Isolation and Diagnosis of *Sclerotinia Sclerotiorum* (Lib) Debary Fungus that Causes White Mold Disease on Eggplant Plants in Iraq and the Effectiveness of Some Elements of Biological Control in Inhibiting It in Vitro

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Abstract

The isolation and diagnosis of the fungal growth growing from the Sclerotia on the PDA culture media showed that several different isolates and different regions of the pathogenic fungus *Sclerotinia sclerotiorum*. The results of migrating the PCR products on agarose gel were shown by giving a band of 500-580bp for *S. sclerotiorum* fungus, 550bp for *Penicillium commune* fungus, and 590 for *Trichoderma. asperellum* fungus, where these two fungi are used in the biological control for the pathogenic fungus *S. sclerotiorum* . The isolates of this pathogen a significant reduction in the percentage of germination of radish seeds grown on the water agar media compared to the control treatment that amounted to 100%, where the percentage of germination ranged between 0.00-53.0%.It was also found that all the tested isolates of the *S.sclerotiorum* were pathogenic to eggplant seedlings, and the severity of infection ranged from 86.1 to 50.0%, compared to the control treatment which its infection severity amounted to 0.0%. The results showed that there was a high Antagonistic Potential between the elements of biological control and the *S. sclrerotiorum* in vitro compared to the control treatment which amounted to 0.0% , where the bacteria treatment of *Pseudomonas fluorescens* gave the highest percentage of inhibition amounted to 100%, followed by the treatment of the *P. commune* fungus which amounted to 86.1% .The treatment of *Trichoderma viride* and *T. asperellum* fungi gave a percentage of inhibition amounted to 72.8% and 70.0%, respectively, where no significant differences were observed between them in the percentage of inhibition compared to the control treatment, which amounted to 0.0%.The percentage of inhibition amounted to 100% using the topsin fungicide, while the treatment of humic acid and folic acid did not give any percentage of inhibition which amounted to 0.0%.

Keywords: Sclerotinia sclerotiorum, DeBary fungus, eggplant plants

1. INTRODUCTION

Eggplant is infected with a number of pathogens, especially the fungi that are endemic to the soil, causing heavy losses to farmers. The *Sclerotinia sclerotiorum* (Lib) DeBary fungus, which causes white mold disease, causes great economic losses in its production (Satyendra et al., 2012). In general,

the percentage of infection with *S. sclerotiorum* range from 50-100%, where it is losing millions of dollars annually around the world due to this fungus (Subbarao, 1998, Tahtamouni et al., 2006). While the percentage of infection increased by more than 85% depending on the planting date and the surrounding environmental conditions (Kamran et al., 2018). Numerous studies have shown that *S. sclerotiorum* contains four types of endo-PGs and two of exo-PGs (Li et al., 2004). It has been proven that these enzymes have different biochemical properties and have necrotic activity on different hosts. This contrast has given the flexibility and ability of the disease to adapt to a wide range of plant hosts (Bolton et al., 2006). Increasing concern about the environmental and human health impact of pesticides in general and fungicides and herbicides, in particular, it has prompted the development of alternative agricultural technologies (McDonald and Linde, 2002). Chemical control in large areas is costly and uneconomical, and its use causes an imbalance in the biological balance for the organisms present inside the soil, where it affects the untargeted micro-organisms (McGrath, 2001). Therefore, attention has focused on treating diseases that affect this vegetable crop by applying biological control methods using some types of biological fungi that have high antagonistic properties and effective metabolic activity against pathogens, as well as using some types of important biological bacteria that work to induce systemic resistance and inhibiting the action of the pathogen. As well as it has the ability to equip the plant with some basic elements for growth and this type of bacteria is called Plant Growth Promoting Rhizobacter. Deepti and Johri, (2003) defined it as a group of bacteria that are attached to the roots and promote plant growth, where they have the ability to interact closely with the roots, thus affect plant health and soil fertility. Due to the importance of white mold disease on the eggplant crop in Iraq and the lack of effective chemicals in controlling it, in addition to its other side effects, the research has focused on finding effective alternative biological methods.

