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EFFECT OF SOME BIOLOGICAL CONTROL AGENTS IN REDUCING THE DISEASE INCIDENCE AND SEVERITY OF WHITE MOLD DISEASE ON EGGPLANT CAUSED BY *SCLEROTINIA SCLEROTIORUM*

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Abstract

The study aimed to isolating and diagnosing the pathogen of white mold on eggplant and evaluating the efficiency of some plant extracts and biological agents against pathogens under field conditions. The results of isolation and diagnosis showed three isolates of *Sclerotinia sclerotiorum*. The results of the field experiment showed that all the treatment used in the experiment, which included *P. fluorescens*, Effective Microorganism EM-1 and the Water hyacinth extract, reduced the negative effects of *S. sclerotiorum* and clearly protected eggplant plants from white mold disease. Resulted in a significant reduction in the percentage of infection and the severity of infection and in different rates compared with the treatment of *S. sclerotiorum* disease alone, which had a treatment rate of 100% and severity of infection 56.67%, where the treatment of integration between the biological product EM-1 and *P. fluorescens* and Aqueous extract of Water hyacinth in the reduction of infection rate, amounting to 16.67% and the severity of the injury 10.00%. Which was positively reflected in the increase in the rate of plant height, wet and dry weight of eggplant plants and the superiority of the treatment of interference between the extract of Water hyacinth and bacteria. *P. fluorescens* and EM-1 increased plant rate of 152.00 cm and wet dry weight 626.67 and 160.42 g, respectively. The total increase of eggplant was 29.96 kg compared with *S. sclerotiorum*, which gave a weight of 11.77 kg.

***Keywords*:** Eggplant, white mold, *Sclerotinia sclerotiorum,* Effective Microorganism, Plant extracts.

Introduction

Eggplant. *Solanum melongena* L is strongly affected by the white mold disease caused by *Sclerotinia sclerotiorum* Bary de (Lib), especially in greenhouses, that attacks the vegetative and causes significant losses of many crops (Barros *et al*., 2015). Despite the use of specialized fungicides to control it, the efforts of researchers in plant protection have focused recently on the search for less dangerous and safer ways to the environment and human health and an alternative to the use of chemical pesticides (Nutsugah *et al*., 2004; Siddiqui and Shaukat, 2003; Rothmann and McLaren, 2018; Smolińska and Kowalska, 2018). The microorganisms that inhibit plant pathogens are also used to increase production. These organisms are a group of bacteria called plant growth promoters Rhizobacteria (*Pseudomonas*, *Bacillus*, etc.) Increase plant growth due to nitrogen, nutrient uptake in soil solution, effect on root growth, ability to resist or reduce the effect of pathogens, formation of Siderophores and some enzymes such as Chitinase and other compounds such as antigens And the ability to build or change the concentration of growth regulators and the ability of bacteria to build the enzyme ACC deaminase, which reduces the concentration of ethylene and then stimulate growth. The bacteria build B-1-3-glucanase enzyme and improve the absorption of nutrients and accelerate the start 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 demonstrated the efficacy of the effective Micro-Organisms (EM-1) against bacterial and fungal pathogens due to the fact that it contains microbiological organisms that compete with the pathogen and produce secondary metabolites, antifungal substances and growth regulators that help to improve growth. (Nira, 2012; Nia, 2015). Researchers are increasingly interested in using plant extracts to control many pathogenic pathogens of plants because they contain these extracts from Merck It is an effective secondary metabolite with desirable properties in the environment such as rapid degradation, high specialization and low toxicity of the organism (Lokendra and Sharma, 1978). Because of the importance of white mold disease on eggplant and to try to control it with using some plant extracts and biological control agent, the study aimed to isolate and diagnose the cause of white mold on eggplant. And evaluation of the efficiency of some plant extracts and biological control agent against the cause of white mold disease on eggplant.

Materials and Methods

Isolation and diagnosis of *Sclerotinia sclerotiorum*

Isolation of *S. sclerotiorum* from the samples of eggplant plants infected with white mold disease collected from the agricultural areas in the province of Babylon/district Mahaweel (Abu Jassim, Al-Badaa and Mussaib), parts of the stem and branches was took, which showed the symptoms of white mold disease and then planted 4 pieces of plants on Potato Dextrose Agar (PDA) medium supplemented with tetracycline at 200 mg/L and then incubated at 25±1 °C for 3 days. *S. sclerotiorum* colonies were purified.

Molecular diagnosis of *Sclerotinia sclerotiorum* using Polymerase Chain Reaction (PCR) technology

(i) DNA extraction of fungus

The genetic material of the fungus (DNA) was isolated after the growth of the *S. sclerotiorum* in petri dishes containing the PDA. The ZR-Fungal/Bacterial DNA MiniPrep™ kit was used by Zymo Research company.

(ii) Measuring the concentration and purity of DNA

Measure the DNA concentration and purity extracted in the previous manner using a photovoltaic Nanodrop device based on optical absorption.

