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Inhibitory Activity of Plant Growth Promoting Rhizobacteria in Control The Pathogens of Cucumber Root Rot Disease in Babylon Province

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Abstract: The study aimed to identify the Cucumber Root Rot Diseases in some areas of Babylon Governorate, isolate and diagnose the pathogen. The evaluation of the efficacy of Azotobacter and Azospierillum in disease control under field conditions. The field survey results showed the presence of cucumber root rot disease in all areas covered by the survey, with infection rates ranging between 70-90% and severity from 30-68%. The isolation results showed the presence of 10 types of fungi associated with the infested roots of the cucumber plant. Fusarium Solani was the most frequent fungi. It appeared in most samples with varying frequency rates of 75.05%, followed by the fungi Macrophomina Phaseolina 68.75% and Rhizoctonia Solani with a frequency of 62.05%. The use of Azotobacter Chrococcum as a biological control agent inhibited the growth of the pathogen Rhizoctonia Solani (Rs-1) and Fusarium Solani (Fs-6) and Macrophomina Phaseolina (Mp-2) in the PDA medium. With a high inhibition rate. The field experiment results showed that the agents used might significantly reduce the adverse effects of the pathogenic fungi. They also provided good protection for cucumber plants from infection with root rot pathogens, with significant differences from pathogenic fungi alone, whose infection rate was 100% and intensity was between 87.67 - 95.67% in the interaction treatment. Chroococcum and Azospirillum Brasiliense were superior in reducing infection incidence and severity with cucumber root rot pathogens under field conditions. It provided good protection from infection so that the disease incidence was between 22.00-27.00% and the infection severity 20.00-21.33%. All the treatments led to a significant increase in the studied cucumber plant growth parameters, increasing plant height, fresh and dry weight, leaf area, and yield weight. Azospirillum and Azotobacter alone and without adding the pathogen to improving plant growth parameters and increasing the yield weight. The interaction treatment between the two types of bacteria surpassed the highest rate of 36.04 kg compared to the control treatment and without any addition, as the yield weight reached 20.12 kgs.

Keywords: cucumber, root rot disease, biological control, pathogens, bacteria

1. INTRODUCTION

The cucumber crop (*Cucumis Satius* L.) belongs to the Cucurbitaceae family, as it is considered one of the economically important summer vegetable crops. Iraq, India, Africa, and China are considered the original home of cucumbers, as it was cultivated in these areas for thousands of years. In Iraq, cucumbers are grown in open fields in two periods (spring and autumn), The site produced by the cucumber crop in Iraq for the year 2019 was 69.502 acres, and the yield was 149.302 tons [1]. The harvest is exposed to many diseases, the most important of which is root rot disease caused by infection with many fungi.

Fusarium Solani is the most common cause of this disease [2]. As this fungus attacks crops in fields and greenhouses, it targets plant roots and stems bases, leading to rotting, plant wilting, and death in advanced stages of the disease. *Rhizoctonia* S*olani* also attacks the plant causing seed rot, seedlings death, and root rot [3]. Root rot disease is a severe threat to agriculture worldwide, constantly reducing production and threatening crop survival [4], [5]. A recent study found that the most common causes of root rot disease are *Macrophomina Phaseolina*, *Rhizoctonia Solani*, and *Fusarium* species, including *Fusarium oxysporum* and *F. Solani*, which cause significant losses in many economic crops [6].