2. MATERIALS AND METHODS

Isolation of the pathogenic fungus *Sclerotinia sclerotiorum* (Lib) DeBary :

Eggplant plants infected with the white mold caused by *S. sclerotiorum* were collected from different regions of the central Euphrates after placing them in bags (polyethylene), where the isolated samples of these isolates were symbolized by (Ss1-Ss12), respectively. The Sclerotia formed inside the stems of the infected plants were collected and surface sterilized with a solution of sodium hypochlorite at a concentration of 1% prepared by the method of (O, Neill, 2009) by applying the equation:

Volume of commercial solution= final required volume x final concentration / commercial concentration

It is also known that the concentration of the commercial solution is 6%, so at the 100 ml of the final solution at a concentration of 1%, the application of the equation is as follows:

Volume of the commercial solution = 100 x 1/6 = 16.66

where It was placed in the solution for 2-3 minutes, then was washed twice with sterile distilled water, dried on sterile filter paper, and then cultured into plastic Petri dishes with a diameter of 9 cm equipped with Potato Dextrose Agar (PDA) added to it the antibiotic Tetracycline at a concentration of (250 $mg.L^{-1}$) after sterilization with an Autoclave device (121 m at a pressure of 5.1 $kg.cm^{-2}$) for 15 minutes, and the dishes were incubated at 24 ° C for 5 days or until the formation of the Sclerotia, then cultured again in the same way, and the

isolates were purified after 4-5 days by transferring part of the edges of the fungal growth Of the colony into a 9 cm Petri dishes containing the culture media (PDA). Isolates were diagnosed from the pathogenic fungi treatments through the formation of Sclerotia for each isolate. The Sclerotia of the isolates were preserved until use in the subsequent study experiments.

Molecular Diagnosis for Isolates of The Pathogenic Fungus *S. Sclerotiorum* using Polymerase Chain Reaction (PCR) Technology. It includes several steps:

1- DNA extraction from *S. sclerotiorum* isolates which Isolated in this study:

DNA was extracted from *S. sclerotiorum* isolates using the kit (Cat. No: FAPGK100) provided by Favorgen Company, Taiwan-China.

2- Estimating the concentration and purity of the DNA extract.

The DNA concentration was estimated using a Spectrophotometer under the wavelength of 260nm, and the concentration of the DNA was known through the following equation:

DNA concentration ($\mu g.ml^{-1}$) = the optical absorption value at the wavelength of 260nm x 50 x dilution factor

The purity of DNA has also been determined by applying the following equation and described by (William et al., 1997):

$$\text{DNA purity} = \frac{\text{The amount of absorption at a wavelength of 260 nm}}{\text{The amount of absorption at a wavelength of 280 nm}}$$

The DNA extracted from *S. sclerotiorum* isolates was then kept at -20 °C until tested using the technique of polymerase chain reaction (PCR).

3- Using polymerase chain reaction (PCR) technique

For the purpose of diagnosing the isolated fungi in this study, a polymerase chain reaction test was performed using the kit (Maxime PCR PreMix (i-Taq), (Cat. No. 25026)) provided by iNtRoN Company, Korean. The polymerase chain reaction was conducted with a total volume of 20 µl containing 1 µl of each of Forward primer (TCCGTA GGTGAACCTGCGG: ITS1) and Reverse primer (TCCTCCGCTTATTGATATGC: TS4) (White et al., 1990) and 1 µL of extracted DNA. All of the above ingredients were placed in the tube prepared by the manufacturer company and the volume was completed with Nuclease-free water to 20 µl. The DNA of *S. sclerotiorum* isolates was replicated using polymerase chain reaction (PCR) steps and conditions (Zhang et al., 2012).

4- Agarose gel electrophoresis

The agarose gel layer was prepared after taking 1 g of the agarose powder and dissolving it in 100 ml of Buffer Solution (Tris boric acid EDTA buffer ×1) until the mixture turned into a clear solution. 5 µl Ethidium bromide dye was added after the solution temperature was reduced to 45-40 °C. The mold was prepared for pouring the agarose containing the comb at one end to make holes in the gel layer,

then pour the dissolved agarose containing Ethidium bromide and leave it to harden at room temperature. When the agarose layer has hardened, the comb was carefully lifted and the mold was then placed in the electrophoresis device. a solution of 1 x TBE was then added to the electrophoresis container, covering the agarose layer, with a height of approximately 1 cm. 10 µL of the DNA replicated by PCR was added to each well of the wells of the previously prepared agarose gel layer. In addition, 5 µL of the DNA ladder was added to the well on the left side of the added samples for the purpose of determining the volumes of the replicated DNA. The power supply electrodes were connected to the current and run at 150 mA for one hour. After completing the sample migration process, the agarose gel layer containing the DNA bands (PCR products) was examined under UV transillumination, and images were taken.

