Effect of some Bio-factors against the Sclerotinia sclerotiorum fungus causing white mold disease on eggplant in vitro

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ABSTRACT

The study aimed to evaluate the efficacy of some plant extracts and Bio-factors against the Sclerotinia sclerotiorum fungus causing white mold disease on eggplant in vitro. The results of isolation and diagnosis showed three isolates of Sclerotinia sclerotiorum. The results of isolation and diagnosis showed three isolates of Sclerotinia sclerotiorum. The results showed that the fluorescens Pseudomonas bacteria had a significant effect in inhibiting the growth of isolate of pathogenic fungi that causing white mold disease of eggplant. The results also showed the Effective microorganism extract (EM-1) in inhibiting the growth of the tested pathogen isolate (S.sclerotiorum (Sc-2)), where the percentages of inhibition amounted to (100.00%). The results showed that all used plant extracts to study their effect against the pathogenic fungus (S. sclerotiorum), which included the leaves of the water hyacinth, Hornwort, and Conocarpus, achieved high effectiveness of inhibitory against the growth of pathogenic fungi on the culture media and in a direct relationship with an increased focus for each treatment. The results indicated that the aqueous extract for the Propolis was inhibiting activity against the pathogenic isolate for the fungi pathogenic (Sc-2), where the percentages of inhibition amounted to (50.77, 52.92 and 74.13%) when adding concentrations of (5, 10 and 15%), respectively. This study showed, for the first time in the world, the efficiency of the cold aqueous extract for propolis and the leaves extract for the water hyacinth, Hornwort, and Conocarpus against the S. sclerotiorum fungus that causing white mold disease on eggplant.

Keywords: White mold, Biological control, Sclerotinia sclerotiorum, Plant extracts, Effective microorganisms.
*Research paper from MSc thesis for the first author.

تأثير بعض العوامل الاحيائية لمقاومة الفطر *Sclerotinia sclerotiorum* المسبب لمرض العفن الأبيض على نبات الباذنجان تحت الظروف المختبرية

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المستخلص

هدفت الدراسة الى تقييم كفاءة بعض المستخلصات النباتية والعوامل الاحيائية ضد المسبب المرضي الفطر *Sclerotinia sclerotiorum*مسبب مرض العفن الابيض على الباذنجان تحت الظروف المختبرية. بينت نتائج العزل والتشخيص الحصول على ثلاث عزلات من الفطر*Sclerotinia sclerotiorum* . وبينت النتائج ان البكتريا *Pseudomonas fluorescens* أحدثت تأثيرا معنويا في تثبيط نمو عزلة الفطر الممرض المسببة لمرض العفن الابيض لنبات الباذنجان. كما اوضحت النتائج فاعلية المستحضر الحيوي EM-1 Effective Microorganisms في تثبيط نمو عزلة الفطر الممرض *S.sclerotiorum* المختبرة (Sc-2), اذ بلغت النسب المئوية للتثبيط 100.00%. اظهرت النتائج ان جميع المستخلصات النباتية المستعملة لدراسة تأثيرها ضد الفطر الممرض *S. sclerotiorum*التي شملت أوراق نبات زهرة النيل والشمبلان والكونوكاربس حققت فعالية تثبيطية عالية ضد نمو الفطر الممرض على الوسط الزرعي وبعلاقة طردية مع زيادة التركيز لكل معاملة، وأوضحت النتائج أن المستخلص المائي للعكبر (البروبوليس) كان ذو

فعاليـة تثبيطيـة ضد عزلة الفطر الممرضة Sc-2، اذ بلغت النسب المئوية للتثبيط 50.77 و 52.92 و74.13 % عند اضافة التراكيز 5 و 10و15% على التتابع. و اوضحت هذه الدراسة لاول مرة في العالم كفاءة المستخلص المائي البارد للعكبر (البروبوليس) و مستخلص أوراق نبات زهرة النيل والشمبلان والكونوكاربس ضد الفطر *S. sclerotiorum* مسبب مرض العفن الابيض على الباذنجان.