(iii) Electrolysis of DNA on Agarose gel electrophoresis of DNA

DNA was transferred to the Agarose gel to confirm its quality after extraction or after polymerase chain reaction (PCR) using different concentrations of gel according to the target of the migration. Concentration of 1.5% of the gel to detect PCR reaction results. The PCR reaction products were carried out with the presence of a volume guide of DNA known as the DNA ladder produced by Kapa USA, With the following molecular weights: 100, 150, 200, 300, 400, 500, 600, 800, 1000, 1200, 1600, 2000, 4000, 5000, 6000, 8000, 1000 When the migration was complete, the beams were observed and photographed using a UV transilumintor device at a 360 nm wavelength. 1XTBE of dilution 10 mL of 10XTBE solution (base solution) in 90 ml sterile distilled water to obtain 1XTBE concentration.

(iv) Polymerase chain reaction

Specialized initiator was used to detect the ITS region of the ITS, the reverse strip called ITS4, which was obtained from the Canadian Integrated Technologies Company, Canada, as shown in table (1). The gene was multiplied by the use of several polymerase reactions from the Korean company INTRON, which consists of the reaction mixture provided by the same company (Table 2) and the reaction program as shown in Table (3).

Table 1 : Sequence of the specialized initiator for detection of the ITS gene in the *Sclerotinia sclerotiorum.*

Primer	Sequence	Tm (□C)	GC (%)	Product size
Forward	5′- TCCGTAGGTGAACCTGCGG -3′	60.3	50 %	500-650
Reverse	5′ TCCTCCGCTTATTGATATGC-3′	57.8	41 %	base pair

Table 2 : Mixture of the specific interaction for diagnosis gene.

Components	Concentration
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl (1 µl)
Reverse primer	10 picomols/µl (1 µl)
DNA	1.5µl
Distill water	16.5 µl
Final volume	25µl

Table 3 : The optimum condition of detection

No.*	Phase	Tm (ºC)	Time	No. of cycle
1-	Initial Denaturation	95ºC	3 min.	1 cycle
2-	Denaturation -2	95ºC	45sec	35 cycle
3-	Annealing	52ºC	1 min	
4-	Extension-1	72ºC	1 min	
5-	Extension -2	72ºC	7 min.	1 cycle

*Each primer (Primers set supplied by IDT (Integrated DNA Technologies company, Canada.). The PCR amplification was performed in a total volume of 25µl containing 1.5µl DNA, 5 µl Taq PCR PreMix (Intron, Korea) was dissolved separately with sterile deionized distilled water as recommended by the company for a concentration of 100 pmol/ ml (base solution). The primers were then diluted to 10 µM / ml by adding 10 bicomol from the base solution to 90 mL distilled deionized sterilized water and then stored in the refrigerator until -20 °C. Transfer 5 microliters of PCR product to 1.5% Agarose gel (90 min under 75 volt and 65 current) using the DNA gel electrophoresis. After completion of the migration, the gel was imaged at UV wavelength at 365 nm.

(v) Determination of sequences of nitrogenous bases sequencing rules

Sequences of the nitrogenous bases of the polymerase chain reaction (ITS) products were determined by sending PCR reaction products with only the front-end stripe (ITS1) to the Korean company Macrogen for the purpose of knowing the DNA sequence of the fungus and determining the fungus.

(vi) Analysis of nucleotide sequence data for fungus genome

The sequences obtained from the Korean company Macrogen were analyzed using the National Center for Biotechnology Information (NCBI) in the search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov) and BioEdit program and the secondary Nucleotide blast was selected.

Test the pathogenicity

(i) Detection of pathogenic isolates using eggplant seeds

The pathogenecity of the *Sclerotinia sclerotiorum* isolated from the affected stems of eggplant was tested according to method of Bolkan and Butler (1974) using water agar medium (20 agar, 1 liter distilled water). Place the inoculated dishes in the incubator at a temperature of 25 ± 1 °C for 2 to 3 days. The seeds of the eggplant are sterile and 25 seeds / plate are placed in a circular manner near the edge of the dish. 3 dishes were used for each isolation as replicates, as well as the treatment of the control without adding the fungus, incubated the dishes under 25 ± 1 °C and the results were taken after 7 days. The percentage of germination was calculated.

Effect of *S. sclerotiorum* pathogen isolates on eggplant seedlings under the conditions of the wooden canopy

The pathogenicity of *S. sclerotiorum* isolates, which included Ss-1, Ss-2, Ss-3, was tested on eggplant seedlings under the conditions of the plastic house at the Technical College/ Mussaib for 2017. In this experiment, a mixture of soil was used after sterilization. The soil was distributed on plastic pots with 1 kg of soil per pot. It was planted with eggplant seedlings 6 weeks old and 3 replicates per isolate with 2 plants per replicate. The agricultural operations of fertilizer and irrigation for a 45 days was carried out. the *S. sclerotiorum* grown on the PDA medium. They wounds were worked a 1 cm long and 1 mm deep on the main stems and branches of each plant and placed a pieces of the fungus isolates on the wound taken from the 7-day fungal colony at an 8 mm diameter, Polyethylene bags was used to covered the plants to maintain moisture and prevent any external contamination, while three replicates remained without inoculated by pathogenic fungi as a control. The readings were followed after the inoculation process. The disease incidence was calculated in the light of the symptoms shown

on the plant after approximately one month and according to the following equation:

Disease incidence = No. infected plants\ sum of plants×100.