5- DNA base sequence analysis of *S. sclerotiorum* isolates.

For the purpose of diagnosing the isolated fungi, the DNA products (PCR amplicons) replicated from the isolates of *S. sclerotiorum* by means of a polymerase chain reaction (PCR) with the Primers (ITS1) and (ITS4) were sent to the Korean company (Macrogen) for the purpose of determining the Nucleotide sequence and In both the forward and backward directions of the replicated DNA. All Nucleotide sequences were analyzed using the BLAST program (Basic Local Alignment Search Tool) to compare them with the data available in NCBI, the National Center for Biotechnology Information, which belongs to the same fungus *S. sclerotiorum* , which is globally diagnosed.

Testing the pathogenicity of *S.sclerotiorum* isolates in vitro, as well as the effect of these isolates on eggplant seedlings under greenhouse conditions.

Pathogenicity of *S. sclerotiorum* isolates was tested using the method of (Bolkan and Butler, 1974). The reading was taken after of 15 days culturing the radish seeds, where the percentage of seed germination was calculated according to the following equation:

$$\text{The percentage of seed germination} = \frac{\text{The number of seeds germinated in a treatment}}{\text{The number of seeds germinated in a control}} \times 100$$

The pathogenicity of *S. sclerotiorum* isolates was also tested under greenhouse conditions. In this experiment, a mixture of loam and peat moss soil (1: 2) was used after sterilization with commercial formalin by preparing a solution of 1: 50 formalin/water (20 ml of formalin per liter of water) (commercial formalin concentration 40), the solution was used at a ratio of 3 liters of water.m^{-3} of soil (Al-Tawajin, 1975). The soil was distributed on plastic pots with a rate of 7 kg of soil per pot with a diameter of 24 cm from the top, 18 cm from the bottom, and a height of 22 cm. it was cultivated with seedlings of the eggplant plant (Barcelona) at the age of one month. Three replicates were made for each isolate, with the rate of three plants per replicates. Pots were placed inside the greenhouse to conducting the necessary agricultural operations, such as irrigation and fertilization. Plants were inoculated with *S. sclerotiorum* isolates, which included isolates isolated from infected areas that were previously grown on PDA media according to the method of (Petzoldt and Dickson, 1996). A wound was made on the main stem of each plant, and a portion of the pathogenic isolate was placed on the wound, taken from the edge of the fungal colony with the age of 5-6 days, by a cork borer (8 mm diameter), while three replicates remained without inoculation with the pathogenic fungus as a control. The readings were taken after observing the cases of wilting and death of most

plants, and the severity of the infection was recorded according to the grade (Dixon and Doodson, 1971) with conducting some modifications on it, where the old grade was based on measuring the fungal growth longitudinally. The grade includes the following:

0 = Non infection.

1 = Surrounding the stem with fungal rot with a rate of less than 1/2.

2 = Surrounding the stem with fungal rot with a rate of 1/2 to less than the full circumference.

3 = Fully enclosing the stem with a fungal rot.

4 = Plant death.

The severity of the infection was calculated according to the formula of (Mckinny, 1923) and as follows:

$$\text{The severity of the infection} = \frac{\text{The number of plants (gade } 0\times 0) + \ldots\ldots + \text{The number of plants (gade } 4\times 4)}{\text{the total number of tested plant} \times 4} \times 100$$

Testing the efficacy of *Pseudomonas fluorescens* and *Trichoderma viride* , *T.asperellum*, and *Penicillium commune* in inhibiting the isolation of the pathogenic fungus *S.sclerotiorum* on the culture media (PDA):

Preparing the suspension of *P. fluorescens* bacteria.

The isolates of the bacteria were obtained from the deteriorate of Agricultural Research, Baghdad, Zaafrana, which was previously isolated from the eggplant plant located in the fungicide laboratory, the biocides section, where they were propagated on a Nutrient broth media in 500 ml glass flasks sterilized in an autoclave at a temperature of 121 °C and a pressure of 1.5 kg.cm^{-2} for 15 minutes. The media was then inoculated with each of the prepared bacteria by taking a swab using a sterile tube from bacterial growth growing on the Nutrient agar media that previously prepared at age of 48 hours. The components of the decanter were well mixed and incubated at 32 ± 3 °C for 3-4 days. After determining the effective concentration of *P. fluorescens*, which inhibits the growth of the *S.sclerotiorum* fungus using the dilution method, the dishes prepared for the experiment were inoculated by taking 1 ml of each dilution of the *P. fluorescens* inoculum with distributing the bacterial inoculum homogeneously to each dish. A disc with a diameter of 0.5 cm was then placed in its center from the edges of the colony of *S.sclerotiorum* isolate grown on the PDA media at age of 5 days, with the rate of four dishes for each dilution. Four dishes of fungi were left without inoculation with the bacteria. 1 ml of sterile distilled water was added to each of them for the control. The dishes were incubated at 24 ± 2 °C until the fungus in the control treatment reaches the edge of the dish. The percentage of inhibition for fungal growth was calculated according to the equation of (Montealegre et al., 2003):