الكلمات المفتاحية: العفن الابيض, المكافحة الاحيائية,*Sclerotinia sclerotiorum* ,المستخلص النباتي, فعالية الكائنات الحية الدقيقة

*البحث مستل من رسالة ماجستير للباحث الاول.

1. INTRODUCTION

Eggplant (Solanum melongena L.) is infected with many agricultural pests, the most important of which is pathogenic of white mold disease and this disease is caused by the Bary de (Lib) Sclerotinia Sclerotiorum fungus, especially in greenhouses, which is a fungus that attacks the total vegetative and causes significant losses to the crop (Barros et al., 2015). It is an alternative to the use of chemical pesticides, so new horizons have been energized, such as genetic engineering and Biological control (Siddiqui and Shaukat, 2003; Rothmann and McLaren, 2018; Smolińska and Kowalska, 2018). where the trend has been made to use microorganisms that work to curb plant pathogens, also work at the same time to increase production, these organisms are represented by a group of bacteria called Plant Growth Promoting Rhizobacteria, which includes the genus of Pseudomonas and Bacillus and others. These bacteria contribute to increasing the plant growth as a result of nitrogen fixation and increasing the availability of the nutrients in the soil solution and the effect on the growth of root and the ability to antagonize or reducing the effect of pathogens and forming chelating compounds for the iron element (Siderophores) and some enzymes such as Chitinase and other compounds such as antibiotics and cyanides, the ability to build or change the concentration of growth regulators and bacteria can build the ACC deaminase enzyme, which reduces the concentration of ethylene, thus stimulating growth, and the bacteria build up the B-1,3,3-glucanase enzyme, improving nutrient absorption and accelerating the onset of stress resistance (Ding et al., 2001; AL-whaib, 2006; Tozlu et al., 2016; Hernández-Salmerón et al., 2017; Manasa et al., 2017; Joshi et al., 2018). Previous studies have shown the efficacy of the Effective Micro-Organisms (EM-1) against bacterial and fungal pathogens due to what the biological extract contains the microorganisms that compete with the pathogen and produce during its growth secondary metabolites and anti-fungicides and growth regulators that help improve growth and inhibit the growth of pathogens. It also plays a role in stimulating plant systemic resistance against many pathogens (Nia, 2015). Researchers have increased interest in the use of plant extracts in controlling many pathogenic fungal pathogens of the plant due what these extracts contain the effective secondary metabolic compounds where they possess desirable traits in the environment such as rapid decomposition, high specialization and low toxicity to the host (Lokendra and Sharma, 1978). Due to the importance of white mold disease on eggplant and to control it using some plant extracts and bio-factors, the study aims to isolate and diagnose the pathogen that causes white mold on eggplant, evaluating the efficiency of some plant extracts and bio-factors against the cause of white mold disease on eggplant under field laboratory conditions.

2. MATERIALS AND METHODS

2.1 Isolation and diagnosis of Sclerotinia sclerotiorum (Lib) DeBary

Sclerotinia sclerotiorum was isolated from samples of eggplant plants infected with white mold disease collected from agricultural areas in Babylon province, Al-Mahawil District (Abu Al-Jasim and Al-Bada'a and Al-Musayyib Project). Parts of the stems and branches that showed symptoms of white mold disease were taken, and then cultured with a rate of 4

vegetative pieces in 9 cm diameter Petri dishes containing a 15-20 cm^3 from the Potato Dextrose Agar (PDA) adding to it the Tetracycline antibiotic at a concentration of (200 $mg.L^{-1}$), the dishes were then incubated at a temperature of (25 ± 1 C) for 3 days and the growth of fungi was continued in the dishes. The colonies of the S. sclerotiorum fungus were purified.