The severity of the infection was calculated according to the following skill which included six degrees as follows:

0 No injuries,1 rot injury does not exceed 2 mm longitudinal length of the wound., 2 Rot exceeds the length of 2 mm to 4 mm longitudinally from the wound, 3 Rot exceeds 4 mm to 6 mm longitudinally from the wound. 4 The rot extends to more than 6-8 mm longitudinally on the wound but is not completed round stem, 5 Rot more than 8 mm longitudinally from the wound with full round the stem. The upper part is sometimes wilt.

The percentage of severity was calculated according to the Mckinney equation (1923) as follows:

Severity (%) = ((Plants in 1 degree ×1+… Plants in 5 degree ×5)/ all plants ×5) ×100%.

Evaluation of the efficacy of micro-organisms EM-1, *Pseudomonas fluorescens*, and Nile flower extracts in the protection of eggplant from *S. sclerotiorum*, causes white mold under field conditions.

The field experiment was carried out in one of the fields of Al-Mahawil district-Al-Azawia area (at the farm of Mr. Hakim Shamran Atallah) on 27/10/2017. After the soil was plowed, cleared and settled well, it was divided into 3 sectors and to 3 m. Experiment treatments that included the following: 1. *Sclerotinia sclerotiorum* (Sc-2). 2. Sc-2+ *Pseudomanas fluorescens* (Pf). 3- Sc-2+ EM-1. 4- Sc-2+ Water hyacinth extract (wh). 5- Sc-2+Pf+EM-1. 6- Sc-2+Pf+wh. 7- Sc-2+EM-1+wh. 8- Sc-2+Pf+EM-1+wh. 9- Sc-2+Topsin. 10- Control. 11- Pf alone. 12- EM-1 alone. 13- wh alone. 14- Pf+EM-1. 15- Pf+wh. 16- EM-1+wh. 17- Pf+EM-1+ wh. The soil was irrigated and planted with seedlings of eggplant (Barcelona variety) at the age of 20 days at the rate of 10 seedlings per replicate and the distance between plant 40 cm repeated 3 replicates per treatment. *P. fluorescens* 5-day-old colony were added at 25 ml on plant before 5 days of cultivating. EM-1 has been added to the soil at 25 mL for treatments that need to be added during cultivation. The chemical pesticide Topsin was added at a concentration of 1 ml/liter a day after the addition of the fungus. The *S. sclerotiorum*, grown on the PDA medium was added where worked a wound length of 1 cm and depth of 1 mm on the main stem of each plant. The treatment of the water extract of the Water hyacinth plant was added by spraying the seedlings after a day of adding the fungus at a concentration of 15%. The soil of the experiment was irrigated according to the need of the plant. Crop Service Operations and fertilization (20 g / m^2) as the first batch after two weeks of seedling and a second batch after 30 days of adding the first batch. The results were calculated by calculating the disease incidence and severity of infection after 190 days of planting with 3 randomly selected plants from each replicate and three replicates / treatment. It also calculated the lengths of plants and the wet and dry weight of plants and the yield weight of the crop.

Results and Discussion

Isolate and diagnose the pathogen of white mold disease on eggplant plant

The results of isolating and diagnosis showed three isolates of the *Sclerotinia sclerotiorum* on the PDA medium. These were taken from the samples of plants that showed the symptoms of white mold disease. The isolation of Sc-1, Sc-2, Sc-3 isolates of Abu Jassem, Al-Badaa and Mashroa Al-Mussaib respectively. The whole of the dish was covered with fungal white growth after 4-5 days of inoculation. Note that the composition of the fungus was attached to the dish cover from the inside. The formation of the sclerotia was observed after 7 days of fertilization with the fungus collecting in the form of white blocks (Figure1), and then to the black color as the composition of the sclerotia at the edge of the dish and marked the These results are consistent with the results of several studies that showed the importance of fungus as a cause of white mold (Paret and Olson, 2010).

Fig. 1 : The cultural and Microscopic characteristics of *Sclerotinia sclerotiorum* on the PDA medium.

Molecular identification

The results of the electrophoreses of the DNA on the Agarose gel 0.8% concentration of the ZR-Fungal/ Bacterial DNA MiniPrep^{TM} kit showed good result in DNA extraction. The results of the PCR transfer on the Agarose gel showed a 1.5% concentration with a package size of 650 bp (Fig. 2). These results correspond to the study carried out by the researchers (Grabicoski *et al.*, 2015; Tok *et al.*, 2016; Ali and Aljarah, 2018). The users are the same as the primer of the fungus and got molecular identified of *Sclerotinia*

sclerotiorum. After the results of the nucleotides sequences of the fungus genome were obtained with the use of the Korean frontal stripe (ITS1) from the Korean company Macrogen, they were matched with the sequential *S. sclerotiorum* isolate recorded in the National Center for Biotechnology Information (NCBI) for determine the species using the https://blast.ncbi.nlm.nih.gov/Blast.cgi window located on the official website of the World Web (NCBI). The results of the analysis of the nucleotide sequences of PCR interaction showed the presence of the *S. sclerotiorum*. The results showed that the Iraqi isolate were identical with LC318720 by 100%. These results were similar to the results of Ali and Aljarah (2018) which appeared the sequences analysis revealed that the four isolates shared 99-100% identities with the equivalent sequences of the fungal isolates conserved international Gen Bank. The results of the molecular diagnosis in this study are the first of its kind in Iraq and which describe the diagnosis of this fungus based on the molecular characteristics of the fungus.