The percentage of inhibit for fungal growth $= \left(1 - \frac{\text{Fungal growth in the treatment of bacteria}}{\text{fungal growth in the control treatment}}\right) \times 100$

Testing the Antagonistic Potential of *Trichoderma viride* , *T.asperellum*, and *Penicillium commune* in inhibiting the isolate of the pathogenic fungus *S.sclerotiorum* in vitro:

The experiment was conducted to study the antagonistic relationship between the pathogen fungus (sclerotiorum S.) and the fungi of biological control (viride T., asperellum. T and commune .p), which was obtained using the double culture technique, with a rate of four replicates per treatment. four dishes were left as a control treatment for any pathogenic fungus alone. The dishes were placed in the incubator at a temperature of 24 ± 2 °C, and after the growth of the pathogenic fungus reached the edge of the dish, the Antagonistic was estimated according to the scale of (Bell et al., 1982), which consisted of 5 grades as follows:

Grade 1 - the antagonist fungus covers the entire dish area.

Grade 2 - the antagonist fungus covers two-thirds of the dish area.

Grade 3 - the antagonist fungus and the pathogenic fungus, each covering half of the dish.

Degree 4- the antagonist fungus covers one-third of the dish area and the pathogenic fungus covering two-thirds of the dish area.

Grade 5 - the pathogenic fungus covers the entire area of the dish.

The biological factor is considered to be antagonistically effective when showing a degree of antagonism of 2 or less with the isolation of the pathogenic fungus under study. The percentage of inhibition for fungal growth was calculated according to the equation (Montealegre et al., 2003) mentioned above.

The percentage of inhibit for fungal growth =

$$\frac{\text{The average diameter of the control colony} - \text{Average diameter of the treatment colony}}{\text{The average diameter of the control colony}} \times 100$$

Statistical analysis

The Completely randomized design (CRD) for the laboratory experiments was used and the Statistical Analysis System (SAS, 2012) program was used in analyzing the data to study the effect of different treatments on the studied traits, and the significant differences between the averages were compared according to the Least Significant Difference (LSD) test.

3. RESULTS AND DISCUSSION

Isolation and diagnosis of the pathogenic fungus *S. sclerotiorum* that cause white mold disease on eggplant plant. The results of isolation and diagnosis of the fungal growths growing from the Sclerotia on the culture media (PDA) showed that several different isolates were obtained from different regions of the pathogenic fungus *S.sclerotiorum* where the

fungus was characterized by a white cotton growth that completely covered the culture media after 4 days of incubation and after 7 days, assemblages of The fungal Hypha in the form of small white blocks at the edge of the dish turned into black Sclerotia. The formation of Sclerotia has also concentrated at the edge of the dish, and this was due to the physical symptoms of the wall of the dish in which the fungi were grown, which stimulated the formation of these Sclerotia. These bodies were distinguished by their different numbers and sizes and they were of irregular shapes, and this was by different isolates. The results of the microscopic examination for the fungal hypha showed that it is divided, transparent, branched and the isolates were taken from plants that had symptoms of infection, where the infection was characterized by the appearance of symptoms on the leaves, necks, and stems of the eggplant plant. These symptoms were also characterized by the presence of pathological signs, which are the formation of Sclerotia and a white cotton growth on the affected areas and the emergence of this disease during the flowering period and shortly before the flowering period as shown in figure (1). These results were similar to the characteristics of the fungus *S. sclerotiorum* that causes white mold disease on the eggplant plant referred to by many studies (Kohn, 1979; Bolton et al., 2005).

Figure 1: The symptoms and pathological signs caused by the Sclerotinia sclerotiorum fungus.

