Role of *Pseudomonas Fluorescens* and Organic Matter in Controlling of Potato Black Scurf Disease Caused by *Rhizoctonia Solani*

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ABSTRACT

Results showed 7 isolates of *R.solani* were isolated from potato tubers that were collected from fields cultivated with crops at 2017 autumn season in Amiriyat Al Fallujah and Saqlawiyah regions in Anbar governorate. The results of the field experiment showed that the use of *Pseudomonas fluorescens* led to an increase in the enzymatic enzyme of amylase and chitinase, which reached 1.10 and 0.92 units / ml, total yield 37.71 tons. The matter organic also achieved a significant effect with the same characteristics of 1.12 and 1.04 units/ml. Total yield 52.15 hectares, the number of microorganisms 0.086 * 10 ^ 6 CFU / g dry soil, and a significant reduction in the degree of infection and pathogenicity of the infected tubers compared with contaminated the pathogen *R.solani* results showed of interaction between the bacteria and the organic matter gave the best results, as the enzymatic activity of amylase and chitinase increased by 2.24 and 1.66 units / ml, and the Total yield reached 54.52 hectares. Pathogenicity and the enzymatic activity increased as a result of the induction process compared with treatment contaminated by pathogen *R.solani*.

INTRODUCTION

Potato *(Solanum tuberosum* L.) is an important crop because of its high nutritional value, as it contains protein, water, carbohydrates, vitamins and some minerals such as potassium, phosphorus and iron (12) Potatoes are exposed to many fungal diseases, the most important of which is the black crust disease caused by the fungus *Rhizoctonia solani*: Several methods were used to control the disease, such as the use of chemical pesticides, the use of which led to the emergence of strains resistant to the influence of some pesticides as well as their effect on the environment and living organisms and disturbing the natural balance of the organisms, so new resistance methods were used, including the use of beneficial microorganisms such as the bacteria *Pseudomonas fluorescens* that stimulate plant growth Plant Growth Promoting Rhizobacteria (PGPR), which is known to be highly antagonistic to many plant pathogens (30, 14, 33). also, the use of organic and biological fertilizers has a role in providing the necessary nutrients for the plant, as well as improving the physical and chemical properties of the soil, reducing pollution in it, increasing the activity, diversity and quantity of micro-soil organisms, especially in the rhizosphere, which has a role in resisting plant diseases known as clean agriculture. Studies show that there are types of bacteria that have positive effects on plant growth, increase soil fertility, increase production on the one hand, and reduce production costs on the other hand (11).

MATERIALS AND METHODS
Samples collection
Amiriyat al-Fallujah and Saqlawiyah regions, and the tubers were placed in sterile nylon bags and brought to the Plant Pathology Laboratory in the Department of Plant Protection, College of Agriculture, University of

Anbar. The infected tubers were isolated, on which the adherent Sclerotia stone objects appeared. On the surface of the tubers and washed with water and with a sterile blade, parts of the tuber tissue containing the stone body were scraped and surface sterilized by immersion in a solution of sodium hypochlorate (1% free chlorine) for 3 minutes, then washed with sterile distilled water for two minutes and dried with filter paper. The stone objects were planted in petri dishes of Qatar. 9 cm container of PSA (Potato Sucrose Agar) was sterilized with autoclaves. The plates were incubated for 2-3 days at a temperature of $25 \pm 2\,°$ C (12). After completing the purification process for R. solani, it was sent to the Agricultural Research Department - Ministry of Science and Technology and was diagnosed based on the diagnostic characteristics that he mentioned (24). The fungus inoculum *R. solani* was prepared by growing it on seeds of local *Millet panicum* miliaceum according to the method (7).

Isolate bacteria from soil
10 random samples (1 kg) were collected from the soils of 9 agricultural fields, in addition to the Saqlawiyah and Ameriyat al-Sumoud fields. The samples were placed in nylon bags and then transported to the laboratory, milled and passed through a sieve with a diameter of 2 mm holes. The sample was divided evenly as a quantity of it was used with the rest of the other samples for the purpose of Isolation of the bacteria (*Pseudomonas fluorescens*). As for the other part, it was used to perform the physical and chemical tests. A specialized food medium was used, Pseudomonas Agar Base. The bacteria were identified in the Center for Biotechnology - Agricultural Research Department - Ministry of Science and Technology, according to (5, 4). A detection test was used to produce fluorescent dyes by preparing King,B

medium only (28).

Antagonistic potential of *Pseudomonas fluorescens* isolates against *R. solani* in PSA culture media.