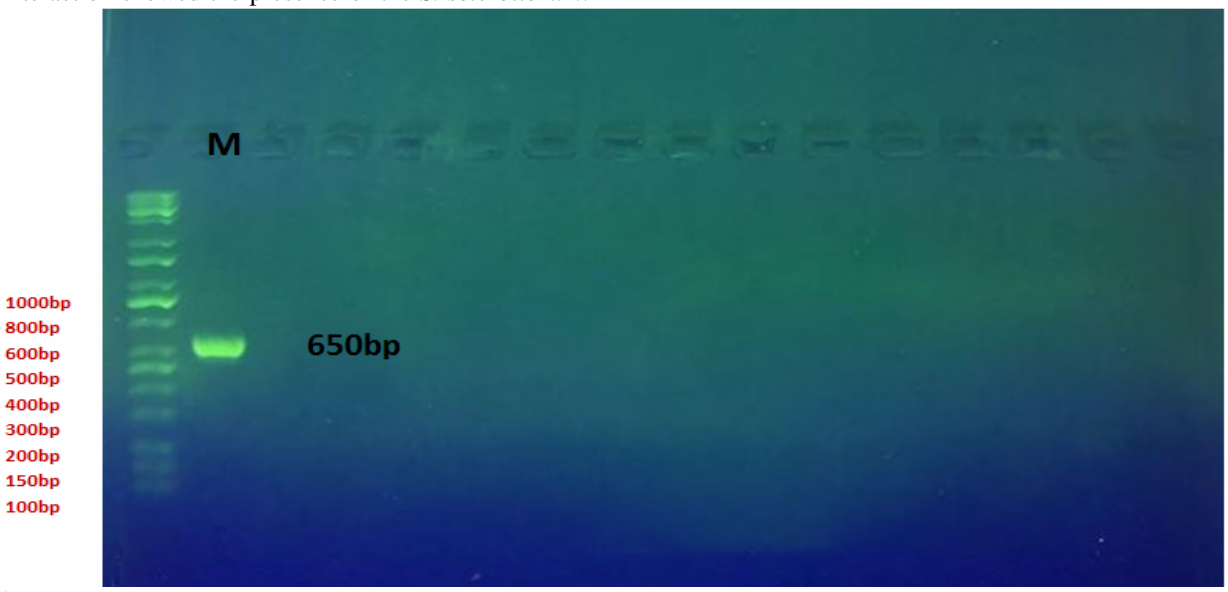

Fig. 2 : Results of the electrical relay of the PCR products of the ITS initiator, which reveals the genetic area confined between the regions ITS1 and ITS4 on the 1.5% Agarose gel (60 min, 70 V, 65 amp current) The beams were seen using a trans illuminator On the ultraviolet light in work, such as M = DNA Marker.

Detection of pathogenic isolates of the *Sclerotinia sclerotiorum* using eggplant seeds

The results showed that the tested isolates of *S. sclerotiorum* showed a significant decrease in the percentage of eggplant seeds germination. It was noted that there was a difference in the pathological potential of fungus isolates (Table 4). Sc-2 isolates isolated from the Badaa district by their pathogenicity to isolate Sc-1 and Sc-3. The effect of reducing the percentage of germination as the number of seeds was 0.67, the percentage of germination was 2.66%. Sc-1 and Sc-3 isolates achieved 53.33% and 65.33% respectively. The reason for the variation of isolates in their effect on the percentage of eggplant seed germination may be due to the genetic difference between the isolates collected from different regions. The decrease in the percentage of eggplant germination in *S. sclerotiorum* treatment is due to its ability to produce the host cell walls, Proteases Pectinases, Hemicellulases, Endo Polygalacturonases, Oxalic Acid (OA) and the toxicity of this acid to host tissues (Riou, 1991, Poussereau *et al*., 2001; Girard *et al*., 2004).

Table 4 : Effect of *Sclerotinia sclerotiorum* isolates on the eggplant seed germination rate.

Germination (%)	Number of germinated seeds	Treatments
53.33	13.33	Sc-1
2.66	0.67	Sc-2
65.33	16.33	Sc-3
100	25.00	Control
6.522	1.630	L.S.D. (P<0.05)

Sc= *Sclerotinia sclerotiorum* The number near the symbol represents the isolate number.

Effect of *S. sclerotiorum* isolates on eggplant seedlings under the conditions of the wooden canopy

The results of Table 5 indicate that all tested isolates were pathogenic to eggplant seedlings with a high infection rate of 100% with a difference in the severity of infection for each isolate, causing a significant increase in the severity of *S. sclerotiorum* infection compared with the control treatment, which had a zero disease incidence of infection. The results showed that isolate Sc-2 achieved the highest values of the percentage severity of eggplant seedlings infection at 82.22%, followed by the isolation of Sc-1, with 42.22% of the severity of infection. These results are in line with the results of detection of the pathogenicity of isolates *S. sclerotiorum* on eggplant seeds under laboratory condition.