2.2 Pathogenicity test

2.2.1 Detecting pathogen isolates using eggplant seeds.

The pathogenicity for the pathogenic isolates of Sclerotinia sclerotiorum fungus, which was isolated from the infected stems for the eggplant, was tested according to the method of (Bolkan and Butler, 1974) using the media and water (20 g Agar, 1 L of distilled water). Dishes were inoculated at its center with a diameter of 0.5 cm from the cultures of fungus isolates that grown on the culture media (PDA) at the age of 5 days for each separately. The dishes were placed in the incubator under a temperature of (25 ± 1 °C) for 2-3 days, a local sterilized eggplant seed was then cultured (sterilized superficially with a sodium hypochlorite solution (1% free chlorine), with a rate of 25 seeds/dish and circularly near the edge of the dish. Three dishes were used for each isolate as replicates in addition to the control treatment without adding the pathogenic fungus. The dishes were incubated after culturing the eggplant seeds in the incubator under a temperature of 25 ± 1 C, the results were then taken after 7 days, and the percentage of seed germination was then calculated.

2.2.2 The effect of isolates for pathogenic (S. sclerotiorum) on eggplant seedlings under the conditions of the lath house

The pathogenicity of S. sclerotiorum isolates, which included Ss-3, Ss-2, and Ss-1, was tested on eggplant seedlings in greenhouse conditions belonging to Al-Mussaib Technical college for the year of 2017. In this experiment, a mixture of loam soil was used after sterilization with the autoclave device and the soil was distributed to plastic pots of 1 kg soil for each pot. It was cultivated with eggplant seedlings at 6 week age, with a rate of three replicates for each isolate, with a rate of two plants per replicate. The pests were placed inside the greenhouse, with conducting the necessary agricultural operations such as fertilization and irrigation for a month and a half. The plants were inoculated with S. sclerotiorum isolates grown on the PDA culture media, Where a wound with the dimensions of (1 cm length and 1 mm depth) made on the main stem of each plant, A portion of the pathogen isolates for the fungus was placed on the wound taken from the edge of the fungal colony at the age of 7 days with a diameter of 8 mm, the pots were covered with polyethylene bags to maintain moisture and prevent any external contamination, while three replicates remained without vaccination with the pathogen as a control treatment. The readings were taken after conducting the inoculation process and the percentage of infection was calculated according to the symptoms appearing on the plant after about a month has passed, according to the following formula:

$$\text{The percentage of infection (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of tested plants}} \times 100$$

The severity of the infection was calculated according to the following pathological index that mentioned in (Dixon and Doodson, 1971) and It includes six grades as follows:

0 = no infection, 1 = Infection with mold not exceeding 2 mm in length from the wound, 2 = The mold is longer than 2 mm to 4 mm longitudinally from the wound, 3 = The mold exceeds 4 mm to 6 mm longitudinally from the wound, 4 = The mold extends to more than 6-8 mm longitudinally on the wound, but it does not complete the full stem circumference, and 5 = The mold more than 8 mm longitudinally from the wound, completely enclosing the stem and

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Sometimes the upper part is droop after it has wilted. The percentage of infection severity was calculated according to the equation of (Mckinney, 1923):

The percentage of infection severity =

$$\frac{(Number\ of\ plants\ in\ degree\ 0\ x\ 0) + (Number\ of\ plants\ in\ degree\ 1\ x\ 1) + \cdots + (Number\ of\ plants\ in\ degree\ 5\ x\ 5)}{Total\ tested\ plants\ x\ 5}\ x\ 100$$

2.3 Preparing the Pseudomonas fluorescens vaccine

P. fluorescens isolate was obtained from the Laboratory of Bio-Resistance, Al-Mussaib Technical college. 250 ml flasks, containing a nutation media (Nutrient Broth), were vaccinated by the loop of a bacterial colony, with the age of 24 hr growing on the Nutrient Agar media, it is incubated at 25 °C for 48 hours.

2.4 Testing the antagonistic capabilities for Pseudomonas fluorescens to inhibit the growth of the pathogenic fungus (Sclerotinia sclerotiorum) in vitro.

