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PERFORMANCE OF ENDOPHYTIC FUNGI AND BACTERIA IN RESISTANCE OF CUCUMBER ROOT ROT DISEASE CAUSED BY PATHOGENS *PYTHIUM APHNIDERMATUM* AND *RHIZCTONIA SOLANI*

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Abstract

The study aimed to test the performance of endophytic fungi and bacteria that were isolated from plants free of any pathological symptoms such as *Cymbopogon citrates*, *Acasia nilotica*, *Thevetia peruviana* and *Cucumis sativus* in inhibiting the pathogens *Pythium aphanidermatum* and *Rhizoctonia solani* causing the cucumber root rot and seedlings damping-off disease in vitro. The results showed the identification of fungi Rosellinia sanctae-cruciana, *Hemicola* sp, *Trichoderma harizianum*, *Nigrosporea* sp, *Aspergillus flavus*, *Aspergillus niger*, *Alternaria alternata*, *Cylindrocarpon* sp, *Ulocladium* sp, *Acremonium* spp, *Cladosporium* sp and *Helminthosporium* spp and bacteria *Pseudomonas fluorescens*, *Azotobacter* spp and *Bacillus subtillis* with varying frequency of isolated plants. Furthermore, the results of its antagonistic in the dual culture method in dishes in vitro showed high efficiency of the fungi (*Rosellinia sanctae-cruciana*) in inhibiting the pathogen (*P. aphanidermatum*), as the percentage of inhibition amounted to 69.35%, followed by the fungi *Trichoderma harizianum*) and (*Alternaria alternata*) with a slight non-significant difference. Besides, the percentage of inhibition was 55.48 and 51.32%, respectively, while the results showed that the highest percentage of inhibition of pathogenic fungi (*R. solani*) by the fungi (*Trichoderma* spp) was 61.55%, followed by the two fungi (*Rosellinia sanctae-cruciana* and *Cylindrocarpon*) with a non-significant difference, as the percentage of inhibition amounted to 60.02% and 54.91%, respectively. The results in the endophytic isolates of bacteria *Pseudomonas fluorescens* recorded an antagonism efficiency against the pathogens *P.aphanidermatum* and *R. solani* were 73.41% and 70.12%, respectively. The use of endophytic in against the cucumber root rot disease is one of the most promising methods to reduce the use of chemical pesticides that have severe harm to human and animal health.

Key words: Endophytic Fungi, pathogens**,** Cucumber Root Rot, *Cucumis sativus*

Introduction

Cucumis sativus is one of the crops of the *Cucurbitaceae* family, which has nutritional importance in countries of the world, including Iraq, because it contains calcium, potassium, phosphorous, protein, carbohydrates and vitamins C, B1, B3 and 6B (Mukherjee *et al.,* 2013). Many vegetable crops are produced in greenhouses, including cucumber that pathogenic fungi have become an important determinant of production, especially the pathogen *Pythium aphanidermatum* and *Rhizoctonia solani* (Gravel *et al.,* 2007). One of the common procedures in managing this disease is the use of chemicals, including pesticides, which have negative effects on human and animal health. As well as, the emergence of strains resistant to pathogens against pesticides, therefore, restrictions emerged from the use of chemicals (Titone *et al.,* 2009). Thus, the biological control has received great attention, especially the fungal species belonging to the genus *Trichoderma* spp, including the fungi *Trichoderma harzianum*. Besides, the bacterial species that belong to the genus *Pseudomonas* spp, including *Pseudomonas* putida and *Pseudomonas fluorescens* (Jabber 1996, Al-Jabbari 1998; Al-Dulaimi 1998). However, the endophytic of fungi and bacteria is a microorganism that can live between plant tissue cells, and sometimes it is within cells, without causing obvious and substantial damage to the host plants (Sturz *et al.,* 2000; Elbeltagy *et al.,* 2005, Arnold, 2005). Fungi and endophytic bacteria play important roles for their host by stimulating them to grow through various

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mechanisms, as well as increasing their resistance to environmental and biological stress (Malinowski *et al.,* 2004; Mandyam and Jumpponen, 2005). Various endophytic fungi produce secondary metabolic compounds, some of which are antifungal such as alkaloids, peptides, steroids, and phenols, where they inhibit the growth of many microorganisms including pathogens of the plant (Gunatilaka, 2006). There are three mechanisms of defense from the plant host by the endophytic fungi are the direct effect where there is the direct interaction between the pathogen and endophytic fungi leading to inhibition of pathogen growth and indirect effect where the endophytic fungi enhance the resistance of the plant and its defenses to the pathogens and environmental impact (Arnold, 2000). (Strobel and Daisy, 2003; Strobel *et al.,* 2004) stated that endophytes of fungi and bacteria are an important source of bioactive natural products because they can occupy unique biological hubs and grow in unusual environments. (Abbamondi *et al.,* 2016) we're capable of isolated some species of endophytic bacteria of the tomato crop was all highly efficient in promoting plant growth and inducing systemic resistance against some diseases affecting tomato. In addition, (Bacon *et al.,* 2001) stated that the endophytic bacteria isolated from maize were successfully used in biological control against the *Fusarium moniliforme*. (Abdallah *et al.,* 2008) indicated that bacteria isolated from the Withania somnifera plant could reduce the infection of Fusarium wilt disease in tomato caused by the Fusarium oxysporum f. sp. *Lycopersici*. Furthermore, (Al-Aidani, 2017) was able to isolate and identify fungi cultivated from the leaves of the Camaldulensis Eucalyptus plant and studied its antifungal activity and obtained positive results in the antagonistic of *Penicillium* sp, *Aspegillus niger*, *Alternaria* sp and *Aspergiilus flavus* with the pathogenic fungi R. *solani*, F. *oxysporum. solani*, F. oxysporum, *F. graminearum* and *F. oxysporum*, respectively. Finally, the study of the endophytic fungi has gained great importance in recent years for its ability to produce effective secondary metabolic compounds with multiple biological activity, including antagonism with the growth of plant pathogenic fungi (Azevedo *et al.,* 2000). However, the endophytic fungi are considered one of the most promising modern applications in the field of biological control of the pathogens for plants, as well as the induction of synthetic and enzymatic defense mechanisms in the plant. Besides, this contributes to reducing the excessive use of chemical pesticides because of their harmful effects on human and animal health as well as their pollution to the environment, as the endophytic fungi can multiply easily and at a low economic cost.

Materials and Methods

The isolation of Pathogens *P.aphanidermatum* and *R.solani* from the cucumber plant

Samples were collected from the cucumber plants that showed symptoms of infection with the pathogen *P.aphanidermatum* and *R. Solani* from experimental fields in the College of Agricultural Engineering Sciences - Baghdad University/Jadriya. These samples were placed in bags of sterile polyethylene and brought to the pathology laboratory in the College of Agricultural Engineering Sciences, and were washed by running water for 15 minutes to remove the suspended soil, then washed with sterile distilled water. The infected roots and the stem base were cut into small pieces of 0.5 cm length and cultivated in 9 cm diameter Petri dishes containing the Potato Dextrose Agar (PDA) medium plus the antibiotic Ampicillin by 100 mg/liter by 3 dishes and each dish contains 4 pieces for both roots and the stem base. Then, they were incubated at a temperature of 25±2ºC for 3-5 days and were purified by transferring the mycelium from the edges of the dishes to new Petri dishes containing the same culture medium. Also, adding the same antibiotic above and incubated at the same temperature for 3-5 days to obtain the pure isolation of the pathogen to be identified morphologically later.