Currently, thinking has begun to find control methods consistent with modern trends as alternatives to chemical pesticides for the significant damage that resulted from the excessive and wrong use of these pesticides in controlling agricultural pests. The most important of these means is the use of natural resistance by using microorganisms against pathogens and improving plant growth, the most prominent of which is the use of microorganisms in biological control programs to reduce the pathogen inoculum and increase production in terms of quantity and quality. Plant Growth Promoting Rhizobateria (PGPR) is at the forefront of these factors, including *Azotobacter Chroococcum* and *Azosperillium* sp. *Azosperillium* sp. It is one of the types of bacteria that stimulate the growth of roots, which have received the attention of researchers and were studied extensively. The importance of this species is not limited to fixing atmospheric nitrogen only but also characterized by its ability to make plant hormones, the most important of which is indole acetic acid. It also contributes to increasing plant tolerance of vital and abiotic stresses [7], [8], [9], [10]. It seems that it shares the pathway of hormones involved in the synthesis pathways of salicylic acid, jasmonic acid, and ethylene, which have a role in stimulating systemic resistance of the plant against pathogens [11],[12]. Due to lack of studies on cucumber root rot disease and to try to find safe control methods that fit modern trends in the world. The current study aims to determine the problem in some areas of Babylon province, isolating and diagnosis the pathogen, and evaluating the effectiveness of some biological control agents in disease control under field conditions.

2. MATERIALS AND METHODS

2.1. Survey of cucumber root rot disease in some areas of Babylon province

The field survey for the agricultural season (2018-2019) was conducted over nine different areas of Babylon province. These areas include fields planted with cucumber plants. These areas included Hilla, Al Mahawil, Al Mussaib, Al Badaa, Al Azzawiyah, Jableh, Al Nil, Al Kifl, and Abu Gharaq, and the area of the fields ranged between 2-8 acres. Samples were collected from plants with symptoms of infection, and the percentage of disease incidence was assessed. The severity of the illness was calculated for the root total according to the pathological index consisting of 5 degrees, as follows: 0 = healthy roots, 1 = secondary root rot, 2 = secondary root rot and part of the primary root, 3 = main root rot without stem base rot, 4 = main root rot and stem base rot and 5 = plant death. The percentage of severity was calculated according to Mckinney's equation [13], as follows:

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

2.2. Isolation and diagnosis of Cucumber root rot disease fungi

Pathogens were isolated from the infested cucumber plant roots. The washed and sterilized roots with sodium hypochlorite were transferred utilizing sterile forceps to Petri dishes of 4 pieces in each petri dish of 9 cm diameter by four replications for each treatment. Each plate contains 15-20 cm^3 of the sterile potato dextrose agar (PDA) which added tetracycline at a concentration of 250 mg/L after sterilization

with Autoclave at 121°C and 1.5kg/cm^2 pressure for 20 minutes and after cooling to 48°C. The dishes were placed in the incubator at a temperature of 25±1°C for four days, after which the plates were examined and diagnosed for fungi. Ahed Abd Ali Hadi based on the cultural and phenotypic characters by following the approved taxonomic keys [14], [15], [16] according to the frequency of the presence of fungi as the following:

repeat the fungus in the sample = (the number of fungi appeared in dishes\total number of pieces used in the sample) x 100 ……..(2)

Then, a purification of the fungi was carried out.

2.3. Detection of pathogenic isolates using radish seeds

The pathogenicity of *F. Solani, R Solani, M. Phaseolina* isolates was tested according to the method of Bolkan and Butler [17] by inoculating with Petri dishes of 9 cm diameter (container with 15-20 cm^3 of more agar culture medium and water agar (WA) with the addition of the antibiotic tetracyclin 250 mg/liter after sterilization with a buffer device under a temperature of 121°C and a pressure of 1.5 kg / cm^2 for 20 minutes with a disk of 5 mm diameter taken by a cork drill from near the edges of the fungus colony at the age of 5 days and the tablet was placed in the center of the plate. 4 plates were used for each isolation. The control has left the container on WA medium only the dishes were placed in the incubator at a temperature of 25 ± 1° C for three days, after which they were planted with local radish seeds (their germination rate was tested in advance). Surface sterilized with sodium hypochlorite solution for two minutes at 25 seeds/plate in a circular motion near the edge of the plate and at approximately equal distances, four vessels were used. For each isolate, in addition to the control treatment without pathogen, the plates were incubated after planting radish seeds in the incubator at a temperature of 25 ±1°C for seven days, after which the percentage of seed germination was calculated .