Petri dishes were prepared with a diameter of 9 cm, with 3 plates for each isolation, 1 ml of the bacteria grown on the special activation medium described before (29) was added to each dish. The edge of the isolation farm of the pathogen. Isolation from the isolated bacterial isolates, then preparing the isolation vaccine by inoculating a 100 ml glass beaker equipped with 50 ml of sterile activation medium, inoculating the beaker with isolate vaccine The flasks were placed in an electric vibrator for 10 minutes and incubated at 28 ± 2 m for 3 days. To obtain a larger amount of inoculum for use in the field experiment, 5 ml of liquid culture was taken for each sex of bacterial genera and added to 1 liter flasks containing 500 ml of medium Aseptic activation, it was incubated at 28 ± 2 ° C for 3 days .

Field experiment

The organic matter (sheep + poultry wastes) (weight: 0.5: 0.5 weight) was added at a rate of 30 tons ha-1 by making a slit along the planting line with a depth of 20 cm and covered with soil. * A network of drip irrigation pipes (G R) was distributed to all treatments in the field. The planting took place on September 25, 2018, when an incision was made along the planting line, at a depth of 12-15 cm, and the pollen for the fungus R.solani loaded on the center of millet seeds was distributed inside the slit at a rate of 50 gm-1 in length according to the order of treatments that include the addition of the pathogen R.solani. Potato tubers, Solanum tuberosum L., cultivar Buren, rank A, were soaked locally in the bacterial inoculum with gum arabic added 10 g L -1) for 30 minutes and then planted at a distance of 30 cm between one tuber (20). Taking into account the cultivation of tubers that are not inoculated with bacteria first to ensure that there is no pollution and after the completion of the cultivation the field was irrigated and the crop was serviced and irrigated when needed and periodically until the stage of harvest. The experiment was carried out according to the RCBD design with three replications per treatment. The coefficients were divided as follows:

1- control treatment = CONT, 2- Treatment contaminated with pathogen = R.solani, 3- Pathogen treatment + organic matter = *R.solan*i + OM, 4- Pathogen treatment + bacteria = R.solani + P.fluorescens 5- Pathogen treatment + organic matter + bacteria = R.solani + M. O + P.flourscens, 6- Organic matter = MO 7- Treatment of bacteria = P.flourscens, 8- Treatment of bacteria + organic matter = *P.Flourscens* + M. O .

Measurements

At the end of the experiment, soil samples were taken from the rhizosphere to estimate the density of microorganisms and measure the enzymatic activity of the soil. The total yield (ha ^{1}) and the rate of infection degree) in each treatment was calculated according to the following pathological evidence and described by (19). As for the severity of the disease (DS), it was calculated according to equation (18), and the numbers of bacteria Pseudomonas fluorescens were calculated in the soil as soil samples were collected and for each treatment from the studied treatments after the end of the experiment, then a series of frightening was prepared for it, and the bacteria were grown on the KingB's special cultivation media and according to (28) and incubated for 3 days at a temperature of 28 + 2 m. The activity of the amylase enzyme was estimated: according to the method (6) and the chitinase enzyme: according to the method (13).

RESULTS AND DISCUSSION

Isolation and diagnosis of *Pseudomonas fluorescens*.

The results of isolation of *Pseudomonas fluorescens* on the specialized medium of Agar Base showed colonies of white to gray color with viscous, mucous, convex in shape, opaque, and uniformly rounded edges. flagella or more, which are arranged in short chains or singly, and no spores were observed (4). Its colonies appeared bright yellow when exposed to ultraviolet rays at a wavelength of 360 nanometers, as a result of testing its ability to produce fluorescent dyes (Pyoverdin dye), according to what he mentioned (3). From these microscopic and biochemical characteristics, we conclude that these isolates belong to the bacteria *Pseudomonas fluorescens*.

The antagonistic potential of *Pseudomonas fluorescens* isolates against *R. solani* Depending on zone inhibition, the results showed the anti-bacterial ability of *Pseudomonas fluorescens,* and the highest inhibition rate was recorded at 68.55%. On this basis, the isolation was chosen. The ability of these bacteria to resist pathogens and stop their growth (bacteriocins, phenazine) as well as their ability to produce chelating compounds called sidrophorse that chelate iron from the culture medium and thus inhibit the growth of pathogenic fungi due to their high competitiveness (26, 8). **The effect of different treatments on the enzymatic activity of soil:**

Table 1 shows the effect of adding Pseudomonas fluorescens, organic matter and *Rhizoctonia solani*, and the interaction between them on enzymatic activity in the soil, where the comparison treatment gave less enzymatic activity for amylase and chitinase, which was 1.08 and 0.61 units. The rate of enzymatic activity increased with the addition of the pathogen *R.solani*. They were 1.24 and 0.66 units, respectively, followed by the addition of bacteria, *Pseudomonas fluorescens,* and they were 1.10 and 0.92 units. Then the organic matter alone amounted to 1.12 and 1.04 units. All the differences were significant and clear and took a higher increase with the mixing coefficients of bacteria and the substance. Organic with pathogen fungi As it gave the highest rates in the effectiveness of these enzymes, and this increase may be due to the induction process by the bacteria and the organic matter that contains many types of organisms, where the mixing treatment between the pathogen *R.solani* and bacteria *Pseudomonas fluorescens* was given 1.43 and 1.50 units. Respectively, and increased with the treatment of pathogenic fungi with organic matter to be 2.26 and 1.30 units. The highest enzymatic activity was reached with the treatment of adding organic matter OM, bacteria and pathogenic fungi and it was 2.26 and 1.66 units. As for the treatment of adding organic matter with the bacteria under study, it was 2.24 and 1.66 units alone.