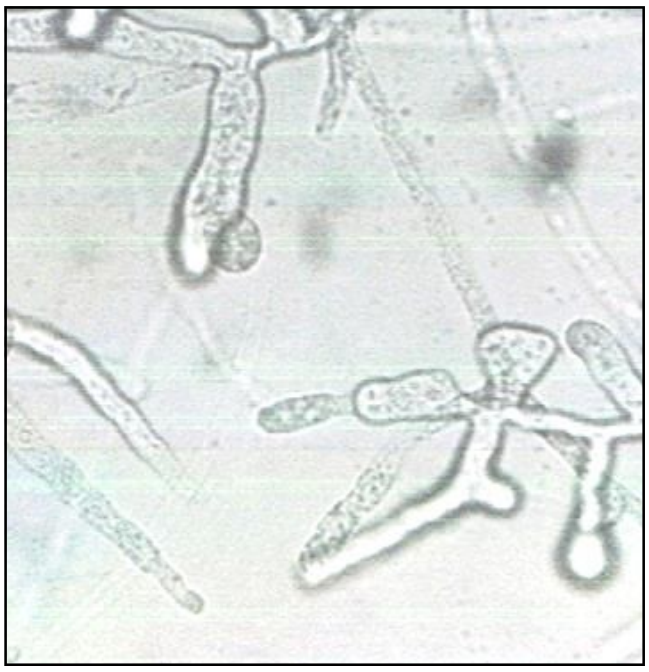
R. solani isolatation from soil

Samples were collected from the soil of a field cultivated with cucumber crops showing symptoms of root rot disease from the same fields mentioned above and placed in a polyethylene bags and transferred to the plant pathology laboratory according to the method (Sneh *et al.,* 2004) for isolation from the soil. Furthermore, a weight of 200 g of soil was taken from each sample, and pure sterile water added to it by 2 liters in a glass jar and shacked well to ensure complete homogeneity between the components of the soil and water. It was left for 10-15 minutes for plankton and mud to deposit, and floats all the soil impurities above the surface of the water, after that the filtration process was carried out with a special sieve size 60mm mesh. Then, the impurities were collected on the surface of the sieve, washed with normal running water for 5 minutes or more, then sterilized by commercial sodium hypo chloride solution with a concentration of 10% by submerging it for 3 minutes, then washed with sterile distilled water for two minutes and then they were dried by sterile filter paper. It was transferred by sterile forceps and cultivated in a 9 cm diameter Petri dishes containing the (PDA) medium plus

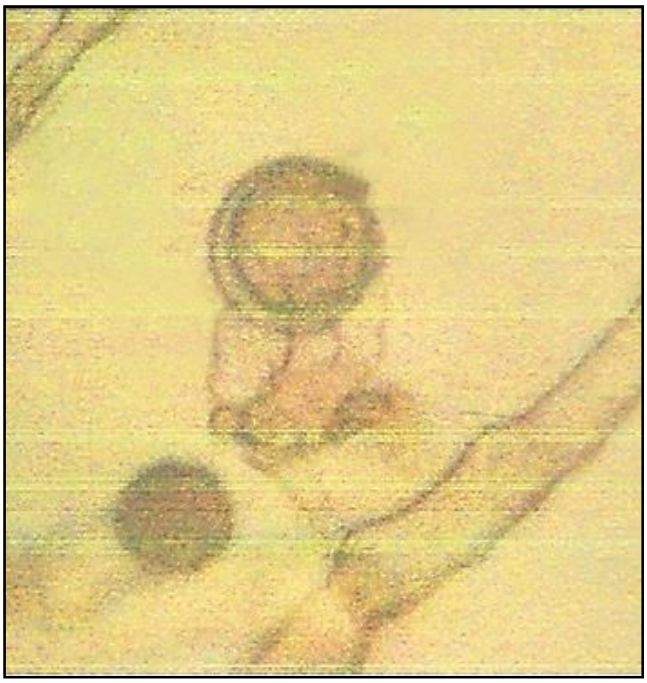
Chloramphenicol antibiotic of 250 µM/1 liter by 4 pieces in each dish and the treatment repeated 3 times and incubated at a temperature of 25±2ºC for 3-7 days. Moreover, it was purified by transferring the mycelium from the edges of the dishes to new glass dishes, containing the same culture medium PDA adding the same antibiotic above and incubated at the same temperature for 3-7 days to obtain pure isolation from the pathogen.

Identification of the pathogen *P. aphanidermatum* and fungi *R. solani*

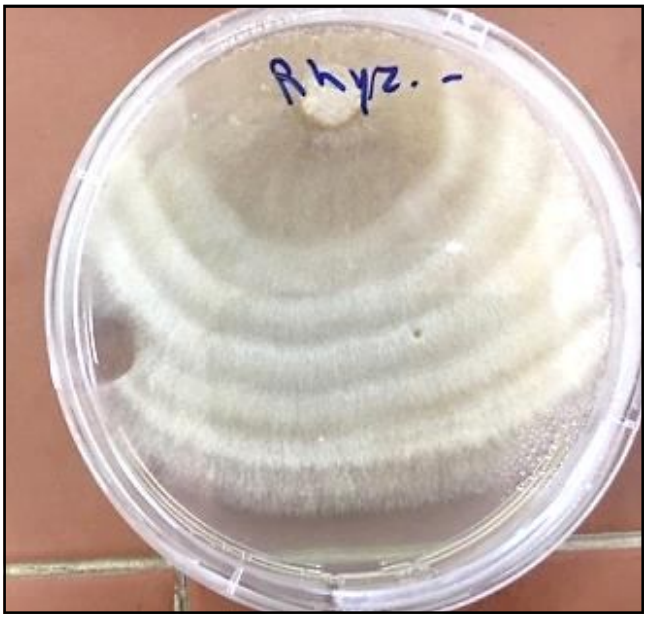
The initial identification of the pathogen *P. aphanidermatum* was performed microscopically of 400X magnification depending on the morphological characteristics of the mycelium as well as the compositions that are made particularly sporangium and the production of spores and the morphological characteristics of oospores. As the identification of the level of the species was carried out using the single-access key and the important characteristics mentioned by (Stamps, 1998). However, the fungi *R. solani* was identified by optical microscopy by X-400 magnification is by its cultural characteristics through the color of the fungal colony that tends from brown to dark brown and the nature of the divided mycelium and thinning of its modern branches. Besides, the presence of barriers with double holes, as well as the ability to produce and form dark Sclerotia which are different in shape and size, and which are not distinguished by the cortex, core, and shape of cells as well (Parmeter and Whitneh, 1970).



Picture 1: Fungi P. *aphanidermatum* (B) compositions Fingers sporangium.



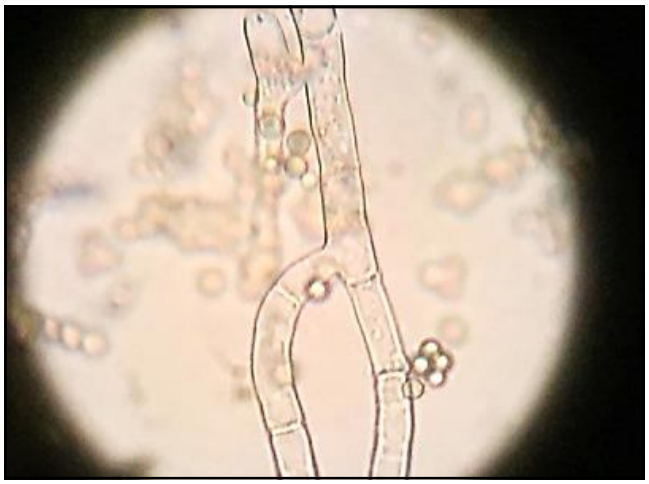
Picture 1: Fungi *P. aphanidermatum* (A) compositions oval oospores.