Table 5 : Effect of *S. sclerotiorum* pathogen isolates on eggplant seedlings.

Severity (%)	Disease incidence (%)	Treatments
42.22	100	Sc-1
82.22	100	Sc-2
31.11	100	Sc-3
0.00	0.00	Control
3.134	6.656	LSD (P<0.05)

The pathogenic fungus produces a group of plant-cell wall degraded enzymes such as Proteases and Pectinases that play an important role in pathogenicity of *S. sclerotiorum*. The hydrolysis of the pectin acts to weaken the cell wall, facilitating the penetration and colonization of the host and

supplying the fungus with the sources of carbon necessary for growth (Agrios, 2005). The result of this experiment was the selection of isolate Sc-2, which achieved the highest percentages of the intensity of the infection of eggplant plants for subsequent experiments.

Evaluation of the efficiency of some biological control agents in reducing the disease incidence and severity of white mold disease on eggplant caused by *Sclerotinia sclerotiorum* and some growth criteria under field conditions.

The results of the field experiment (Table 6) showed that all the treatments used in the experiment, which included the *P. fluoresescens*, EM-1 and the water extract of the Water hyacinth plant, reduced the negative effects of the *S. sclerotiorum* and clearly protected the eggplant from infection by white mold disease, which resulted in a significant reduction in the percentage of infection and severity and in varying rates compared with the treatment of *S. sclerotiorum* disease alone, which had a treatment rate of 100% and severity of infection 56.67%, where the treatment of integration between the biological product EM-1 and *P. fluorescens* and Water hyacinth extract decreased the disease incidence rate into 16.67% and the severity was 10.00%. The treatment *P. fluorescens* significantly reduced the infection rate by 50.00% and the severity was 33.33%. Regarding the addition of single or integrated biological agents. The results showed the efficiency of the water extract of the Water hyacinth plant and the EM-1 significantly reduced the disease incidence and severity of infection by 33.33% and 26.67% respectively. approach of treatment of overlap between the water extract of the Water hyacinth and *P. fluorescens* was 33.33% and 23.33%. The interaction between *P. fluorescens* and EM-1 has demonstrated a high efficiency in reducing the disease incidence and severity compared with the treatment of single fungus. The results showed that the efficiency of the chemical pesticide Topsin in reducing the incidence of pathogenic fungi, thus reducing the disease incidence and severity of infection.

Table 6 : Effect of some biological control agents on *Sclerotinia sclerotiorum* causing of white mold disease on eggplant c under field conditions.

Severity (%)	Disease incidence (%)	Treatments*
56.67	100.00	Sc-2
33.33	50.00	Sc-2+ Pf
35.00	41.66	Sc-2+ EM-1
28.33	66.67	Sc-2+ wh
13.33	25.00	Sc-2+ Pf+ EM-1
23.33	33.33	Sc-2+ Pf+wh
26.67	33.33	Sc-2+ EM-1+wh
10.00	16.67	Sc-2+Pf+ EM-1+wh
8.33	33.33	Sc-2+Topsin
6.67	8.33	Control
0.00	0.00	Pf
0.00	0.00	EM-1
0.00	0.00	wh
0.00	0.00	Pf+ EM-1
0.00	0.00	Pf+wh
0.00	0.00	EM-1+wh
0.00	0.00	Pf+ EM-1+wh
3.44 *	5.36	LSD (P<0.05).

*Each number represents the rate of 3 replicates. Sc=*Sclerotinia sclerotiorum*, Pf = *Pseudomonas fluorescens*, EM-1 = Effective microorganisms, wh = water extract of Water hyacinth plant.