The antagonistic capabilities for P. fluorescens were tested against the isolate of the pathogenic fungus (S. sclerotiorum S.) (Sc-2) on the PDA culture media by adding 1 ml of bacterial isolate suspension growing on the liquid activation media (N.B), with the age of 3 days in the center of Petri dish on the PDA culture media with moving it circularly to distribute the suspension homogeneously before adding a disc with a diameter of 0.5 cm from the pathogenic fungus culture, with the age of 7 days in the center of the dish leaving three control dishes without adding Bacteria (pathogenic fungus only). After the completion of the fungus growth in the control treatment, the growth rate of the pathogenic fungus and the percentage of inhibition were calculated according to the following formula:

$$\text{The percentage of inhibition} = \frac{\text{Fungi growth in control} - \text{growth in treatment}}{\text{Fungi growth in control}}\ \text{x } 100$$

2.5 Preparing Effective microorganisms (EM-1) and testing its antagonistic activity against the sclerotiorum S. fungus

The basic raw EM-1 solution (the Iranian Emakanpazir pars company) was activated by mixing it with molasses and sterile water free of chlorine, with a ratio of 95: 5:5, respectively, where it was placed in a glass jar and its nozzle was closed and placed in a warm place away from sunlight for 10 days at a temperature of 35-40 ° C, with conducting a ventilation every 3 days, it is then observed the precipitated pieces at the bottom of the dish and it is evidence of the readiness of the combination products for use, 1 ml of EM-1 solution was added to the Petri dishes containing a PDA media. The dishes were shaken circularly, Thereafter, it was vaccinated with a 0.5 cm diameter disc of pathogenic isolates from the S. sclerotiorum S. fungus, with the age of 5 days in the center of the dish, with the rate of 3 replicates, a control treatment was conducted by vaccinating the center of the dish with the fungi of the pathogenic fungus (sclerotiorum S.) only, The dishes were then incubated at a temperature (1 25 °C) for 7 days (castro et al., 1995), the colony diameter and percentage of inhibition were measured based on the control treatment to evaluate the efficacy of EM1 and according to the equation mentioned in the previous paragraph.

2.6 plant extracts

2.6.1 Collecting plants and preparing samples

Three plants were chosen to study their effect against S. sclerotiorum, which included the leaves of the water hyacinth, Hornwort, and Conocarpus as shown in Table (1). Plant samples were dried in the air under the sun, by spreading them in thin layers over broad

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surfaces of fabric and exposing them to sunlight for an appropriate period while continuing to overturn the samples and expediting their drying to prevent them from rotting. The vegetative samples were then crushed using an electric grinder, then the powder of each plant was placed in polyethylene bags, labeled with the name of the plant and the weight of the sample and stored in the refrigerator until use.

Table 1: Plants that their extracts used in the study

English name	Scientific name	The used part	Collection area
Water hyacinth	*Eichhornia crassipes* (Mart) Solms	Leaves	Tigris River in Al-Akilat region, Baghdad province
Hornwort	*Ceratophyllum demersum*	Leaves	Project area / Al-Mussaib
Conocarpus	Conocarpus lancifolius	Leaves	Gardens of Al-Mussaib Technical college

2.6.2 Testing the effect of the aqueous extract for some plants on the growth of pathogenic fungi (S.sclerotiorum) on the culture media (PDA)

The extraction was conducted according to (Shekhawat and Prasada, 1971) method, where a specific weight of each powder was taken from the used plants (Water hyacinth, Hornwort, Conocarpus) and placed in a 250 ml glass flask, distilled water was added to it with a ratio of 1:10. The flask nozzle was closed at room temperature (24 +1 ° C) with manual shaking every half hour for 24 hours, a clean piece of cloth was then taken to filtrate the solution to remove the large particles, and the extract was then filtered through Whatman No.1 Type Filter Paper placed in a funnel, the total filtrate resulting from the extraction was concentrated in a water bath at a temperature of 45 °C to get rid of water. A dense liquid was obtained, the extracts were weighed and stored in marked, sealed glass bottles and placed in the refrigerator until use. The effectiveness of different plant extracts was tested using the method of food poisoning. a 5, 10, 15 ml of the extract was taken and add to 95, 90, 85 ml of sterile and cooled PDA culture media, respectively, with implementing the control treatment by leaving a culture media without adding the extract. The media were poured into sterile dishes (9 cm in diameter). After the media was hardened, the dishes were vaccinated at the center with a 0.5 cm diameter disc from the culture media containing the growths of pathogenic fungi (S. sclerotiorum), with the age of 5 days. The experiment was conducted using a completely random design. Three dishes were used for each treatment as replicates. The dishes were placed in the incubator at a temperature of 25 +1 °C and when the fungal culture diameter for the control treatment (without adding an extract) reached to the edge of the dish, the results were taken by calculating the average of two orthogonal from the growth of each colony and the percentage of inhibition was then calculated.