Picture 2: Colony of fungi *R.solani.*

Pathogenicity test of pathogen *Pythium aphanidermatum*

The pathogenicity of the pathogen *Pythium aphanidermatum* was tested according to the (Stephens *et al.,* 1981) method, where nine plastic pots of 30×15 cm with a depth of 10 cm were prepared and sterilized by formaldehyde and placed in an airtight place for two days. Then, completed with loam soil with a depth of 8 cm containing the peat moss and in a ratio of 1:2 that sterilized thermally twice between the first sterilization



Picture 3: Mycelium *R.solani* 400 × magnification.

and the second 48 hours by the steam sterilizer device (121 m and a pressure of 1.5 kg/cm^2 for one hour). Cucumber and muskmelon seeds were planted on two equal lines of length and meet at the point of the head with a length of 25 to form two equilateral triangular sides and its base at the width of the plastic pot with a number of 10 seeds from each crop. After 7 days of germination, the seedlings were polluted by placing the pathogen vaccine in the form of a disc of 0.5cm diameter of the PDA medium contains the pathogen of each isolate by 3 replicates at the point of convergence of the two equilateral triangles sides. The comparison treatment was carried out in the same way in the other pots, the discs from the medium PDA with a diameter of 0.5 cm free of the pathogen were placed at the point of convergence of the two sides of the equilateral triangle. Finally, after 10 days, the infection percentage of seedling was calculated, and on this basis, the infection percentage of seedling was calculated according to the following equation:

Infection percentage of seedling % =

$$\frac{Number\ of\ \text{inf}\ ected\ seedling}{Total\ number\ of\ seedling} \times 100$$

Pathogenicity test of *Rhizoctonia solani in vitro*

The pathogenicity test for fungi R. *Solani* was carried out in the Plant Pathology Laboratory - Department of Plant Protection/College of Agricultural Engineering, using cucumber seeds (Var Inad). The Petri dishes were prepared with a diameter of 9 cm, containing approximately 20 ml of the culture medium (Water Agar) prepared previously by dissolving 15 g of agar in 1 liter of water that sterilized by the Autoclave. Besides, the Chloramphenicol antibiotic was added to it with 250 mg / 1 ml, and vaccinated at the center by the pathogen in the form of a disc of 0.5 cm diameter; it was taken from the ends of the mycelium colony from the petri dish containing the fungi prepared for research employing a sterile cork borer by flame. The dishes were incubated at a temperature of 25±2ºC for 72 hours, after that the seeds were planted after being surface-disinfected by commercial sodium hypo chloride solution at a concentration of 10% for 3 minutes. Then washed with sterile distilled water and dried with sterile filter paper; in dishes at a rate of 20 seeds/dish in a circular way on the growth of the mycelium in 3 replicates for each isolation (Bolkan and Butler, 1974). The comparison treatment was carried out in the same way above without pathogenic fungi, and the dishes were incubated at 25±2ºC for 3-5 days, and the percentage of seed damping-off after 10 days of planting was calculated according to the following equation:

Percentage of damping - off seed % =

$$\frac{Number\ of\ damping-off\ seed}{Total\ number\ of\ planted\ seed} \times 100$$

Isolation and identification of Endophytic fungi from some plants

Leaves of intact plants and free from any spotting or pathogenic symptoms were collected from *Cucumis sativus*, *cymbopogon citrates*, *Acasia nilotica* and *Thevetia peruviana* from different areas of Baghdad during the period of November-December 2018. It was put in clean plastic bags and transferred to the laboratory to isolate endophytic fungi and bacteria and followed the following method, as the leaves were washed with a large quantity of tap water to remove the soil and dust, then the leaves were cut into small pieces of 5.0-1 cm. Then, they were surface-disinfected first with ethyl alcohol at a concentration of 75% for one minute, and then were submerged in a solution of sodium hypochlorite at a concentration of 5.2% for 4 minutes, and re-submerged it with ethyl alcohol at a concentration of 75% for 30 seconds. Furthermore, it was washed with sterile distilled water three times and dried with sterile filter papers. Furthermore, the pieces were cut by 3 pieces for each dish with homogeneous dimensions in 10 Petri dishes containing the PDA medium and added anti-chloramphenicol (5.0g/l), which was added to the medium after sterilization. Besides, the edges of the dishes were wrapped by Parafilm, incubated at a temperature of 25 ± 2 °C for 3-4 days, with the observation of colonies growth daily, and then purified by transferring the mycelium from the ends of the newly developed colonies to new Petri dishes containing the PDA medium added to it. The same antibiotic above and in the same proportion and incubated at the same temperature above then tested by optical microscopy on 400X magnification and the fungi species

were identified according to morphological characteristics depending on colony shape, shape and type of mycelium and the shape and composition of sporophore mentioned by (Domsch, 1980). The frequency percentage C.F percentage was calculated using the following equation (Chhetri, 2013).

Colonization Frequency C.F % =

$$\frac{Number\ of\ colonies\ in\ each\ piece}{Total\ number\ of\ pieces} \times 100$$

Endophytic bacteria isolation from some plants

The bacteria were isolated from the leaves pieces of plants in the same previous way in terms of washing and sterilization and were cultivated in Petri dishes of 9 cm in diameter. They containing the Nutrient Agar medium, by 3 pieces in each dish distributed homogeneously and with equal dimensions from each other inside the dishes and by 10 dishes for each plant type, and were incubated at a temperature of 27±2ºC for 3-5 days with continuous checking of bacterial isolates growth. The C. Fpercentage of endophytic bacteria colonies was calculated according to the following equation (Chhetri, 2013).

Colonization Frequency C.F % =

$$\frac{Number\ of\ pieces\ in\ which\ bacteria\ colonies\ appeared}{Total\ number\ of\ pieces} \times 100$$

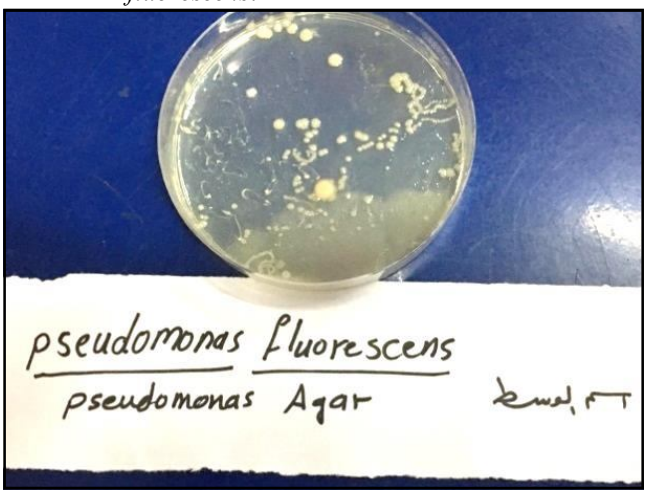
Identification of endophytes bacteria

Bacterial isolates were purified separately by transferring a smear from the surface of a single colony for each of them on their medium. The types of endophytes bacteria were identified in the laboratories of the Ministry of Science and Technology/Biotechnology Department / Microbiology Division using the different selective culture medium, and observe the nature of growth and shape of the bacteria colonies, among which are:

Pseudomonas* Agar medium for the diagnosis of bacteria *P. fluorescens

The *Pseudomonas* Agar consisting of 10 g enymic hydrolysate Casein, 10g, Pancreatic digest of gelatin 16g, Magnesium Chloride anhydrous 1.4g Potassium Sulphate, g11 Agar and 500 ml water. The medium was inoculated with bacteria growing on Nutrient Agar N.A by streak method, the dishes were incubated at 30°C for 24 hours, then the dishes were tested under the UV device was performed to reveal the Luminescence. As the bacteria, *P. fluorescens* belong to the fluorescent group, which shows it's luminous when growing in the deficient media of iron element, as it produces a green-yellow fluorescent stain in the medium on which it grows. However, these

Picture 4: *fluorescens* test under UV device of bacteria *P. fluorescens*.