These results were consistent with several studies that showed that the overlap between biological control agents was more effective in reducing the severity of plant diseases than if a single factor was used alone (Nandakumar *et al*., 2001; Saravanakumar *et al*., 2007; Young Cheol *et al*., 2008; Latha *et al*., 2009). The efficacy of *P. fluorescens* is due to the control of the pathogen and the reduction of the rate and severity of infection to the ability to produce various types of antibiotics such as Oomycin, Pyrroles, Phloroglucinal and Pyrolnitrin against pathogenic fungi (Voisard *et al*., 1994; Sharma *et al*., 2002). This bacteria also stimulate systemic resistance, The resulting plants produce an inhibitory pathogenic compounds such as Phytoalexin (Van Peer *et al*., 1991; Bakker *et al*., 2007). The effective effect of EM-1 is to reduce the severity of the disease because it contains a corresponding group of beneficial microorganisms that inhibit the growth of pathogenic fungi (Surgeon, 2011). The results showed that the efficiency of the use of biological control agents alone or the interaction between the bacteria *P. fluorescens* and EM-1 and the water extract of Water hyacinth in the protection of eggplant did not show any infection with pathogen *S. sclerotiorum* due to the superiority of control agents in the elimination of injury, The percentage of severity of infection in all these transactions was 0%. The results (Table 7) showed the positive effect of the coefficients on increasing the plant height, wet and dry weight of the eggplant plants, as all the treatments achieved a significant increase in the measured growth parameters. The treatment of the interaction between the Water hyacinth extract and the biological resistance factors, which included *P. fluorescens* and EM-1, was the most effective in increasing the plant length of 152.00 cm and wet dry weight 626.67 and 160.42 g respectively, compared to the treatment of pathogenic fungus alone, The average length of the plant was 94.00 cm and the wet and dry weight was 310.67 and 70.77 g respectively. The ratio of the Water hyacinth extract and the *P. fluorescens* was increased in the length which was 143.33 cm while the wet and dry weight was 566.67 and 151.35 g respectively. The treatment of EM1 with the presence of fungus caused a significant increase in plant height of 127.00 cm where the wet weight was 455.00 g and dry weight was 131.00 g. The interaction coefficients between the Water hyacinth extract and the *P. fluorescens* and the EM-1 with *S. sclerotiorum* showed a significant increase in plant height Where it was 139.32 cm and the weight was wet and dry 466.67 and 139.50 g on the relay. These results show that the bioprotective agents, which included *P. fluorescens* and EM1, were highly effective in protecting eggplant plants from *S. sclerotiorum* infection and increased growth parameters. The results agree with Chang *et al*. (1986) that the efficiency of *P. fluorescens* increase in plant growth is due to the ability to release growth substances such as organic acids and growth regulators such as Indol Acetic Acid (IAA), which helps to increase germination rate and improve plant growth. Bacteria colonize treated plant roots, thus forming a strong, Stress factors and induced systemic resistance in plants (Bakker *et al*., 2003; Vanloon and Bakker, 2003). *P. fluorescens* play an important role in providing additional amounts of N element by means of various mechanisms such as N air fixation or analysis of mineral rocks and the release of elements that help the

growth of roots and deepening in the soil, and thus increase its ability to absorb water and nutrients.

Table 7 : The efficiency of some biological control and Topsin pesticide in plant length, wet and dry weight and weight of eggplant yield under field conditions.

Plant yield (kg)	Weight (g)		Plant length (cm)	Treatments
	Dry	Wet		
11.77	70.77	310.67	94.00	Sc-2
22.68	122.09	428.67	121.00	Sc-2+ Pf
23.99	131.00	455.00	127.00	Sc-2+ EM-1
21.31	116.91	416.67	115.33	Sc-2+ wh
23.15	124.17	431.67	122.67	Sc-2+ Pf+ EM-1
23.42	132.06	446.67	132.33	Sc-2+ Pf+wh
23.12	128.67	456.67	129.33	Sc-2+ EM-1+wh
24.05	139.50	466.67	139.32	Sc-2+Pf+ EM-1+wh
18.37	93.67	393.33	119.32	Sc-2+Topsin
20.42	89.17	383.33	113.67	Control
23.80	135.49	456.00	134.67	Pf
24.43	143.13	463.00	137.67	EM-1
23.55	131.21	453.33	124.33	wh
28.71	147.96	500	138.00	Pf+ EM-1
26.24	151.35	566.67	143.33	Pf+wh
25.36	138.35	613.33	140.67	EM-1+wh
25.36	160.42	626.67	152.00	Pf+ EM-1+wh
1.481*	4.418*	22.16 *	2.012 *	LSD (P<0.05).

*Each number represents the rate of 3 replicates. Sc=*Sclerotinia sclerotiorum*, Pf = *Pseudomonas fluorescens*, EM-1 = Effective microorganisms, wh = water extract of Water hyacinth plant.

Table 7 also showed a positive effect on the increase in the total number of eggplant plants since all the treatments achieved a significant increase in the weight of the yield compared to the single fungus treatment. We note that all the interaction factors between the biological factors and the presence of pathogen achieved a significant increase in weight and the treatment of the interaction between the water extract of the Water hyacinth, EM-1 and the bacteria *P. fluoresescens*, where the weight of 29.96 kg compared with the treatment of fungus *S. sclerotiorum*, which gave a weight of 11.77 kg and the results showed the efficiency of interference coefficients between the factors of biological control increase growth measures of eggplant plant, which consisted of wet and dry weight and yield. All biological control agents gave good results in increasing vegetative growth parameters. The results are in line with Lavania *et al*. (2006) and AL-Kaim (2015), that the EM-1 has increased the growth parameters of the tested plants in general. The wet and dry weight. The microorganisms contained in this Bio-formula are due to several species of aerobic and anaerobic bacteria such as photosynthesis *Rodopseudomonas* spp. Which secrete various substances such as amino acids and carbohydrates that promote plant growth and increase the fertility of the soil and contains bacteria capable of converting sugars to lactic acid, and the formation of lactic acid to reduce the degree of pH, which helps to dissolve the nutrients, as well as Lactic acid itself accelerates the decomposition of complex organic matter and has a strong inhibitory effect that resists the growth of certain pathogenic fungi, including *S. sclerotiorum*.