Molecular Identification

The results of DNA electrophoresis on agarose gel showed the efficiency of the extraction kit produced by Favorgen Company, Taiwan-China, in extracting the DNA, where the results of the electrophoresis of PCR products on agarose gel showed a concentration of 1.5% by giving a band size of 500-580bp for the pathogenic fungus *S. sclerotiorum* , 550 for *P. commune* bp, and 590 for *T. asperellum*, and The last two fungi are used in the biological control of the pathogenic fungus *S. sclerotiorum* as shown in figure (2). The beams were seen using a UV transilluminator that relies on ultraviolet rays in its work. After the results of the

nucleotide sequences of the fungus genome were obtained when using the frontal Primer (ITS) sequencing that reveals the genetic region confined between ITS1 and ITS4 regions used from the Korean company (Macrogen). The alignment was matched with the global isolate sequences of *S. sclerotiorum* and *P. commune*. *T. asperellum*, registered in the National Center for Biotechnology Information (NCBI) database, to determine the type of fungus diagnosed by using the "https://blast.ncbi.nlm.nih.gov/Blast.cgi" located within The official website of the global organization NCBI on the World Wide Web. The results of the analysis for the nucleotide sequences of the PCR reaction showed the presence of *S. sclerotiorum* in the first 12 isolates, and the isolate (9) differed from the rest of the isolates according to some Nucleotide Sequencing for the *S. sclerotiorum* fungus, which gave a percentage of matching about 99%. The results also showed that the Iraqi isolate is identical with the rest of these isolates, with a ratio of 100% as shown in the appendix (1). The results also showed a genetic linkage between isolate (6) and isolate (9) as shown in Figure (3), which shows the tree of genetic analysis, which shows the genetic relationship between *S. sclerotiorum* isolates isolated in this study, while the rest of the studied isolates were similar in terms of the genotypes for these isolates. Table (1) shows the similarity ratios between the Nucleotide Sequencing of the DNA products duplicated by PCR-amplified products from *S. sclerotiorum* isolates isolated in this study. These results agree with (Tok et al., 2016; Ali and Aljarah, 2018) who used the Primers in the molecular diagnosis of the pathogenic fungus *S. sclerotiorum*. The results of the analysis also showed the nucleotide sequences of the PCR reaction to isolate the fungus used in biological control. The diagnosis of *T. asperellum* in one of the samples was diagnosed. The tree of the genetic analysis (Figure 4) shows the genetic relationship between *T. asperellum* isolates isolated in this study with the same fungi isolates registered in the National Center for Biotechnology Information.

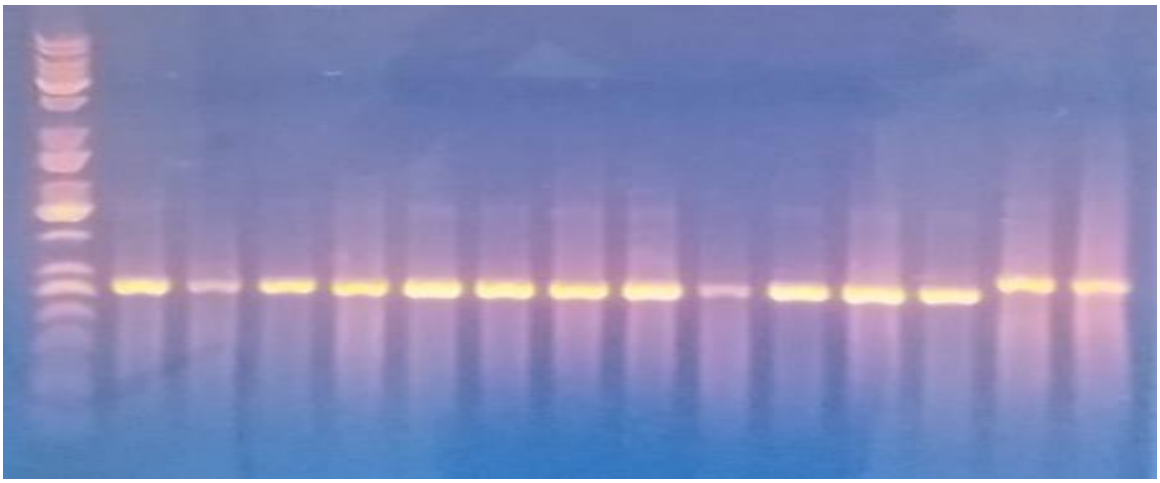
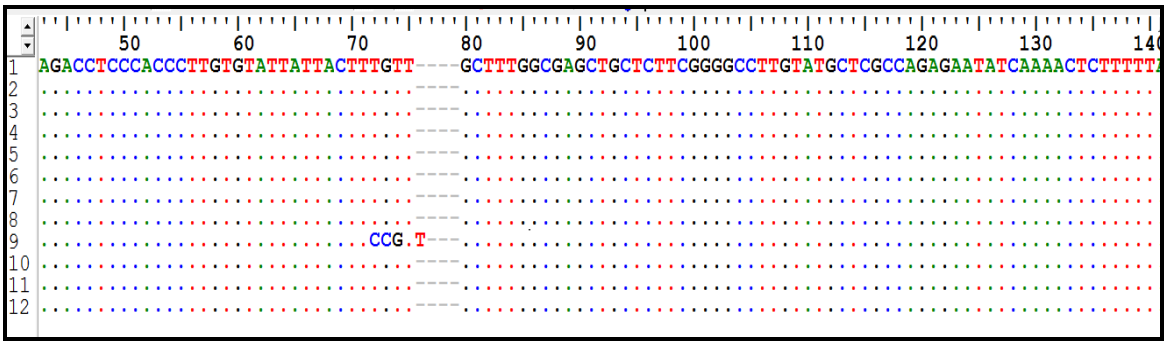