2.6.3 Testing the effect of aqueous extract for propolis on the growth of S. sclerotiorum growing on the culture media (PDA).

The aqueous extract of the propolis was prepared using the method of (Contari, 1987) with some modifications, where 10 g of the propolis (obtained from some apiaries in Baghdad province) were cut into small pieces and placed in a 250 ml flask, 100 ml of sterile distilled water was then added to it and leaving it at room temperature for 5 days with continuous manual shaking every half hour. The solution was filtered with a clean, sterile cloth to remove large particles and then filtered using Whatman No.1 filter paper placed in a funnel. The total filtrate resulting from the extraction process was concentrated in a 45 °C water bath to get rid of the excess water. A

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dense liquid was obtained, the extracts were weighed and stored in marked, sealed glass bottles and placed in the refrigerator until use. The effectiveness of aqueous extract for propolis against an isolate of pathogenic fungi (S. sclerotiorum Sc-2) was tested using the method of food poisoning. a 5, 10, 15 ml of the extract was taken and add to 95, 90, 85 ml of sterile and cooled PDA culture media, respectively, with implementing the control treatment by leaving a culture media without adding the extract. The media were shaken and poured into sterile dishes, Three dishes were used for each treatment as replicates. After the media was hardened, the dishes were vaccinated at the center with a 0.5 cm diameter disc from the culture media containing the growths of pathogenic fungi (S. sclerotiorum). The dishes were placed in the incubator at a temperature of 25 +1 °C. The experiment was conducted using a completely random design. when the fungal culture diameter for the control treatment (without adding an extract) reached to the edge of the dish, the results were taken by calculating the average of two orthogonal from the growth of each colony and the percentage of inhibition was then calculated according to what followed in paragraph (2.4).

3. RESULTS AND DISCUSSION

3.1 Isolating and diagnosis of the pathogen for white mold on eggplant.

The results of isolation and diagnosis showed that three isolates of Sclerotinia sclerotiorum on the culture media (PDA) were taken from plant samples that showed symptoms of white mold disease, which are the isolates (Sc-1, Sc-2, Sc-3) from Abu Al-Jasim and Al-Bada'a and Al-Musayyib Project, respectively. where the whole dish was covered with a white fungal growth after 4-5 days of inoculation, with observing the formation of anaerobe Hypha in the cover of the dish from the inside, it was observed that the formation of sclerotia bodies after 7 days of inoculation assembling Hypha in the form of white blocks (sclerotia) that turned into a yellow-green color, then to the black color. The composition of stone objects was also centered at the edge of the dish, sclerotia bodies were distinguished by their different sizes, numbers, and irregular shapes, with different isolates, where they were distinguished by the hardness of their outer shell and their black color. These results agree with the specifications of the Sclerotinia sclerotiorum, and these results agree with the results of several studies that have demonstrated the importance of the fungus as a cause for white mold disease (Paret and Olson, 2010).

