogens (Nawary, 2011).

Table (7): Suppression the infection by *R. solani* and stimulate the growth of cucumber plants treated with some isolated Actinomyces.

Treatments	Plants height cm	Wet Weight (g)		DRY Weight (g)	
		Shoot system	Root system	Shoot system	Root system
control/ free from fungi and bacteria	13.27	3.000	0.767	0.267	0.0667
control / pathogen	6.90	1.467	0.667	0.163	0.0533
S. grisues	15.20	3.967	0.933	0.300	0.0833
Actinomycetes 10	14.33	4.633	0.900	0.400	0.0833
Actinomycetes 14	15.53	4.467	0.733	0.333	0.0600
Actinomycetes 24	15.20	4.967	0.800	0.333	0.0633
Actinomycetes 44	15.77	5.100	0.700	0.433	0.0567
B. celere	15.97	4.967	0.867	0.367	0.0867
L.S.D 0.05	1.938	0.7445	0.4883	0.0880	0.03199
Treatments	Plants height cm	Wet Weight (g)		DRY Weight (g)	
		Shoot system	Root system	Shoot system	Root system
control/ free from fungi and bacteria	13.27	3.000	0.767	0.267	0.0667
control / pathogen	6.90	1.467	0.667	0.163	0.0533
S. grisues	15.20	3.967	0.933	0.300	0.0833
Actinomycetes 10	14.33	4.633	0.900	0.400	0.0833
Actinomycetes 14	15.53	4.467	0.733	0.333	0.0600
Actinomycetes 24	15.20	4.967	0.800	0.333	0.0633
Actinomycetes 44	15.77	5.100	0.700	0.433	0.0567
B. celere	15.97	4.967	0.867	0.367	0.0867
L.S.D 0.05	1.938	0.7445	0.4883	0.0880	0.03199

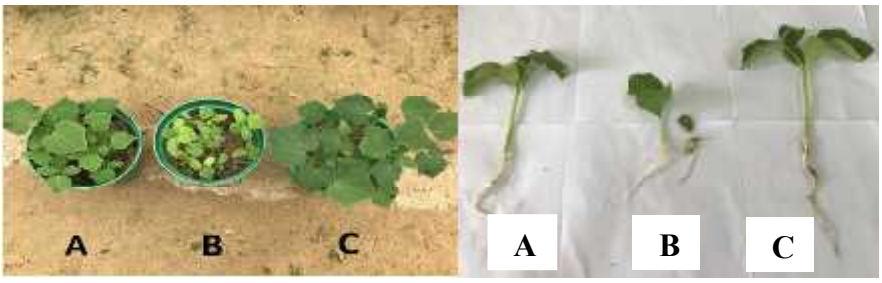


Fig (4): The inhibitory effect of *Streptomyces griseus* in damping off disease caused by *R. solani.*
A- Untreated cucumber plants(free from fungi and bacteria).
B- Control cucumber plants in soil treated by *R. solani.*
C- Control cucumber plants in soil treated by *R. solani* and *S. griseus.*

Table (8): Suppression the infection by *Pythium* sp. and stimulate the growth of cucumber plants treated with some isolated Actinomyces.

Treatments	Plants height cm	Wet Weight (g)		DRY Weight (g)	
		Shoot system	Root system	Shoot system	Root system
control / free from fungi and bacteria	10.0	2.000	0.300	0.200	0.0433
control / pathogen	10.40	2.200	0.433	0.200	0.0600
S. grisues	15.17	3.933	0.600	0.367	0.0767
Actinomycetes 10	14.43	3.700	0.433	0.367	0.0733
Actinomycetes 14	14.77	4.700	0.433	0.400	0.0533
Actinomycetes 24	13.83	4.933	0.733	0.400	0.0633
Actinomycetes 44	15.10	4.600	0.533	0.467	0.0567
B. celere	14.50	4.433	0.967	0.400	0.0600
L.S.D 0.05	1.997	0.8413	0.4105	0.1588	0.03722

Conclusions

This study showed the possibility of isolating Actinomycetes from different environments sources, as well as its efficiency to reduce damping off disease of cucumber caused by *R. solani* and *Pythium* sp., and also its efficiency to improve plant growth.

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