Table 1. Effect of different treatments on the activity of enzymes induced in the soil

Treatment	Activity of amylase unit.ml^{-1}	Activity of catanase unit.ml^{-1}	Treatment	Activity of amylase unit.ml^{-1}	Activity of catanase unit.ml^{-1}
Cont.	1.08	0.61	*R.solani+ P.flourscens +O.M*	2.62	1.66
R.solani	1.24	0.66	organic matter (O.M)	1.12	1.04
R.solani+ organic matter (O.M)	2.26	1.30	*P.flourscens*	1.10	0.92
R.solani+P.fiousciens	1.43	1.50	*P.flourscens* +O.M	2.24	1.66
L.S. D	0.08	0.07	L.S. D	0.08	0.07

The Effect of different treatments on inhibiting the activity of *R.solani* pathogen:

Table 2 shows the effect of the treatments under study on the activity of the pathogen, expressed by the degree of infection and the evidence of pathogenicity, where the degree of infection is higher and the evidence of pathogenicity on potato tubers with the addition of the pathogenic fungus alone, where the infection severity was 3.73 and the pathogen index was 87%, with a high significant difference from other treatments, and the fungus activity decreased. With the addition of organic matter and Pseudomonas fluorescen, the degree of integrity was decreased to To 1.7 and 1.3 with the addition of bacteria and organic matter, respectively. As for the disease index, it also decreased to 29 and 30% when adding bacteria and organic matter, respectively, with clear significant differences, and the activity of the pathogen ceased completely with the treatment of mixing the pathogenic fungi with the bacteria under study and the organic matter. It was carried out by (25) that *P. fluorescens* possesses the characteristics of plant growth stimulating bacteria (PGPR) and that they have a high ability to produce IAA (Indole acetic acid) and raise the content of dissolved phosphorous in the soil, as well as their production of compounds with low molecular weights called Siderophore that work on chelating iron and making it non Available for many plant pathogenic fungi.

Table 2. Effect of different treatments in inhibiting the activity of the pathogen

Treatment	Severity of infection%	Degree of infection	Treatment	Activity of catanase unit.ml-1	Activity of amylase unit.ml-1
Cont.	0	0	*R.solani+ P.flourscens +O.M*	0	0
R.solani	87	3.73	*O.M*	0	0
R.solani+O.M	29	1.30	*P.flourscens*	0	0
R. solani+ P.flourscens	31	1.70	*P.flourscens +O.M*	0	0
L.S. D	2.31	0.09	L.S. D	2.31	0.09

Table 3 shows the effect of the different treatments on the total yield and the density of the number of bacteria, *Pseudomonas fluorescens* × 10^6. The mixing treatment of organic matter and bacteria achieved the highest amount of yield, reaching 54.25 tons. Hectare, and this value decreased with the addition of the pathogenic fungus with organic matter and bacteria, and it was 53.53 tons. Hectare and by difference Significant, followed by treatment of organic matter alone, which was 52.15 tons. Hectares, and the amount of yield decreased when treating the addition of pathogenic fungi with organic matter to 50.44 tons. Then followed by treatment of bacteria, Pseudomonas fluorescen, amounting to 37.71 tons, which in turn decreased when adding the pathogen to 35.12 tons. As for the treatment of pathogenic fungi, the total yield was 30.32 hectares. This decrease in the total yield is the result of the biological stress that the pathogen exerts on the plant. Several studies have indicated such results as (1, 30)

Table 3. Effect of different treatment in total yield and density of *P. fluorescens*

Treatment	The amount of yield in tons. Hectare^{-1}	Total number of *P. flourscenscfu*$\times 10^6$	Treatment	The amount of yield in tons. Hectare^{-1}	Total number of *P. flourscenscfu*$\times 10^6$
Cont.	33.23	0.053	*R.solani+ P.flourscens +O.M*	53.53	3.590
R.solani	30.32	0.043	O.M	52.15	0.086
R.solani+ organic matter (O.M)	50.44	0.067	*P.flourscens*	37.71	1.950
R.solani+P.fiousciens	35.12	1.851	*P.flourscens +O.M*	54.25	3.753
L.S. D	0.28	0.032	L.S. D	0.28	0.032

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