Picture 5: *P. fluorescens* colony growing on the medium *Pseudomonas* Agar.

bacteria are characterized by the production of the Siderophore compound, which works to withdraw iron from the soil, and these are the most important characteristics of these bacteria (Holt, Krieg, 1980).

***Azotobacter* for identification *Azotobacter sp*:**

The use of the selective media N-Free Media Agar and consists of 10g Glucose, 1g K2HPO4, 0.2 g MgSo4.7H2o, 0.05g 7H2O.FeSo4, 0.01g $Na_2MoO_4.2H_2o$, 0.1g $CaCL_2$, 15Agar g. The medium components were dissolved in a liter of distilled water, where the PH was adjusted to 7.2-7.3 and sterilize by the Autoclave at a temperature of 121 °C and pressure 1.5 kg/cm^2 for 20 minutes. However, the medium was inoculated with bacteria in Petri dishes and the dishes were incubated at a temperature of 28±2°C for 48-72 hours with an observation of the shape of the bacterial colony and its characteristics on the dish from convex. As well as, it strength and color by the optical microscopy at a magnification of 400 × 400 degrees to identify the cell

Picture 6: bacteria *Azotobacter* spp.

forms and composition of the (Cysts) or the outer shell, and the bacteria appeared on the surface of the nutrient medium in a creamy white color mucous textures. These characteristics are the most important characteristic of these bacteria (Becking, 1981).

Nutrient Agar to isolate and identify *Bacillus subtilis*

The NA medium was prepared according to the manufacturer's instructions and sterilized by the Autoclave with a temperature of 121°C and a pressure of 5.1 kg/cm² for 15 min. The bacteria growing on the N.A medium was identified, as the colonies appeared after a 24-48 hour incubation period at a temperature of 28±2°C and they were of various sizes in a solid wrinkled form. Then, the colony was taken and tested with the Gram stain test, where the bacteria appeared when tested under a light microscope x-100 magnification, it is a Gram-positive type forming the endospores, and this is what distinguishes these bacteria that belong to the group of Gram-positive Bacillus forming the endospores.

The efficiency test of the endophytic fungi in inhibiting the two pathogens (*Pythium aphanidermatum* and *Rhizoctonia solani*) *in vitro*

This test was carried out using a dual culture method on the solid medium in Petri dishes of 9 cm diameter, by taking a disc of fungal growth for both Endophytes and the pathogens *P. aphanidermatum* and *R. solani* from modern farms in the age of seven-day-old by a sterile Cork borer with a diameter of 3 mm. The discs were put in a dish containing the PDA medium with a distance of about 7 cm between them, each disc was placed at a distance of 7 mm from the edge of the petri dish, and left a comparison dishes for the two pathogens alone with three replicates of the two treatments separately. The dishes were incubated at a temperature of 25±2°C and the results were taken three days after incubation, and the diameter of the two pathogens colonies was measured and compared to the diameter of the pathogens in the comparison treatment dishes. Then, the antagonistic potential of the endophytic fungi was calculated according to the following equation (Suryanarayanan, 2003; Gomathi, 2011).

$$\text{Inhibition percentage \%} = \frac{D1 - D2}{D1} \times 100$$

where:-

D1 =the diameter of the pathogenic fungi colony in the comparison.

D2 =the diameter of the pathogenic fungi colony on the dual farm.

The efficiency test of the endophytic bacteria in inhibiting the two pathogens (*Pythium aphanidermatum* and *Rhizoctonia solani*) *in vitro*

The same previous method was carried out of the dual culture on the solid medium in Petri dishes of 9 cm in diameter. A smear was taken from the endophytic bacteria that does not exceed 5 days old and was placed in a line of 2 cm length and 1 cm away from the edge of the dish. They placed on the opposite side of the dish a disc from each of the pathogens *P. aphanidermatum* and *R. solani* separately and from a modern farm at the age of seven-day by a sterile Cork borer with a diameter of 3 mm. The disc placed in the same dish containing the PDA with a distance of about 7 cm between them and a distance of 7 mm from the edge of the petri dish, and left a comparison dishes for the two pathogens alone with three replicates of the two treatments separately and in the same method above. The dishes were incubated at a temperature of 25±2°C and the results were taken three days after incubation. The diameter of the two pathogens colonies was measured separately in the comparison, and the diameter of the pathogens colonies was measured in the dishes in which the endophytic bacteria were cultivated in the same dish, then the antagonistic potential of the endophytic bacteria was calculated according to the following equation (Suryanarayanan, 2003; Gomathi, 2011):

$$\text{Inhibition percentage \%} = \frac{D1 - D2}{D1} \times 100$$

Where:-

D1 = the diameter of the pathogenic fungi colony alone.

D2 = the diameter of the pathogenic fungi colony on the dual farm.

Results and Discussion

Isolation of Pathogens *P. aphanidermatum* and *R.solani* from the cucumber plant

The results of the laboratory isolation of the pathogen *P.aphanidermatum* showed that the appropriate period of the isolate begins during February and March with the beginning of high temperatures because the fungi are active during this period and the recorded cases of root rot and the damping-off of cucumber plant in greenhouses increase. Moreover, the results in table 1 showed that the highest C.F% of the pathogen *P. aphanidermatum* in the isolation from the cucumber fruits (83.3%), followed by 58.3 and 66.6% in isolation from the roots and the stem bases respectively. As for the R. *solani*, the results showed a variation in the C.F%, where the highest frequency of fungi isolates from soil samples was 66.6% compared to the isolates obtained from the pieces taken from the roots and the stem bases and reached 58.3 and 41.6% respectively. Accordingy, the characteristics of all these isolated isolates correspond to the fungi R. *solani*, which causes cucumber root rot.

Table 1: Frequency percentage of the pathogen colonies P. *aphanidermatum* and R. *solani* isolated from cucumber pieces infected with the disease.

P. aphanidermatum		R. solani	
Isolation source	**Frequency %**	**Isolation source**	**Frequency %**
Fruits	83.3	Soil	66.6
Roots	58.3	Roots	58.3
Stem bases	66.6	Stem bases	41.6

* Each number in the Table represents three replicates.

Table 2: Pathogenicity test for the P. *aphanidermatum* isolates on muskmelon and cucumber plants.

Isolate symbol	Source	Muskmelon plant	Percentage of infection	Cucumber plant	Percentage of infection
Pa_1	Fruits		78.5		82.7
Pa_2	Roots		93.1		92.8
Pa_3	Stem bases		85.1		86.2
Comparison	/		3.33		3.44
LSD (P= 0.05)		8.026 *		7.941*	

* Each number in the Table represents three replicates.

Pathogenicity test of pathogen *Pythium aphanidermatum*

The results in table 2 showed the pathogenic test of isolates obtained from the fruits, roots and stem bases of the cucumber plant infected with the pathogen *P.aphanidermatum* (which was named according to the source of the plant part taken from it) that there is a convergence in the pathogenicity of muskmelon and cucumber plants.