Figure 2: Results of the electrophoresis for the PCR products of the Primer (ITS) which reveals the genetic region confined between ITS1 and ITS4 regions on a 1.5% agarose gel (for 60 minutes, 70V, 65A current). The bands were seen using a UV .transilluminator that relies on ultraviolet rays in its work

where: M = DNA Marker.



Appendix 1: The similarity and difference in some regions of the sequence alignments of the multiplexed PCR-amplified products from *S. sclerotiorum* isolates isolated in this study and their numbers are confirmed on the left side of the figure. Similar nitrogenous bases are represented by points.

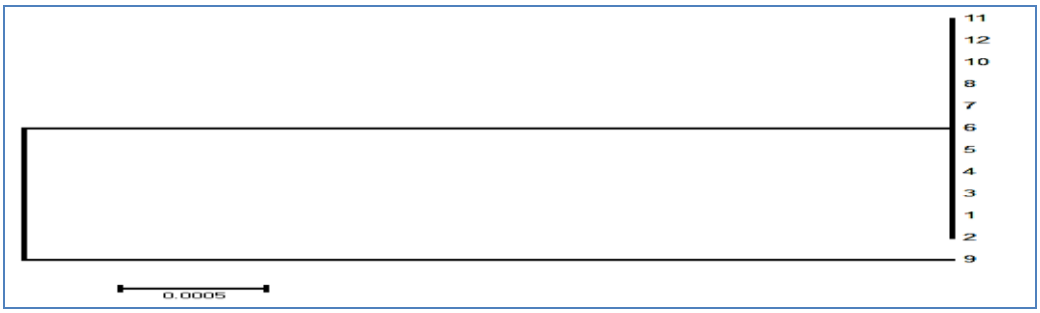


Figure 3: The Neighbor-joining tree shows the genetic relationship between *S. sclerotiorum* isolates that isolated in this study.

Table 1: The percentage of similarity between the nitrogenous base sequences of the DNA products multiplied by PCR-amplified products from *S. sclerotiorum* isolates that isolated in this study.

No. of isolate												
1	-											
2	100	-										
3	100	100	-									
4	100	100	100	-								
5	100	100	100	100	-							
6	100	100	100	100	100	-						
7	100	100	100	100	100	100	-					
8	100	100	100	100	100	100	100	-				
9	99	99	99	99	99	99	99	99	-			
10	100	100	100	100	100	100	100	100		-		
11	100	100	100	100	100	100	100	100	100	100	-	
12	100	100	100	100	100	100	100	100	100	100	100	-
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