3.2 Pathogenicity test

3.2.1 Detection of the pathogen isolates for the pathogenic fungus (Sclerotinia sclerotiorum) using eggplant seeds.

The results showed that the tested S. sclerotiorum isolates caused a significant decrease in the percentage of eggplant seed germination, and there was a variation in the Pathogenicity for the fungi isolates as shown in Table (2), where Sc-2 isolates (Al-Bada'a region) have excelled in its Pathogenicity on isolates (Sc-1 and Sc-3), which was evident in the effect of reducing the percentage of germination, where the number of germinated seeds amounted to (0.67). The percentage of germination was 2.66%, while the isolates (SC-1 and Sc-3) achieved the percentage of germination amounted to (53.33 and 65.33%), respectively. The reason for the variance of isolates in their effect in the percentage of germination for eggplant seeds may be due to the genetic difference between the fungus isolates collected from different regions. The decrease in the percentage of germination for eggplant seeds in the treatment of S.sclerotiorum is also due to its ability to produce enzymes that analyze the walls of host cells such as Cellulases, Proteases Pectinases, Hemicellulases, Endo Polygalacturonases and the production of fungus for oxalic acid (OA) and the toxicity of this acid to host tissue (Rioueau 1991; Et al., 2001; Girard et al., 2004).

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Table 2: The effect of studied treatments on the number of seeds and the percentage of germination.

Treatments		The number of seeds	The percentage of germination
1	Sc-1	13.33	53.33
2	Sc-2	0.67	2.66
3	Sc-3	16.33	65.33
4	Control	25.00	100
LSD value		**1.630 ***	**6.522 ***
* (P<0.05).			

*Sc = Sclerotinia sclerotiorum and the number near the symbol represents the isolate number

3.2.2 The effect of isolates of pathogenic fungus (S. sclerotiorum) on the seedlings of eggplant under the conditions of the lath house.

Table (3) indicates that all the tested isolates were pathogenic to eggplant seedlings with a high infection rate amounted to 100% with a difference in the severity of infection for each isolate, It significantly increased the severity of infection with the pathogenic fungus (S. sclerotiorum) compared to the control treatment in which the severity of the infection was zero%. The results showed that the sc-2 isolate achieved the highest values in the percentage of infection severity for eggplant seedlings which amounted to (82.22%), followed by the sc-1 isolate in which the percentage of the infection intensity amounted to (42.22%) compared to the control treatment and these results agree with the results of the detection of Pathogenicity for fungus isolates (S. sclerotiorum) on eggplant seeds. The fungus produces a group of plant-cell enzymes such as Proteases and Pectinases that have an important role in the pathogeny of the pathogenic fungus (S. sclerotiorum). The hydrolysis of pectin weakens the cell wall where facilitates the host penetration and colonization, It also supplies the fungus with the carbon sources necessary for growth (Favaron et al., 1994), From the results of this experiment, the sc-2 isolate that achieved the highest percentages of eggplant infection severity was selected for subsequent experiments.

Table 3: Effect of isolates of pathogenic fungus (S.sclerotiorum) on eggplant seedlings.

Treatments		The percentage of infection (%)	The severity of infection
1	Sc-1	100	42.22
2	Sc-2	100	82.22
3	Sc-3	100	31.11
4	Control	0.00	0.00
LSD value		**6.656 ***	**3.134 ***
* (P<0.05).			

*Sc = Sclerotinia sclerotiorum and the number near the symbol represents the isolate number

3.3 Antagonistic capability test for Pseudomonas fluorescens against the pathogenic fungus (Sclerotinia sclerotiorum) on the culture media (PDA).

Table (4) shows that the tested fluorescens P. bacteria had a significant effect on the growth of isolate for pathogenic fungi that cause white mold disease for eggplant compared to the control treatment (pathogenic fungi individually), where the percentage of inhibition for the isolate of pathogenic fungus (S. sclerotiorum) (Sc-2) amounted to (75.92%). The inhibition action of fluorescens P. is due to its production of the B-1,3-glucanase enzyme which inhibits many fungi and its ability to produce iron-chelating siderophores and then

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compete with the fungi for iron (Al-Whaibi, 2006). The results agree with (Ellis et al., 1999) that bacteria possess the ability to inhibit pathogenic fungi and producing several enzymes such as Chitosanase and Chitinase, which destroy Chitin in the cells of Higher fungi walls. These results agree with what some researchers mentioned about the ability of bacteria to encourage plant growth to inhibit the growth of many pathogenic fungi in nutrient media, where Saad, (2006) emphasized that the inhibition mechanism for P. fluorescens is due to the production of bacteria to Chitinase and 1.4 glucanase-B, 1,3– B, Lipase and Protease enzymes that inhibiting various pathogenic fungi. The results agree with (Sharma et al., 2017) who found the ability of P. fluorescens to inhibit the growth of pathogenic fungi (S.sclerotiorum) with a high rate of inhibition on the culture media. The effect of the P. fluorescens bacteria used in the biological control field exceeds the direct effect in the pathogen only to include improvement of the plant health condition and then control of pathogens (Bakker et al., 2003; Vanloon and Bakker, 2003; Bakker et al., 2007).