Samiyappan *et al*. (2011) suggest that the use of PGPR, which includes bacteria *P. fluorescens* leads to a significant increase in the quality and quantity of production, as *P. fluorescens* produce plant growth promoters such as Gibberellins and Auxins that increase their growth and productivity (Thomashow and Weller, 1996). *P. fluorescens* also have the ability to produce compounds Such as Siderophores, which have low molecular weight and have high binding ability to iron ions, which compete with the iron element and make it unsuitable for other microorganisms, including plant pathogens, which have been shown to inhibit many pathogens. (Mavrodi *et al*., 2001; Landa *et al*., 2002). The use of biological control agents, as well as the use of bacteria, Water hyacinth extracts by adding them to the soil of the field (without pathogen) showed a significant increase in aggregate weight. This is due to the fact that these biological control agents have different mechanisms that enable and work with them to stimulate the growth of the plant and then increase the yield of plants.

References

Agrios, G.N. (1997). Plant pathology. 4th edition Academic press. London. 635.

Ali, H.A. and Aljarah, N.S. (2018). Isolation and identification of *Sclerotinia sclerotiorum* the casual agents of white mold in eggplants by polymerase chain reaction (PCR) technique. J. Bio. Env. Sci. 12(4): 30-38.

AL-Kaim, F.A.A. (2015). Evaluate the efficacy of some bio-agents and chemical inducers to reduce sore skin disease on cotton seedling caused by *Rhizoctonia solani* Kühn. A Thesis, Technical college / Al- Musaib.119pp.

Al-Whaibi, M.H. (2006). Role of diazotrophic bacteria in some non – leguminous plant. J. Saudi Soc. For Agric. Sci. 5(2).

Bakker, P.A.H.M.; Ran, L.X.; Pieterse, C.M.J. and Vanloon, L.C. (2003). Understanding the involvement of rhizobacteria-mediated induction of systemic resistance in biocontrol of plant disease Can. J. Plant Pathol., 25: 5-9.

Bakker, P.A.H.M.; Pieterse, C.M.J. and Van Loon, L.C. (2007). Induced systemic resistance by fluorescent *Pseudomonas* spp. Phytopathology. 97: 239-243.

Bolkan, H.H. and Butler, E.E. (1974). Studies on heterokaryosis virulence of *Rhizoctonia solani*. Phytopathology 64: 513 – 522.

Chang, Y.C.; Baker, R.; Kleifeld, O. and Chet, I. (1986). Increased growth of plant in presence of the biological control agent *Trichoderma harzianum* . Plant Dis., 70: 145-148.

Ding, Z.; Zhang, J.; Chen, Z.; Hang, D. and Li, J. (2001). Some biological characteristical genetically engineered insecticidal *Pseudomonas fluorescens*. Wei. Sheng. Wu. Xue. Bao. 41:3-8.

Girard, V.; Fevre, M. and Bruel, C. (2004). Involvement of cyclic AMP in the production of the acid protease Acpl by *Sclerotinia sclerotiorum*.

Grabicoski, E.M.G.; Filho, D.S.; Pileggi, M.; Henneberg, L.; Pierre, M.L.C.; Vrisman, C.M.; Dabul, A.N.G. (2015). Rapid PCR-based assay for *Sclerotinia sclerotiorum* detection on soybean seeds. Sci. Agric., 72(1): 69-74.

Hernández-Salmerón, J.E.; Moreno-Hagelsieb, G. and Santoyo, G. (2017). Genome Comparison of *Pseudomonas fluorescens* UM270 with Related Fluorescent Strains Unveils Genes Involved in Rhizosphere Competence and Colonization. Journal of Genomics. 5: 91-98.

Joshi, M.L.; Tewari, R.; Tripathi, R. and Adhikari, R.S. (2018). Potentialities of Pseudomonas fluorescens for Management of Fusarium Wilt Disease of Tomato in Central Himalayas. Int. J. Curr. Microbiol. App. Sci., 7(7): 1167-1174.

Landa, B.B.; Dewerd, H.A.E.; Mespadden-Gardener, B.B. and Weller, D.M. (2002). Comparison of three methods for monitoring populations of different genotypes of 2,4- diacetylphloroglucinol producing *Pseudomonas fluorescens* in the rhizosphere. Phytopathology. 92: 129-137.

Latha, P.; Anand, T.; Ragupathi, N.; Prakasam, V. and Samiyappan, R. (2009). Anti-microbialactivity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and zimmu leaf extract against *Alternaria solani*. Biological Control. 50: 85–93.

Lavania, M.P.; Chauhan, S.; Chauhan, S.V.S.; Singh, H.B. and Naautiyal, C.S. (2006). Induction of plant Defense Enzymes and Phinolics by treatment with plant Growth Promoting Rhizobacteria Serratia marcescens NBRI 1213. Current Microbiology. 52: 363- 368.

Lokendra, C. and Sharma, B. (1978). Antifungal Properties of some plant extracts . Geobios. 5: 49–53.

Manasa, K.; Reddy, R.S. and Triveni, S. (2017). Isolation and characterisation of *Pseudomonas fluorescens* isolates from different rhizosphere soils of Telangana. Journal of Pharmacognosy and Phytochemistry, 6(3): 224-229.

