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ORIGINAL ARTICLE

The Biological Activity of Some *Pseudomonas Sp* Isolates on Growth of Three Plant Pathogenic Fungi under Incubator Conditions.

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ABSTRACT

This report contains some experiments conducted under incubator conditions to test the ability and efficiency of some species of *Pseudomonas* bacteria as biocides to inhibit some plant pathogenic fungi like *Pythium, Alternaria and Fusarium,* in Complete Randomized Design (CRD) with three replicates. For this purpose five *Pseudomonas* species 1) *Pseudomonas putida1*; (2) *Pseudomonas putida2*; (3) *Pseudomonas fluorescens3*; (4) *Pseudomonas fluorescence4*; and (5) *Pseudomonas chlororaphis*, were chosen as a bacteria produced siderophores compounds which were collected previously from Iraqi soils. To test the ability of these bacterial isolates to inhibit the growth of 3 different plant pathogenic fungi (*Alternaria sp, Pythium sp,* and *Fusarium sp*) under the incubator conditions and to find out the best among the five isolated bacteria in inhibition growth of the three plant pathogenic fungi. Secondly, to make a comparison between effects of *Pseudomonas* as Biocides and Dithen fungicide in inhibition fungi growth. Results showed that *Pseudomonas putida2* and *Pseudomonas fluorescens3* were the best among the five isolates in their ability to inhibit growth of the three fungi significantly. *Pseudomonas putida2 & Pseudomonas fluorescens3* were significantly higher effect as Biocides on fungi growth more than Dithen fungicide.

Key words:

Introduction

Many plant pathogenic fungi are involving in the infection of plant with diseases like *Pythium* and *Fusarium, Alternaria*. Infection by fungi causes: 1) high reduction in germination percentage because the high reduction in embryo activity of seeds by fungus; (2) seedlings death at the first stage of growth; (3) death of the whole plant at flowering stage; and (4) reduction in production. Iraqi soils are contaminated by fungi diseases.

Use the pesticide chemicals in Agriculture cause contamination problems to the ecological resources and components like soil, water, plant, foods etc… The Biological control depends on some Biological organs to control diseases and pests which are dispersing among the crops. Biological control is one from many methods to protect crops and keep the ecological resources in the safe side.

Many recent studies mentioned that a lot of species from *Pseudomonas sp*. can produce siderophores compounds which can inhibit growth of plant pathogenic fungi [19,14,13,15,16,2]. Indeed, James & Gutterson [12] used *Pseudomonas fluorescens* to inhibit *Pythium ultimum* fungi which caused damping of on cotton crop.

Pseudomonas isolates can also produce some antibiotic compounds which can inhibit fungi growth [17]. Al-Dulaimy [3] found that 20 isolates belonging to *Pseudomonas fluorescens* and 4 isolates belonging to *Pseudomonas putida* showed the ability to produce siderophores compounds. Recently, Ganeshan and

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Kumar [11] found that Pseudomonas fluorescens, a potential bacterial antagonist to control plant diseases.

This research project will investigate the biological effects of some isolated bacteria from Iraqi soils like *Pseudomonas sp* on growth of some fungi involving in germination, seedlings death and wilting disease of crops.

The main aims of this study are:

To investigate the activity and ability of some Bacteria sp like *Pseudomonas sp.* as a Biocides to inhibit fungi growth like: *Fusarium, Pythium, Alternaria* under the incubator conditions.

To compare the effects of the best selected *Pseudomonas sp* as Biocide with Fungicide like Dithen on growth of the three fungi.

Materials and methods

Experiment no.1:

This experiment was conducted to test the ability and activity of five isolates bacteria species from Iraqi soils. This experiment will test the activity and growth of some plant pathogenic fungi under incubator conditions. To achieve this goal the following isolated identified bacteria were chosen: *Pseudomonas putida1, Pseudomonas putida2, Pseudomonas fluorescens3, Pseudomonas fluorescens4 and Pseudomonas chlororaphis*

Cultural Media:

King B (KB) medium was prepared to activate bacteria growth [7]. The medium was sterilized in autoclave before using. Also Potato Dextrose Agar (PDA) was prepared to activate fungi growth as the following [1]:

Sterilization:

- Autoclave used to sterilize culture media under 121 C^0 , pressure 15P/inch for 15 minutes.
- Oven used to sterilize all glasses under 180 C^0.

Sterilization by Chemicals:

Alcohol 70 % was used to sterilize benches, tables, others.

Bacteria Isolates:

They are supplied from Biology Department, College of Sciences, Al-Anbar University.

Activity of isolated bacteria (Bacterial Vaccines):

Isolated bacteria were activated in liquid media. It was prepared from each isolated bacteria in conical flask size 250 ml, contains 100 ml of KB liquid culture media, after contamination with bacteria each conical flask was incubated at 28 C^0 for 24 hours to activate the 5 isolates bacteria, and then were kept in the fridge at 4 C^0.