2.4. Preparation of Azosperillium sp. and Azotobacter sp. inoculum

Isolates of the bacteria *Azosperillium* sp. and *Azotobacter* sp were obtained from the College of the Agriculture/University of Baghdad. The collected bacteria were grown on the culture medium (Nutrient broth NB) by placing 50 ml of this medium in a 100 ml conical flask and after sterilization with inoculum from a modern culture of this isolation using a carrier and incubated. The flasks were in the incubator at 30°C for a period of 2-3 days, and to prepare a sufficient amount of inoculum for field experiments, 250 ml conical flasks were prepared, each containing 100 ml of the liquid activated culture medium, and after sterilizing them with the inoculum of each of them by adding 1 ml of the prepared liquid culture using sterile pipettes, these flasks were incubated in an incubator at 30 ° C for 2-3 days [18].

2.5. Calculate the numbers of bacteria

The Plate Count Technique (The Plate Count Technique) was followed to calculate the total number of *A. Chroococcum* and *Azosperillium* sp. Relevancy 10^1-10^7 was prepared by transferring 1 ml of the growing bacterial culture onto a liquid activation medium at three days to a series of sterile distilled water tubes using a sterile micropipette. Transfer 1 ml, starting from the fourth dilution, to a petri dish containing the medium (Nutrient broth) while moving the plate. The plates were incubated (28 ± 1m^o) for 1-3 days, then the number of bacterial cells was calculated as follows:

The number of bacteria/ml of the original sample = the number of colonies in the dish x the reciprocal of the sample dilution ……..(3)

2.6. Efficiency of plant growth-promoting rhizobacteria and the complementarity between them in protecting cucumbers from infection with root rot disease under field conditions.

The experiment was conducted in Babylon Governorate - Al Mahawil district in the spring period of agricultural season (2019-2020). The soil was prepared and irrigated, and the seeds of the cucumber plant (Super Faris variety) were snowed. The following treatments were added :1-The fungus *Rhizoctonia Solani* (R.s) alone و 2- R.s + *A. Chroococcum*, 3- R.s + *Azosperillium Brasilense* , 4- R.s + *A. Chroococcum* + *A. Brasilense*, 5 -R.s + Beltanol, 6-The fungus *Fusarium Solani*(F.s) alone., 7- F.s + *A. Chroococcum*, 8- F.s + *Azosperillium brasilense* و 9- F.s + *A. Chroococcum* + *A. Brasilense*., 10 -F.s + Beltanol, 11-The fungus *Macrophomina Phasiolina* (M.p) alone, 12- M.p + *A. Chroococcum*, 13- M.p + *Azosperillium Brasilense* , 14- M.p + *A. Chroococcum* + *A. Brasilense*, 15 -M.p + Beltanol, 16 -Bacteria *A.chroococcum* alone, 17 Bacteria *A. Brasilense* alone, 18-Bacteria *A.chroococcum* + *A. Brasilense* and 19- Control. The bacterial inoculum (2 x 10^7 CFU / ml) was added to *Azotobacter* and (8 x 10^6 CFU / ml) *Azosperillium* bacteria at a 20 ml/treatment write Then, the pathogenic fungi inoculum was added, loaded on the seeds of local millet *Panicum miliaceum*, which was prepared according to the method of Dewan [9] in the slit and on the sides of each according to its treatment at the rate of 250ml beaker containing 50 g of fungal inoculum for each breed [19]. The experimental land was irrigated according to the plant's needs. Also, butanol was added at a rate of 1 ml/liter with a pathogen. The results recorded the disease incidence and severity after 100 days of planting by removing three plants randomly from each replicate and for three replications/treatments. The height of the plants, the yield weight, and the wet and dry weight of the treated plants was also calculated.