The percentage of muskmelon infection ranged between 78.5-93.1%, while the percentage of infection of cucumber plants ranged between 82.7-92.8% without a significant difference between them. Nonetheless, it was significant compared to the comparison treatment that its percentage of infection reached 3.33 and 3.44% on muskmelon and cucumber plants respectively. The highest pathogenicity of isolate Pa_2 isolated from roots was 93.1 and 92.8% on muskmelon and cucumber respectively, while the percentage of infection for isolates Pa_1 and Pa_3 85.1 and 78.5% on muskmelon with a slight difference from the isolate Pa_2. Besides, cucumber plant ranged 82.7, 86.2% for isolates P_1 and P_3 respectively, while the difference was significant with the comparison treatment, as the percentage of plant infection reached 3.33, 3.44% for muskmelon and cucumber respectively. This slight difference between isolates in the pathogenicity may be due to genetic differences between fungi isolates collected from different sites, and on this basis the Pa_2 isolate was chosen to conduct all the experiments in the subsequent greenhouse, these results are consistent with (Stephens *et al.,* 1981) findings.

Pathogenicity test for *Rhizoctonia solani*

The results of the pathogenicity test in table 3 showed that all the isolates of *R. solani* have the ability to cause disease infection on cucumber plants, but there are slight and insignificant differences between isolates. Furthermore, the percentage of cucumber seed damping-off ranged between 91.9-96.6%, with a significant difference compared to the comparison, 3.3% and the reason for this may be due to the failure of the seed on germination as a result of their spoilage (Stephens *et al.,* 1981). The Rs_2 isolate recorded the highest percentage of seed damping-off 96.6% isolated from the roots of the cucumber plant, while the Rs_1 and Rs_3 isolates isolated from the soil and the stem bases of cucumber plants 93.3 and 91.6% respectively. However, the slight difference between these isolates in the percentage of infection may

Table 3: Pathogenicity test of *Rhizoctonia solani* isolates on cucumber seeds.

Isolate symbol	Source	Cucumber seed damping-off %
Rs_1	Soil	93,3
Rs_2	Roots	96.6
Rs_3	Stem bases	91.6
Comparison	/	3.3
LSD P=(0.05)	11.174 *	

* Each number in the Table represents three replicates.

be attributed to that there are differences in its ability to secrete pectin and cellulose-degrading enzymes in the early stages of the infection, on this basis Rs_1 isolate was chosen to conduct all subsequent field experiments, and this result is consistent with (Naif, 2014) findings.

Endophytic fungi isolation from the plant

The results of isolating and identifying the endophytic fungi of the leaves of *Thevetia peruviana* plant in table 4 showed the presence of 30 colonies belonged to 7 species of fungi with a total C.F% of 99.97% for all colonies and this is agreed with (Contia, 2012) findings.

Table 4: The endophytic fungi isolated from *Thevetia peruviana* plant, the number of colonies per fungi and the frequency percentage of each fungi.

Endophytes	No of Colonies	C.F%
Rosellinia *sanctae-cruciana*	7	23.32
Hemicola sp	6	20
Trichoderma harizianum	5	16.66
Nigrosporea sp	4	13.33
Aspergillus flavus	3	10
Aspergillus niger	3	10
Alternaria alternata	2	6.66
Total	30	99.97

* Each number in the Table represents three replicates.

The highest C.F% was 332.3% for *Rosellinia sanctae-cruciana*, while the lowest C.F% was 6.66% for *Alternaria alternata*, whereas the C.F% of the rest fungal isolates were distributed between the highest and lowest percentage according to the number of its colonies as in the table below:

Whereas, the results of isolating and identifying the endophytic fungi obtained from the leaves of the *Acasia nilotica* plant in table 5 showed that there are 30 colonies belonged to 6 species of endophytic fungi with a total C.F% of 99.98% for all colonies (Contia, 2012). The highest C.F% was 26.66% for *Rosellinia sanctae - cruciana* and the number of its colonies was 8 colonies, while the lowest C.F% was 6.66% for *Aspergillus flavus*

Table 5: The endophytic fungi isolated from the *Acasia nilotica* plant, the number of colonies per fungi and the frequency percentage of each fungi.

Endophytes	No of Colonies	C.F%
Rosellinia sanctae-cruciana	8	26.66
Nicrosporea sp	6	20
Alternaria alternata	5	16.66
Cylindrocarpon sp	5	16.66
Aspergillus niger	4	13.33
Aspergillus flavus	2	6.66
Total	30	99.98

* Each number in the Table represents three replicates.

and the number of its colonies was only two colonies. As well as, C.F% of the rest of the fungal isolates were distributed between the highest and the lowest percentage according to the number of its colonies. While the results of isolating and identifying the endophytic fungi from the leaves of the *Cymbopogon citrates* plant in table 6 showed the presence of 30 colonies belonged to 7 species of fungi with a total C.F% of 99.97% for all colonies.

In addition, this is consistent with (Contia, 2012), the highest C.F% reached 23.33% for fungi *Aspergillus*

Table 6: The endophytic fungi isolated from the Cotabopogon citrates plant, the number of colonies per fungi and the frequency percentage of each fungus.

Endophytes	No of Colonies	C.F%
Aspergillus flavus	7	23,32
Aspergillus niger	5	16.66
Alternaria alternata	5	16.66
sp Ulocladium	4	13.33
Acremonium spp	3	10
Nicrospora sp	3	10
Cladosporium sp	3	10
Total	30	99.97

* Each number in the Table represents three replicates

flavus and the number of its colonies was 7 colonies, while the lowest C.F% was 10% for the fungi *Acremonium* spp and *Nicrosporea* and *Cladosporium*. The number of colonies for each fungus was only 3 colonies, and the C.F% of the rest of fungal isolates were distributed between the highest and lowest percentage according to the number of its colonies.

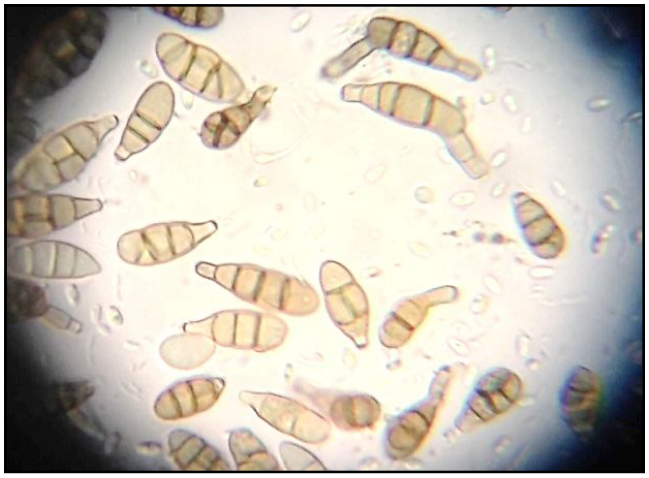
The results of isolating and identifying the endophytic fungi of cucumber leaves in table 7 showed the presence of 30 colonies belonged to 5 species of fungi with a total C.F% of 99.98% for all colonies. These results agreed with (Contia, 2012) and the highest C.F% was 26.66% for fungi *Helminthosporium* spp and *Trichoderma harizianum*, and the number of colonies for each fungus

Table 7: The endophytic fungi isolated from the cucumber plant, the number of colonies per fungi and the frequency percentage of each fungus.