Mavrodi, O.V.; Mespadden, B.B.; Thomashow, L.S.; Mavrodi, D.V.; Bonsall, R.F. and Weller, D.M. (2001). Diacetyl phloroglucinol producing fluorescent *Pseudomonas* spp. Phytopathology, 91: 35-43.

Mckinney, H.H. (1923). Influence of soil teM.pherature and moisture on infection of wheat seedling by *Helminthosporum sativum*. J. Agric. Research 26: 195–217.

Nandakumar, R.; Babu, S.; Viswanathan, R.; Raguchander, T. and Samiyappan, R. (2001). Induction of systemic resistance in rice against sheath blight diseases by *Pseudomonas fluorescence* Soil Biology biochemistry 33: 603-612.

Nia, E. (2015). Seed yield, some yield components and morphological traits of wheat as affected by Azotobacter and Pseudomonas bacteria inoculation. International Journal of Biosciences. 6(2): 1-5.

Paret, M.L. and Olson, M.S. (2010). Integrated Management of white Mold On Vegetables in Florida. uf University of Florida Ifas extension.276pp USA.

Poussereau, N.; Gente, S.; Rascle, C.; Billon-Grand, G. and Fèvre, M. (2001). .aspSeccoding an unusual aspartyl protease from *Sclerotinia sclerotiorum* is expressed during phytopathogenesis FEMS Microbiol. Lett., 194: 27-32.

Riou, C.; Freyssinet, G. and Fèvre, M. (1991). Production of cell wall-degrading enzymes by the phytopathogenic fungus *Sclerotinia sclerotiorum*. Appl. Environ. Microbiol. 57: 1478–1484.

Rothmann, L.A. and McLaren, N.W. (2018). *Sclerotinia sclerotiorum* disease prediction: A review and potential applications in South Africa. South African Journal of Science, 114, 3/4:1-9.

Samiyappan, R.; Jonathan, E.; Mohankumar, S.; Raguchander, T. and Karthikeyan, G.. (2011). PGPR mediated IPM for tropical vegetables in south India. Phytopathology. 101. No.6. (Abstract).

Saravanakumar, D.; Vijayakumar, C.; Kumar, N. and Samiyappan, R. (2007). PGPR induced defense responses in the tea plant against blister blight disease. Crop Protection 26: 556–565.

Sharma, R.C.; Vasal, S.K.; Fernarido Gan zalez, B.K. and Singh, N.N. (2002). Redress al of Banded Leef and Sheath blight of Maize through breeding chemical and biocontrol agent. Proceeding of the 8th Asia Regional Maize Workshop, Bankok, Thailand. August 5-8, 2002.

Siddiqui, I.A. and Shaukat, S.S. (2003). Combination of *Pseudomonas aeruginosa* and Pochonia *chlamydosporia* for control of Root-infecting fungi in Tomato. Journal of Phytopathology, 151: 215-222.

Smolińska, U. and Kowalska, B. (2018). Biological control of the soil-borne fungal pathogen *Sclerotinia sclerotiorum* – a review. J. Plant Pathol. 100:1–12.

Thomashow, L.S. and Weller, D.M. (1996). Current concepts in the use of Introduced Bacteria for Biological Disease Control : Mechanisms and Antifungal Metabolites. In: Plant-Microbe Interaction. Stacey, G. Keen, M. (Eds.). Chapman and Hall, New York Vol.1:187-235.

Tok, F.M.; Derviş, S. and Arslan, M. (2016). Analysis of genetic diversity of *Sclerotinia sclerotiorum* from eggplant by mycelial compatibility, random amplification of polymorphic DNA (RAPD) and simple sequence repeat (SSR) analyses, Biotechnology & Biotechnological Equipment, 30(5): 921-928.

Tozlu, E.; Mohammadi, P.; Senol Kotan, M.; Nadaroglu, H. and Kotan, R. (2016). Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary, the causal agent of white mould disease in red cabbage, by some bacteria. Plant Protect. Sci., 52: 188–198.

Van Loon, L.C. and Bakker, P.A.H.M. (2003). Signaling in rhizobacteria-Plant interaction. Ecological Studies. 168: 297- 330.

Van Peer, R.; Niemann, G.J. and Schippers, B. (1991). Induced resistance and phytoalexine accumulation in biological control of Fusarium wilt of carnationation by *Pseudomonas* sp strain WCS417. Phytoppathology 81: 728-734.

Voisard, C.; Bull, C.T.; Keel, C.; Laville, J.; Maurhofer, M.; Schnider, U.; Défago, G. and Haas, D. (1994). Biocontrol of root diseases by *Pseudomonas fluorescens* CHA0: Current concepts and experimental approaches. Pages 67-89 in: Molecular Ecology of Rhizosphere Microorganisms. F. O'Gara, D.N. Dowling, and B. Boesten, eds. VCH, Weinheim, Germany.

Young, Cheol, K.; Hyunchae, J.; Kil Yong, K. and Seur Kee, P. (2008). An effective biocontrol bioformulation against Phytophthora blight of pepper using growth mixtures of combined chitinolytic bacteria under different field conditions. European Journal of Plant Pathology 120: 373–382.