3. RESULTS AND DISCUSSION

3.1. Field survey for cucumber root rot disease

The results of the field survey (Table 1) showed the presence of cucumber root rot disease in all areas covered by the study in Babylon province, with varying rates of infection ranging between (70-90%) and severity (30-68%), and the highest infection severity was in samples of areas Jableh, Abu Gharaq, Al Mahawil, Al Mussaib, and Al Azzawiyah, respectively with disease incidence rates of 80, 85 and 90%, respectively. The reason for the high incidence in these areas may be due to the places specializing in the cultivation of cucumber as this crop is grown in them annually, which led to the accumulation of the pathogenic fungi inoculum, especially Sclerotia that remain in the soil for an extended period of up to five years [20]. The reason for the difference in infection rates may be the difference in agricultural operations and in the type and method of adding organic and chemical fertilizers.

Table 1. Field Survey of Cucumber Root Rot Disease in Some Fields in Babylon Governorate for the 2018-2019 Agricultural Season.

Sample No.	Distract	Field area \ acres	Disease incidence (%)	Severity (%)
1	Al Azzawiyah	3	90	66.0
2	Al Badaa	4	70	31.0
3	Al Nil	2	75	33.0
4	Al Mahawil	1.5	85	40.1
5	Al Mussaib	3	90	68.0
6	Jableh	4	80	44.5
7	Al Kifl	5	70	32.0
8	Abu Gharaq	3	70	50.0
9	Hilla	2.5	80	30.0

It is known that environmental factors such as humidity and temperature have a great effect on increasing the fungus inoculum, as well as increasing the pathogenic capacity of fungi as all these factors affect plants, making them more sensitive to the response to plant pathogens and the results also showed that the lowest percentage and severity of infection appeared in the samples in the Al-Kifl area. And the innovation and the center of Hilla. The reason is attributed to the fact that these areas were planted with crops for the first time and the interest in soil and crop service operations.

3.2. Isolation and diagnosis of infected cucumber root fungi

The isolation results showed the presence of 10 types of fungi associated with the infested roots of the cucumber plant, which showed symptoms of the disease represented by yellowing of the leaves, weakness of the plant, the appearance of necrotic on the bases of the stems, and the rot of the primary root and the secondary roots (Table 2). *Fusarium Solani* was the most frequent fungi as it appeared in most samples with frequency rates variable and at a rate of 75.05%, followed by the *Rhizoctonia Solani* with a frequency of 62.05% and *Macrophomina Phaseolina* in pieces from the Mahawil area, Jabla, and Mussaib, at a rate of 68.75%. The *F. Solani*, *R. Solani*, and *M. Phaseolina* were the most visible and were recorded in most samples. The results are consistent with that of Rahim et al. [21] from the fungi *R.solani*, *F.solani*, and *M. Phaseolina* were the primary pathogens of cucumber root rot. Al-Fadhal et al. [3] found that the pathogenic fungi *Rhizoctonia Solani* and *Fusarium Solani* were the leading causes of root rot and seedling damping-off in cucumbers.

Table 2. The Percentages of Fungi Associated with the Infested Roots of the Cucumber Plant, Their Locations, and Their Frequency in Samples.

Fungi	Sample No.	Rate of appearance (%)	The highest ratio of appearance (%)
Fusarium solani (Mart.) Sacc.	1-9	75.05	96.00
Rhizoctonia solani Kuhn	1-7	62.05	93.05
Macrophomina phaseolin (Tassi) Goid.	1,2,4,6	68.75	85.00
Aspergillus niger Van Tieghem	8,6,5,2,1	5.00	15.00
Fusarium sulphureum schlecht.	1-5	27.05	50.00
Fusarium oxysporum Schlecht	1-8	21.00	68.00
Rhizobus sp.	2,3,4	13.75	15.00
Aspergillus flavus	2-6	12.05	12.06
Pythium sp.	1-3	6.25	8.00
Alternaria alternata (Fres.) Keissler	2-4	11.00	17.00

The isolation results showed some fungi associated with the roots of the cucumber plant, such as *F.oxysporum* and *F. Sulphureum*, which may have a parasitic ability on the plant, and some fungi such as

(1)

Aspergillus flavus, *Aspergillus niger*, and *Rhizopus* sp. The presence of these types of fungi may be attributed to their growth and penetration of their primary fungi. Within the decaying plant tissues that had previously been infected with the fungi that cause root rot, which provided them with protection from the surface sterilizer, as well as found another group of fungi with less frequency, including *Alternaria alternata* and *Pythium* sp.