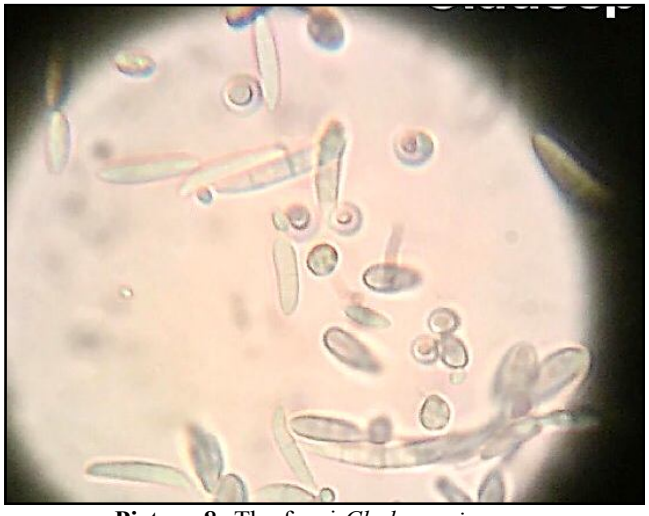
Endophytes	No of Colonies	C.F%
Helminthosporium spp	8	26.66
Tricoderma harizianum	8	26.66
Aspergillus niger	6	20
sp Nicrosporea	5	16.66
Aspergillus flavus	3	10
Total	30	99.98

* Each number in the Table represents three replicates.

was 8 colonies. The lowest C.F% was 10% for the fungi *Aspergillus flavus* and the number of its colonies was only 3, while the C.F% of the rest of fungal isolates were



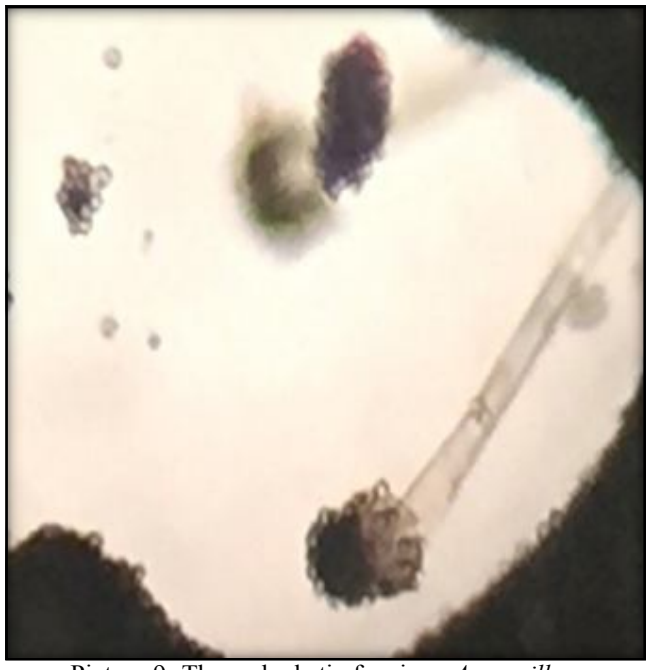
Picture 7: The fungi *Alternaria alternata*.



Picture 8: The fungi *Cladosporium* sp.

distributed between the highest and lowest percentage according to the number of its colonies.

These results obtained from isolates of the endophytic fungi species and their C.F% are consistent with several studies in which the endophytic fungi are isolated, and most of the isolated fungi consistent with what (Ali, 2014 and Jeffrey, 2008 and Khan, 2015) result.

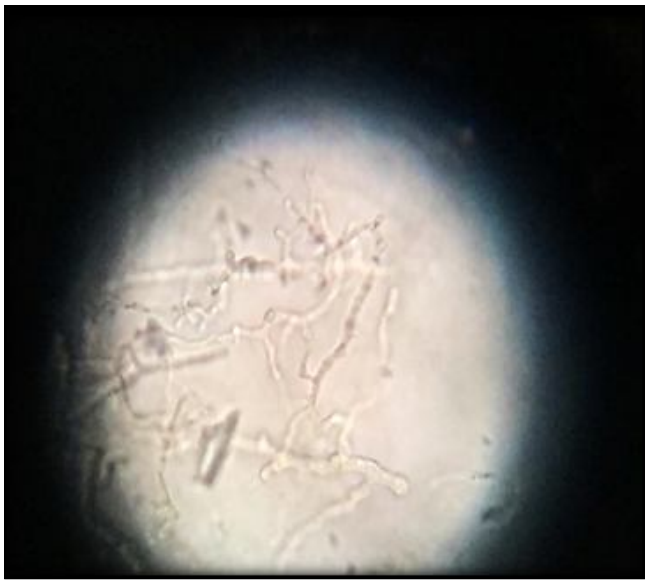


Picture 9: The endophytic fungi sp. *Aspergillus*.

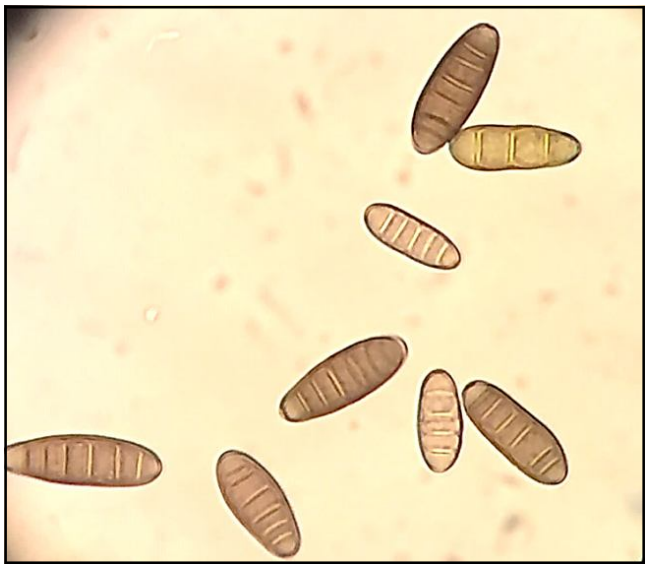
Picture 10: The endophytic fungi sp Nigrospora.

The efficiency test of endophytic fungi in inhibiting the pathogens (*Pythium aphanidermatum* and *Rhizoctonia solani*)

The results in table 8 showed that there was a significant difference in the inhibitory efficiency of the endophytic fungi among them towards the pathogens *P. aphanidermatum* and *R. solani* with a different endophytic fungi species. The laboratory experiments in the method of dual culture in dishes that were conducted in the post-graduate laboratories of diseases in the College of Agricultural Engineering Sciences, University of Baghdad showed the highest inhibitory efficiency of the endophytic fungi is for the fungi (*Rosellinia sanctae-*



Picture 11: The endophytic fungi *Rosellinia sanctae-cruciana*.



Picture 12: Endophytic fungi *Helminthosporium* sp.