Table 4: Effect of Pseudomonas fluorescens on the growth of Sclerotinia sclerotiorum in PDA culture media.

Treatments		Colony diameter (cm)	The percentage of Inhibition (%)
1	Sc-1	9.00	0.00
2	Sc-2	2.16	75.92
LSD value		**0.462**	**5.13**
* (P<0.05).			

*Sc-2= Sclerotinia sclerotiorum represents isolate 2, P.f = Pseudomonas fluorescens.

3.4 Testing the antagonistic capability for EM-1 to inhibit the growth of the Sclerotinia sclerotiorum in the culture media (PDA)

Table (5) shows the effectiveness of the EM-1 inhibiting the growth of isolate for the tested pathogen (S.sclerotiorum) (Sc-2), where the percentages of inhibition amounted to (100.00%) when adding 1 mL of the EM-1/ dish. These results agree with a previous study that showed that EM1 is highly effective in inhibiting the growth of bacterial and fungal pathogens in vitro (Castro et al., 1995). The mechanism of inhibiting the EM-1 is due to the microorganisms that contain anti-pathogen or its production of fungicide-inhibiting substances during their growth, such as antibiotics that stop or inhibit the growth of many pathogens (Higha, 2006).

Table 5: Effect of Effective microorganisms (EM-1) on the growth of Sclerotinia sclerotiorum in PDA culture media.

Treatments		Colony diameter (cm)	The percentage of Inhibition (%)
1	Sc-1	9.00	0.00
2	Sc-2	0.00	100.00
LSD value		**1.070**	**5.00**
* (P<0.05).			

*Sc-2= Sclerotinia sclerotiorum represents isolate 2, EM-1 = Effective microorganisms (EM-1).

3.5 Testing the effect of the aqueous extract for some plants on the growth of the pathogenic fungus (Sclerotinia sclerotiorum) on the culture media (PDA)

Tables (6, 7, 8) indicate that all extracts used for the laboratory plants achieved inhibiting activity against the growth of the pathogenic fungus (S.sclerotiorum) on the culture media and with a direct relationship with increasing concentration

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for each treatment and with significant differences at the level (5%) for the control treatment. where the treatment of the Hornwort plant extract achieved a significant decrease in all its concentrations added to the culture media, with a fungus growth rate amounted to (3.33, 0.83 and 0.00 cm), and the percentage of inhibition ranged between 63.33 -90.74-100.00%), respectively, with increasing concentration as shown in Table (6). Table (7) shows the efficiency of the leaves extract for the water hyacinth plant in reducing the growth rate of a pathogenic fungus, where the growth rate for the fungus amounted to (5.33, 2.33, 0.00 cm) and the percentage of inhibition amounted to (40.73, 74.07, 100.00%), While the treating with the extract of the Conocarpus plant achieved a decrease in the growth rate for the pathogenic fungus, which amounted to (4.33, 3.58, 0.00 cm) and the percentage of inhibition ranged between (51.84, 60.18, 100.00%) and a direct increase with increasing concentrations of (5, 10, 15%), respectively, as shown in Table (8). The results of the statistical analysis showed that there were significant differences between the used concentrations for each extract (5, 10, 15%) and the highest percentage of inhibition for each treatment was at the concentration of (15%). The results agree with (Thobunluepop et al., 2007) that some plants contain effective compounds that inhibit the growth of microorganisms, where these compounds have chemical compounds and efficacy different from the traditional fungicides used to control the growth and survival of these microorganisms. The effective inhibitory compounds found in plant antimicrobial extracts reduce carbohydrates and total content of protein, It also increases the effectiveness of the Malik dehydrogenase, Fumaras, and Succnic dehydrogenase enzymes. At the same time, it works to reduce the effectiveness of the Catalase enzyme in fungal cells, which leads to increased poisoning and then works to reduce their growth rates (El-Mehalawy, 2006). Wen-Bao et al., (2000) explain that the inhibitory effect of these plant extracts may be due to their effect on changing the permeability of cell walls or their effect on preventing spore germination or their effect on preventing Hypha growth in its early stages, which inhibits the growth of these fungi. Hornwort extract contains nutrients, salts, and minerals nourishing the plants and stimulating them. The Hornwort extract also contains many compounds such as Alkaloids, Ketones, Flavonoids, Aldehydes, Phenols, Glycosides, etc., where these compounds were found to have the effectiveness of inhibitory for the growth of fungi and other pathogens (Bankora et al., 1995; Singh et al., 2007; Tabassum and Vidyasagar, 2013).