3.3. The pathogenicity of fungi

The results showed that all *R. Solani*, *F.Solani* , and *M. phaseolina* tested isolates caused a significant reduction in the percentage of radish seed germination, as it was revealed that there was a variation in the pathogenicity of isolates of fungi (Fig 1), as the isolates outperformed Rs-1, Rs-3, Rs-6, Fs-1, Fs-5, Fs-8 and Mp-1, Mp-2, with their pathogenic potential on the other isolates, which was evident in the effect of reducing the percentage of germination that was 0% in its treatments compared to the control treatment in which the germination rate was 96%. The reason for the variation of isolates in their effect on the percentage of radish seed germination may be due to the genetic difference between the fungus isolates collected from different regions and thus the difference in isolates in their ability to secrete enzymes that dissolve pectin and cellulose in the early stages of infection and these enzymes play a role host penetration and analysis of its components, including Pectinase, Pectin methylesterase, Pectin lyase, Cellulase, and Phosphatase, which have a significant impact on fungus pathogenesis[22]. The results are consistent with that found by Hussein [23] that *Fusarium Solani* was the most common fungus in samples of cucumber plants affected by root rot disease and negatively affected seed germination.

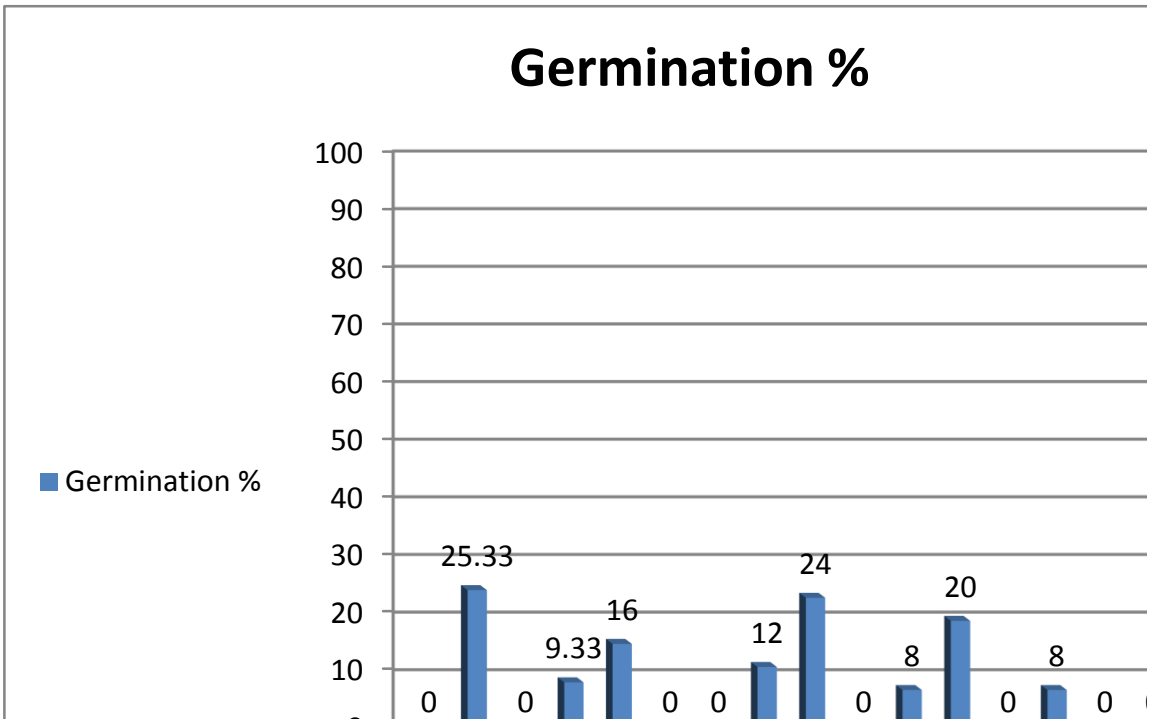

Figure 1. Detection of pathogen isolates associated with cucumber roots infected with radish seeds. LSD (*P.*: 0.05)= 5.12 Each number represents one treatment rate with three replicates

3.4. Evaluation of the efficacy of Azotobacter and Azospirillum in reducing the incidence and severity of cucumber root rot diseases under field conditions.