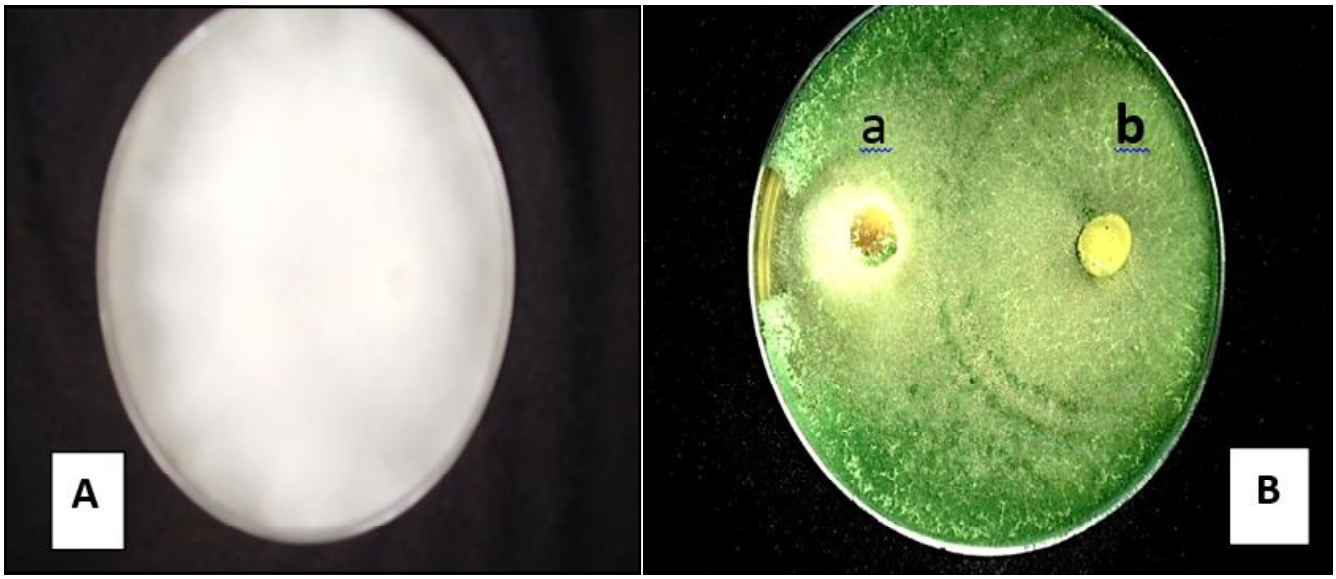
cruciana) in inhibition the pathogen (*P. aphanidermatum*). Furthermore, the inhibition percentage reached 69.35% followed by the fungi *Trichoderma harizianum*) and (*Alternaria alternata*) with a slight insignificant difference, as the inhibition percentage amounted to 55.48 and 51.32%, respectively, while the rest of the endophytic fungi isolates, their inhibition percentage ranged between 43.75% for the fungi (*Nicrosporea*) and 25.22% which is the lowest inhibition percentage for the fungi (*Ulocladium* spp). While the experiment showed that the highest inhibition percentage of pathogenic fungi (*R. solani*) by the fungi (*Trichoderma* spp) was 61.55%, followed by fungi (Rosellinia sanctae-cruciana and *Cylindrocarpon*) with non-significant differences, as the inhibition percentage reached 60.02% and 54.91% respectively, as for the rest of the endophytic

Table 8: the efficiency test of the endophytic fungi in inhibiting pathogens P. *aphanidermatum* and R. *solani* in vitro.

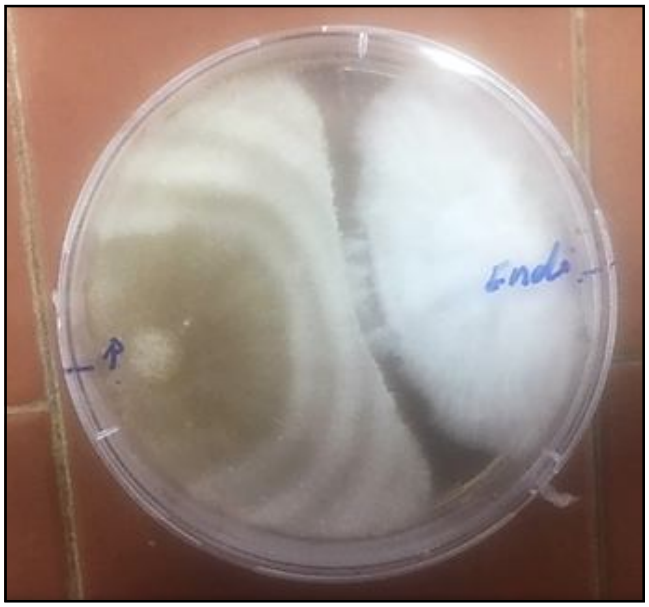
Endophytic fungi isolates	Inhibition percentage %	
	P. aphani-dermatum	***R. solani***
Trichoderma harizianum	55.48	61.55
Rosellinia sanctae-cruciana	69.35	60.02
Helminthosporium sp	28.62	51.46
Cylindrocarpon sp	35.68	54.91
Nicrosporea sp	43.75	34.86
Alternaria alternata	51.32	29.75
Ulocladium sp	25.22	40.48
Acremonium sp	42.87	43.03
Cladosporium sp	25.59	29.37
Hemicola sp	39.47	36.14
Aspergillus flavus	33.16	42.52
Aspergillus niger	35.30	45.08
(P = 0.05) LSD	8.532 *	9.668 *

*Each number in the Table represents three replicates.

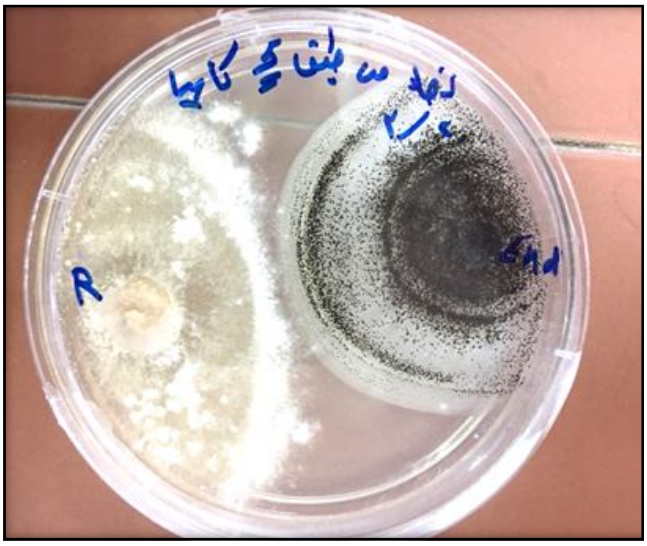
fungi isolates. However, their inhibition percentage ranged between 29.37- 51.46% for the fungi *Cladosporium* spp and *Helminthosporium* sp. respectively and is considered the lowest percentage. The efficiency of the endophytic fungi in inhibiting the pathogens by a dual culture method indicates the efficiency of (Mycoparasitism) for these fungi on the mycelium of pathogen and wrapping around it, where the endophytic fungi secrete secretions and substances that decompose the walls of the hyphae of the pathogenic fungi on the dual farm and may kill it. As well as secretions of substances and antibiotics that prevent its growth and development, in addition to enzymes that decomposed the components of the cell wall or through several mechanisms, such as parasitism on the mycelium and secretions of antibiotics (El -Nagerbi, 2013). From this test, it can be concluded the efficacy of some endophytic fungi in inhibiting the growth of pathogens by competing for food or place, as well as their ability to produce anti-growth for pathogens such as Antibiotic such as Pyrrocidines AB, which is a strong antifungal against Aspergillus spp (Wicklow, 2005). As well as its ability to produce toxins such as Podophyllotoxins aryl tetralin lignans which have been used to treat cancerous diseases and as antioxidants (Puri SC, 2006). All the efficiency of the endophytic fungi indicates its efficiency in Biological control and plant disease control caused by various pathogens including P. *aphanidermatum* and R. *solani* (Garoe, 2013). Based on the highest inhibition percentage, the endophytic fungi of spp *Trichoderma* and *Rosellinia sanctae-cruciana* was selected to conduct experiments under greenhouse conditions.



Picture 13: The inhibitory efficiency of the endophytic fungi *T. harzianum* fungus against *P.aphanidermatum.* A - *P.aphanidermatum* B - antagonism test (a: pathogen, b: endophytic fungi).



Picture 14: The inhibitory efficiency of the endophytic fungi R. sanctae-cruciana against pathogenic fungi R. *solani.*



Picture 15: The inhibitory efficiency of the endophytic fungi *Aspergillus niger* against the fungi R. *solani.*

Endophytic bacteria isolation from the plant

The results of isolating and identifying the endophytic bacteria obtained from the leaves of cucumber plants, *Acasia nilotica* , *Cymbopogon citrate* and *Thevetia peruviana* , which are 120 species. Table 9 showed the identification of three species of the endophytic bacteria, where the experiment showed 89 colonies belonged to 3 bacterial pieces *Pseudomonas* fluorescens, *Azotobacter* spp, and Bacillus subtillis, with a total C.F% for all bacterial colonies reached 74.16%. The highest C.F% was is for *Pseudomonas* fluorescens, as it reached 26.66% of all colonies obtained from the leaves pieces of the plants referred to above, and the total number of their colonies was 32 colonies distributed as follows:

12 colonies of cucumber leaves with a C.F% of 40%.