Table 6: Effect of Hornwort plant in inhibiting the growth of pathogenic fungus (S.sclerotiorum).

Treatments		**Concentration**	**Colony diameter (cm)**	**The percentage of Inhibition (%)**
1	Sc-1+ aqueous extract	5	3.33	63.33
2	Sc-2+ aqueous extract	10	0.83	90.74
3	Sc-3+ aqueous extract	15	0.00	100.00
4	Control		9.00	0.00
LSD value			**0.384 ***	**5.67 ***
* (P<0.05).				

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Table 7: Effect of water hyacinth plant in inhibiting the growth of pathogenic fungus (S.sclerotiorum).

Treatments		Concentration	Colony diameter (cm)	The percentage of Inhibition (%)
1	Sc-1+ aqueous extract	5	5.33	40.73
2	Sc-2+ aqueous extract	10	2.33	74.07
3	Sc-3+ aqueous extract	15	0.00	100.00
4	Control		9.00	0.00
LSD value			**0.390 ***	**4.332 ***
* (P<0.05).				

Table 8: Effect of Conocarpus plant in inhibiting the growth of pathogenic fungus (S.sclerotiorum).

Treatments		Concentration	Colony diameter (cm)	The percentage of Inhibition (%)
1	Sc-1+ aqueous extract	5	4.33	51.84
2	Sc-2+ aqueous extract	10	3.58	60.18
3	Sc-3+ aqueous extract	15	0.00	100.00
4	Control		9.00	0.00
LSD value			**0.275***	**3.83 ***
* (P<0.05).				

3.6 The effect of aqueous extract for propolis on the growth of the fungus (Sclerotinia sclerotiorum) on the culture media (PDA).

Table (9) shows that the aqueous extract for propolis was highly inhibiting efficacy against the isolate of pathogenic fungus (S. sclerotiorum), where the percentages of inhibition amounted to (50.77, 52.92 and 74.13%), respectively, when adding concentrations (5, 10 and 15) from the extract to (95, 90, 85), respectively, from the culture media (PDA). It is clear from the above results that there are significant differences in the fungus treatments with propolis extract when using the three concentrations, where the percentages of inhibition differed significantly from the control treatment (without extract) in which the growth rate of the fungus amounted to (9.0 cm) and the percentage of inhibition was (0.0%). The reason for propolis efficiency may be due to inhibition because it contains many chemical compounds, resinous materials, waxy materials, flavones, essential oils, elements, organic materials, etc. Which are against many harmful microorganisms (Kaal, 1991; Wali et al., 2017).

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Table 9: Efficacy of an aqueous extract for propolis against Sclerotinia sclerotiorum in vitro.

Treatments		Concentration	The percentage of infection (%)	The severity of infection
1	Sc-1+ propolis extract	5	4.41	50.77
2	Sc-2 + propolis extract	10	4.25	52.92
3	Sc-3 + propolis extract	15	6.33	74.13
4	Control		9.00	0.00
LSD value			0.775 *	2.611 *
* ($P<0.05$).				

*Sc-2= Sclerotinia sclerotiorum represents isolate 2

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