The results of this experiment (Table 3) were shown that used agents reduced the adverse effects of R. solani, F. solani significantly, and *M. phaseolina* pathogenic fungi, and provided good protection for cucumber plants from infection with root rot pathogens, with significant differences compared to the pathogenic fungi alone, which was 100% and severity 87.67- 95.67% The interaction treatment between *A. chroococcum* and *Azospirillum brasilense* was superior in reducing the incidence and severity of infection of cucumber root rot pathogens under field conditions so that the infection rate was between 22.00-27.00% and the severity 20.00-21.33%. *A. chroococcum* bacteria also achieved high efficiency in reducing the rate of plant infection with the target fungi, which was 44.4, 55.50, and 55.5%, respectively, and the infection severity was 40.00, 46.33, and 49.00%, respectively, without significant differences, as its effect was similar in all pathogenic fungi. After that, *Azospirillum brasilense* bacteria were treated in effect, where the percentage and severity of the pathogenic fungi were reduced by 41.00, 44.00, and 44.4%, and their intensity was 35.55, 40.00, and 48.67%, respectively. These results are consistent with Benuzzi et al. [24], who proved that *Azotobacter* bacteria are among the critical inhibitors of pathogenic fungi with their essential role in increasing plant growth and development.

Table 3. Evaluation of the efficacy of *Azotobacter* sp and *Azospirillum* sp in reducing the disease incidence and severity of infection with *F.solani, M. Phaseolina,* and *R.solani* pathogenic fungi of cucumber root rot under field conditions.

Treatments*	Disease incidence (%)	Severity (%)
Rs-1	100.0	90.0
Rs-1+Azot	55.50	40.00
Rs-1+Asp	44.4	35.55
Rs-1+AA	25.00	20.66
Rs-1+Belt	27.00	23.33
Mp-2	100.0	87.67
Mp-2+ Azot	55.5	46.33
Mp-2+ Asp	44.00	40.00
Mp-2+AA	27.00	20.00
Mp-2+Belt	28.33	17.67
Fs-6	100.0	95.67
Fs-6+Azot	49.00	44.4
Fs-6+Asp	48.67	41.00
Fs-6+AA	22.00	21.33
Fs-6+Bel	25.00	18.00
Azot	0.00	0.00
Asp	0.00	0.00
AA	0.00	0.00
Cont	18.00	9.00
LSD (*P.*: 0.05)	3.284	2.827

Each number represents one treatment rate with three replicates, Rs= *Rhizoctonia solani*, Fs= *Fusarium solani*, Mp= *Macrophomina phaseolin*, Azot = *Azotobacter chroococcum*, Asp=*Azospirillum brasilense*, and Bel=Beltanol the number next to the symbol represents the isolate number.

It showed the results of Table (4) that all treatments led to a significant increase in the parameters of cucumber plant growth studied, and a considerable superiority was found for the treatment of integration between *A. chroococcum* and *Azospirillum brasilens* bacteria over all other equations in increasing plant height in the presence of pathogenic fungi that ranged between 3.07-3.29 cm.

Table 4. The effect of biological control agents on some parameters of cucumber plant growth under field experiment conditions.