8 colonies of *Acasia nilotica* leaves, with a C.F% of 26.66%.

6 colonies of *Cymbopogon citrate* leaves, with a C.F% of 20%.

6 colonies of *Thevetia peruviana* leaves, with a C.F% of 20%.

Whereas, *Azotobacter* spp. recorded a C.F% of 24.16% of all colonies obtained from the leaves pieces of plants referred to above, and the total number of their colonies were 29 colonies distributed as follows:

6 colonies of cucumber leave with C.F% of 20%.

6 colonies of *Acasia nilotica* leaves, with a C.F% of 20%.

8 colonies of *Thevetia peruviana* leaves, with a C.F% of 26.66%.

9 colonies of *Thevetia peruviana* leaves, with a C.F% of 30%.

The lowest C.F% was for the Bacillus subtillis, as it reached 23.33% of all colonies obtained from the leaves pieces of plants referred to above, and the total number of their colonies was 28 colonies distributed as follows:

5 colony of cucumber leaves, with a C.F% of 16.66%.

5 colonies of *Acasia nilotica* leaves, with a C.F% of 16.66%.

7 colonies of *Cymbopogon citrate* leaves, with a C.F% of 23.33%.

11 colonies of *Thevetia peruviana* leaves, with a C.F% of 36.66%.

Table 9 indicates that C.F% of the three bacterial species colonies in the leaves of cucumber plants has reached 76.66%, as the total number of their colonies was 23, distributed as follows:

Colonies of *Pseudomonas fluorescens* with a C.F% of 40%.

6 colonies of *Azotobacter* spp, with a C.F% of 20%.

5 colonies of *Bacillus subtillis* with a C.F% of 16.66%.

While the table indicated that the C.F% of the three bacterial species colonies in the leaves of the *Acasia nilotica* plant has reached 63.33%, as the total number of their colonies was 19 colonies distributed as follows:

8 colonies of *Pseudomonas fluorescens* with a C.F% of 26.66%.

6 colonies of *Azotobacter* spp, with a C.F% of 20%.

5 colonies of *Bacillus subtillis* with a C.F% of 16.66%.

While the C.F% of the bacterial species colonies in the leaves of *Cymbopogon citrate* plant reached 70%, as the total number of their colonies was 21 colonies distributed as follows:

6 colonies of *Pseudomonas fluorescens* with C.F% of 20%.

8 colonies of *Azotobacter* spp, with a C.F% of 26.66%.

7 colonies of *Bacillus subtillis* with a C.F% of 23.33%.

The table showed that the with a C.F% of the three bacterial species colonies in the leaves of *Thevetia peruviana* reached 86.66%, as the total number of their colonies was 26 colonies distributed as follows:

6 colonies of *Pseudomonas* fluorescens, with a C.F% of 20%.

9 colonies of the *Azotobacter* spp. with C.F% 30%.

11 colonies of *Bacillus subtillis* with C.F% of 36.33%.

The efficiency test of endophytic bacteria in inhibiting the pathogens *Pythium aphanidermatum* and *Rhizoctonia solani*

The results showed that there was a variation in the antagonistic potential of endophytic bacteria isolates in inhibiting the pathogens by calculating the inhibition percentage. As the endophytic bacteria, *Pseudomonas*

Table 9: Isolation of the endophytic bacteria from the cucumber plants *Acasia nilotica*, Cymbopogon citrate and *Thevetia peruviana* and the F.C% for each species of bacteria in each plant type.

Bacterial isolates species	No. of colonies in Cucumber plant samples	C.F% in cucumber pieces	No. of colonies in Cassia plant samples	C.F% in Cassia pieces	No. of colonies in cymbopogon citrates plant samples	C.F% in cymbopogon citrates pieces	No. of colonies in *Thevetia peruviana* samples	C.F% in Thev. pieces	Total colonies for all plant samples	C.F% for the total No of pieces
Pseudomonas fluorescens	12	40	8	26.66	6	20	6	20	32	26.66
Azotobacter spp	6	20	6	20	8	26.66	9	30	29	24.16
Bacillus subtillis	5	16,66	5	16.66	7	23.33	11	36.66	28	23.33
Total number of	23	76.66	19	63.33	21	70	26	86.66	89	74.16
pieces produced isolates									F.C%	
Total used pieces	30		30		30		30		120	100

*Each number in the Table represents three replicates.

Table 10: The efficiency test of endophytic bacteria in inhibiting the pathogens P. *aphanidermatum* and R. *solani.*

Endophytic bacteria isolates	Inhibition percentage %	
	P. aphani-dermatum	***R. solani***
Pseudomonas fluorescens	73.41	70.12
Azotobacter spp	56.58	54.51
Bacillus subtillis	60.37	52.31
(P=0.05) LSD	7.337 *	7.164 *

* Each number in the Table represents three replicates.

Picture 16: Antagonistic potential of bacteria P.*fluorescens* against pathogenic fungi R. *solani* on the culture medium P.D.A.

Picture 17: Antagonism between the pathogen P. *aphanidermatum* and bacteria *P. fluorescens* after 48 hours.

fluorescens showed the highest inhibition percentage of the pathogen *P.aphanidermatum* reached 73.41%, followed by bacteria *B. subtillis* with insignificant difference, as the inhibition percentage was 60.37%, while the bacteria *Azotobacter* spp recorded the lowest inhibition percentage amounted to 56.58%. As for the fungi *R. solani*, the bacteria *Pseudomonas fluorescens* showed the highest inhibition percentage amounted to 70.12%, followed by *Azotobacter* spp, with a slight insignificant difference, and the inhibition percentage was 54.51%, while *Bacillus subtillis* recorded the lowest inhibition percentage of 52.31%. Based on these results, which appeared in table 10, that bacteria *Pseudomonas fluorescens* recorded a high antagonistic potential against the pathogen *P.aphanidermatum* and *R. solani* fungi in the PDA medium, where the bacteria caused the highest inhibition of the two pathogen reached 73.41% and 70.12%, respectively, as in the Pictures (16-17). The high antagonistic potential of *P. fluorescens* may be due to its production of antibiotics that can inhibit the growth and activity of plant pathogens. The most important of these compounds are Phenazins and Hydrogen Cyanide (Voisard *et al.,* 1989), Siderophores, Pterines, Pyrroles (Shanahan *et al.,* 1992) and enzymes Protases and Chitinases (Nielsen *et al.,* 1998) and Peptidase (Nielsen, 2000). These findings are consistent with what some researchers have mentioned the ability of bacteria *P. fluorescens* in inhibiting the growth of many pathogenic fungi in nutrition media. (Andersen *et al.,* 2003) stated that *P. fluorescens* inhibited the growth of *R. solani* and *Pythium* ultimum in the culture medium by producing some antibiotics such as lipopeptide cyclic and Amphisin, while the bacteria. *P. fluorescens* showed a high antagonistic potential to inhibit the growth of the pathogens *P.ultimum*, *R.solani* in culture medium by producing some enzymes that break down fungal cell walls such as endochitinase (Nielsen *et al.,* 1998). Therefore, it has been selected as endophytic bacteria to conduct field experiments as a pathogen control agent.

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