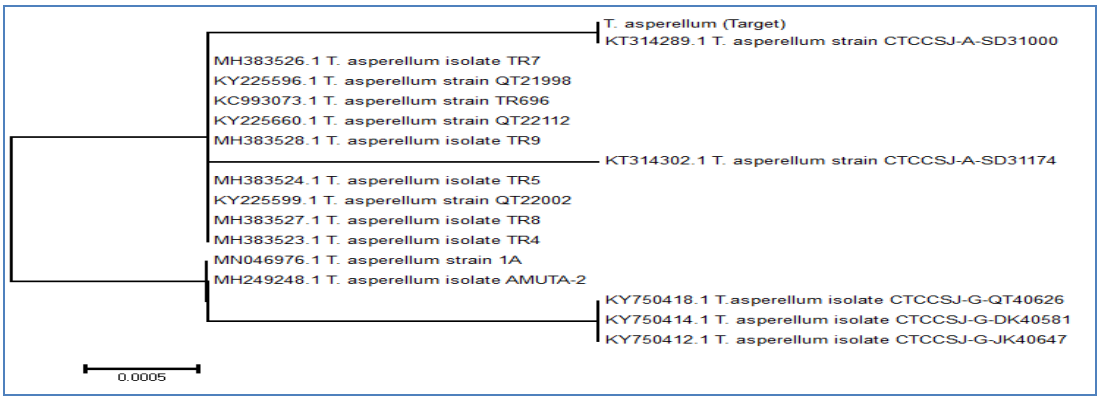


Figure 2: the Neighbor-joining tree shows the genetic relationship between *T. asperellum* isolates isolated in this study with the same fungus isolates registered in the National Center for Biotechnology Information.

Testing the pathogenicity of *S.sclerotiorum* isolates in vitro, as well as the effect of these isolates on eggplant seedlings under greenhouse conditions.

Table (2) shows that *S.sclerotiorum* isolates caused a significant reduction in the percentage of germination for radish seeds grown on the water agar media compared to the control treatment that amounted to 100%, where the percentage of germination ranged between 0.00 - 53.0%. The isolates Ss4, Ss5, Ss6, Ss9, and Ss11 gave the lowest percentage of germination amounted to 0.0%, due to their high pathogenicity, while Ss6 and Ss12 isolates were less pathogenic and gave a percentage of germination amounted to (39.0 and 53.0%), respectively. The reason for the difference in the pathogenicity between these isolates is due to the difference in their genotypes between these studied isolates. These results agree with (Al-Tameemi, 2019) who found that the difference in pathogenicity varies according to the different fungal isolates grown on nutrition media in vitro and on eggplant seeds and also Radi, (2019) in its study on radish seeds. Radish seeds were used to detect the pathogenicity of different pathogens (Sneh et al., 2004). The reason for infecting radish seeds with the pathogen in this experiment is possibly due to the production of enzymes that degrade the cell walls of the plant host such as, Pectinases, Hemicellulases, Proteases, Cellulases Endo polygalacturonases. The activity of these enzymes increases under acidic conditions, which is achieved by the fungus production of oxalic acid, as well as the toxicity of this acid to the host's tissues, which leads to the decomposition of the middle walls of the plant cells for this tissue (Poussereau et al., 2001 and Girard et al., 2004). Table (2) also indicates the effect of different *S.sclerotiorum* isolates on the percentage of infection on eggplant seedlings under lath house conditions, where it was found through this experiment that all tested isolates were pathogenic to eggplant seedlings and the severity of infection ranged from 86.1 to 50.0% compared to the control treatment which their severity of infection amounted to 0.0%, with the difference in the severity of infection for each isolate. The isolates Ss4 and Ss9 gave the highest severity of infection which amounted to (86.1 and 83.3%), respectively, while Ss6 and Ss12 isolates gave the lowest severity of infection which amounted to 50.0% for both isolates. these results agree with the results of the percentage of germination for radish seeds, which represents the pathogenicity of the fungus isolates *S.sclerotiorum* . This may be due to

the fact that *S.sclerotiorum* produces a group of enzymes that degrade plant cell walls, and it is also known that a plant cell consists of more than 10% of a wall of proteins. Pectin is considered the main component of plant cell walls, so the enzyme Protease and Pectinase have an important and main role in the disease development of this pathogen or the hydrolysis of pectin. It works to weaken the cell wall to facilitate penetration and colonization of the host as well as to provide the fungus with the necessary carbon sources for growth. The pathogenic fungus produces several forms of pectinolytic enzymes that have the ability to analyze plant host cells, thus are important factors in the pathogenicity of this fungus (Favaron et al., 1994).

Table 2: Testing the pathogenicity of *S. sclerotiorum* isolates in vitro, as well as the effect of these isolates on eggplant seedlings under greenhouse conditions.