Treatments	Plant length (cm)	Weight (g)		Leaf area Cm^2	Plant yield (kg)
		Wet	Dry		
Rs-1	0.90	261.33	21.33	8.00	13.66
Rs-1+Azot	2.87	489.00	45.00	15.33	23.41
Rs-1+Asp	2.86	479.33	43.33	15.00	22.47
Rs-1+AA	3.29	499.67	63.33	19.00	28.85
Rs-1+Belt	2.62	343.67	32.00	14.33	15.41
Mp-2	1.17	258.33	24.00	10.67	12.26
Mp-2+ Azot	2.59	475.33	44.67	16.00	24.82
Mp-2+ Asp	2.62	474.67	46.00	15.00	23.95
Mp-2+AA	3.31	501.00	60.33	19.67	29.43
Mp-2+Belt	2.44	368.67	33.67	14.00	16.45
Fs-6	0.94	277.67	23.00	11.00	12.68
Fs-6+Azot	2.66	479.33	44.00	15.00	22.74
Fs-6+Asp	2.67	482.33	49.00	15.00	24.58
Fs-6+AA	3.07	445.33	70.33	18.66	28.72
Fs-6+Belt	2.82	362.00	32.00	14.00	19.55
Azot	3.62	374.00	57.00	20.00	31.37
Asp	3.65	489.00	58.34	19.00	33.61
AA	3.99	501.33	75.67	23.00	36.04
Cont	2.60	367.00	37.67	17.00	20.12
LSD (*P*.: 0.05)	0.228*	4.681*	2.21*	1.076*	2.960*

Each number represents one treatment rate with three replicates, Rs=*Rhizoctonia solani*, Fs=*Fusarium solani*, Mp= *Macrophomina phaseolin*, Azot=*Azotobacter chroococcum*, Asp=*Azospirillum brasilense*, Bel= Beltanol. The number next to the symbol represents the isolated number.

It also improved and increased the wet and dry weight, reaching 445.33-501.00 gm and 60.33-70.33 gm, respectively. The results showed a significant increase in plant height when treated with biological agents A. chroococcum and Azospirillum brasilense (without adding the pathogen), which differed from the control treatment. Without any addition, the plant had a height of 2.60 cm. The results of Table 4 showed that the use of biological agents represented by bacteria, *Azospirillum* and *Azotobacter*, and the interaction between them a significant increase in the leaf area in the presence of the pathogenic fungus and essential compatibility of the interaction treatment between the two bacterial species, which amounted to 19.00, 19.67 and 18.66%. On the other hand, all biological factors contributed to improving total weight yield and a significant superiority of the interaction treatment between *Azospirillum* and *Azotobacter* in the presence of the pathogen *R. soloni* , *F. soloni* and *M. phaseolina*, which had yield weight averages of 28.85, 29.43 and 28.72%, respectively, compared to the treatment of pathogenic fungi alone, and for

which the yield weight rates were lowest, ranging between 13.66,12.26,12.68%. The results are consistent with Parveen et al. [6] found that the most common root rot pathogens are *Macrophomina phaseolina, Rhizoctonia solani*, and *F. solani*, which cause significant losses in many economic crops. Using the biological bacteria *Azospirillum* and *Azotobacter* alone and without adding the pathogenic fungi improved the plant growth parameters. It increased the yield weight as the Azotobacter bacteria increased to 31.37 kg and Azospirillum 33.61kg.

In comparison, the interaction between the two types of bacteria exceeded the highest rate of 36.04 kg. In contrast with the control treatment and without any addition, the yield weighed 20.12 kg. The importance of this species fixing atmospheric nitrogen could make plant hormones, the most important of which is indole acetic acid, and it also contributes to increasing plant tolerance of vital and abiotic stresses [7], [8], [9], [10], [19].

4. CONCLUSIONS

Our current study concludes that cucumber root rot disease existed in all the surveyed areas in Babylon province. The primary pathogens of cucumber root rot most frequently are *Fusarium Solani, Rhizoctonia Solani*, and *Macrophomina Phaseolina*. Efficient use of *A. Chroococcum* and *Azospirillum Brasilense* was influential as a biological control agent against pathogenic fungi. The integrated two biological control agents, A. chroococcum and Azospirillum brasilense achieved the best control of pathogenic fungi. They provided good protection for cucumber plants from infection with root rot pathogens. Efficient use of the biological bacteria alone and integrated increased the plant growth parameters compared to the control treatment.

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