Isolates	The percentage of germination for radish seeds	The number of seeds germinated from radish seeds	The severity of the infection
Ss1*	**5.0	**1.25	***55.6
Ss2	27.0	6.75	58.3
Ss3	39.0	9.75	50.0
Ss4	0.0	0.00	86.1
Ss5	0.0	0.00	66.7
Ss6	3.0	0.75	66.7
Ss7	13.0	3.25	58.3
Ss8	29.0	7.25	63.9
Ss9	0.0	0.00	83.3
Ss10	23.0	5.75	55.6
Ss11	0.0	0.00	71.8
Ss12	53.0	13.25	50.0
المقارنة	100	25	0.0
L.S.D. (5%)	19.17	4.793	12.83

Sc* = Sclerotinia sclerotiorum and the number near the symbol represents the number of isolates

** Each number in the column represents a rate of four replicates

*** Each number in the column represents a rate of three replicates

Testing the Antagonistic Potential of some elements of biological control against th *S. sclerotiorum* fungus, using the dual culture technique in vitro.

The results of this test showed the presence of a high Antagonistic Potential between the factors of biological control and the pathogenic fungus *S. sclrerotiorum* compared to the control treatment which amounted to 0.0%, which included some fungi, including *Trichoderma virid*, *T. asperellum* and *Penicillium. commune* as well as one type of bacteria that is *Pseudomonas fluorescens*. In addition, the fungicide Topsin, humic acid, and folic acid were used to know their effect on the growth of the pathogenic fungus in vitro. Table (3) shows the results of the investigation of the fungi used in biological control with a high

Antagonistic Potential, where it amounted according to the scale set by (Bell et al., 1982) within the second grade, which is the grade to which the fungus is considered biological within this scale and after four days of cultivation of the pathogenic fungus, until it arrives The pathogenic fungus to the edge of the dish, where *P. fluorescens* had a significant effect in obtaining the highest percentage of inhibition for the growth of the pathogenic fungus, where the percentage of inhibition for the pathogen amounted to 100%, compared to the control treatment which amounted to 0.0%. This is due to the fact that the mechanism of *P. fluoresens* in resistance is through the production of antibiotics, which are responsible for inhibiting plant pathogens in general and Among the antibiotics produced by *P. fluorescens* are (PLT) Pyuluteorin, (Prn) Pyrolnitrin, (PCA) Carboxylicacid, Phyenazin, and (DAPG) Diacetylphloroglycinol-2,4 (Deepti and Johri, 2003). The treatment of *P. commune* fungus with the pathogenic fungus gave a percentage of inhibition amounted to (86.1%). This is due to the production of this type of fungi to antibiotics, which is one of the important mechanisms of the fungus in biological control. There is evidence of lovastatin production from the environment in which *P. commune* besides its ability to improve the performance of oxacillin antibiotics. The environmental isolate for the fungus has shown that it produces statin and anti-disease products, and this species has proven to be a promising new source in the production of anti-disease products for medical applications (Diblasi et al., 2015). It was followed by the treatment of *T.viride* and *T. asperellum*, which amounted to 72.8% and 70.0% , respectively, where no significant differences were observed between them in the percentage of inhibition. The antifungal activity of *Trichoderma* spp. is due to the production of secondary and volatile metabolites such as ethylene, hydrogen cyanide, aldehydes, and ketones that have an effect on controlling plant pathogens (Barbosa et al., 2001; Vey et al. 2001). Shabir and Rubina, (2010) also mentioned that *T.viride*'s effectiveness in inhibiting the pathogenic fungus may be due to its production of secondary metabolites such as gliotoxin, viridian and glioviridin as shown in Table (3). In addition, 100% inhibition was obtained by using the fungicide Topsin for the pathogenic fungus, while the treatment of humic acid and folic acid did not give any percentage of inhibition, where the percentage of inhibition amounted to 0.0%, where the diameter of the pathogenic colony amounted to 9.00 cm. This is due to the humic and folic acid which made the medium acid and be suitable for the growth of the pathogenic fungus.

Table 3: Testing the Antagonistic Potential of some elements of biological control against the Sclerotinia sclerotiorum fungus, using the dual culture technique in vitro.

No.	Treatment*	Diameter of Colony (cm)	The percentage of inhibition
1	*T.viride* + Ss	**2.450	**72.8
2	*T. asperellum*+ Ss	2.700	70.0
4	*P. commune* + Ss	1.250	86.1
5	*P.fluorescens*+ Ss	0.00	100
6	HA + Ss	9.00	0.0
7	fungicide Topsin +Ss	0.00	100
8	Fungus Ss alone (control)	9.00	0.0
9	L.S.D. (5%)	0.4446	4.940

Sc * represents the fungus *Sclerotinia sclerotiorum*,
P.f = The bacterium *Pseudomonas fluorescens*
** Each number in the table represents a rate of four replicates

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