Preparing Fungi Isolates:

Three pure fungi isolates: *Fusarium Sp, Pythium sp, Alternaria sp* were supplied previously from Department of Plant Protection, College of Agriculture, Baghdad University to Biology Department, College of Sciences, Al-Anbar University.

Small mycelium as a sample from each plant pathogenic fungi was cultured in sterilized Petri-dishes contain PDA culture media, then all dishes were incubated in the incubator at 25 C^0 to activate the growth of each fungi for 24 hours. All fungi isolates were kept in the fridge at 4 C^0 .

Testing the inhibition activity of isolated bacteria against the pathogenic fungi:

Solid media culture was used in sterilized Petri dishes. Each Petri dish was contaminated completely with 0.1 ml of bacterial vaccine by using sterilized pipette (Micropipette). Then small disc from each fungus was taken by cork borer from the previous cultures, each 4 mm diameter. Then each disc was cultured in the center of each Petri dish to test the inhibition activity of each isolated bacteria. All units of the experiment were incubated in the incubator at 25 C^0 for six days.

Completely Randomized Design was used with five replicates in this experiment. Control treatment for each fungus was left without contamination with bacterial vaccine.

Experiment no.2:

The Biological activity of some *Pseudomonas sp* isolates on growth of three plant pathogenic fungi.

This experiment was conducted and repeated again under incubator conditions to test the ability and activity of five isolates bacteria species from Iraqi soils. The aims were to make sure to select the best bacteria sp isolate in its efficiency as Biocide to use it in our further experiments. To achieve this goal the following isolated identified bacteria were chosen: *Pseudomonas putida 1, Pseudomonas putida 2, Pseudomonas fluorescens 3, Pseudomonas fluorescens 4 and Pseudomonas chlororaphis.*

Testing the inhibition activity of the five isolated bacteria against the plant pathogenic fungi:

The procedure of this section was similar to that in Experiment 1.

Experiment no.3:

Comparison between activity of *Pseudomonas putida2* & *Pseudomonas fluorescence3* as Biocides with Dithen Fungicide to inhibit growth of three plant pathogenic fungi under incubator conditions.

Activity of two isolated bacteria (Bacterial Vaccines):

The procedure of this section was similar to that in Experiment 1.

0.5 gm from Dithen fungicide was mixed well in 1 liter of sterilized and distilled water. Then 200 ml from the mixture were put in conical flask size 500ml contain KB nutrient media, and mixed homogenously. After preparing media, it dropped and distributed in sterilized Petri dishes size 9cm. Then all treatments contaminated with 0.4cm in diameter from *Pythium*, *Alternaria* and *Fusarium* according to the experimental design. Control treatments for each fungi were left without contamination with bacterial vaccine or chemical fungicides [2]. All Petri dishes were kept in the incubator at 28 C^0 for 7days. Completely Randomized Design was used with three replicates in this experiment. Table (5) shows all treatments used in this experiment. Inhibition percentage for fungi growth was calculated according to the following equation [10]:

$$\text{Inhibition \%} = \frac{\text{mean of fungi growth in control- mean of fungi growth in bacteria treatment}}{\text{Mean of fungi growth in control treatment}} \times 100$$

Results and discussion

Experiment 1:

The results of this experiment showed a clear variation in the ability of the five isolated bacteria in their inhibition to fungi growth under incubator conditions cultured in KB and PDA media (Tables, 1 and 2). *P. putida2 and P. fluorescens3* gave high significant inhibition in fungi growth compared with control and other treatments (Table, 1). In respect of *Pseudomonas putida2* the diameter mean of fungi growth was 4.6, 5.6, 6.6 mm for *Pythium, Alternaria and Fusarium respectively.* And for *Pseudomonas fluorescens3* was 4.8, 6.6, 4.8 mm respectively, while the growth mean for control treatment was 50, 55, 43 mm respectively.

Pseudomonas putida 2 and Pseudomonas fluorescens3 gave a high significant inhibition against growth of the three fungi compared with control treatment and other isolates under incubator conditions.

Pseudomonas putida2 scored inhibition in fungi growth by 90.8, 89.8, 84.7 % for *Pythium, Alternaria and Fusarium* respectively compared with control treatment, while *Pseudomonas fluorescens3* scored inhibition in fungi growth by 90.4, 89.0, 88.8 % respectively compared with control treatment (Table, 1). *PDA* media gave similar results to KB media under similar conditions (Table, 2).

Results also showed no significant differences in growth between KB and PDA media, this agree with [2,4]. However, the fungi growth was 5, 5.7, 6.0 mm in diameter for *Pythium* , *Alternaria*, *Fusarium respectively* with *Pseudomonas putida2.* And for *Pseudomonas fluorescens3 was* 6, 6, 4 mm respectively. *P. putida2 & P. fluorescens3* gave a high significant inhibition against growth of the three fungi compared with control treatment and other isolates under incubator conditions respectively, while the growth mean for control treatment was 43, 42, 40 mm respectively.

These results gave an indicator that the two isolates inhibited fungi growth significantly much higher compared with control and other treatments under incubator conditions. Indeed, *Pseudomonas putida* and *Pseudomonas* f*luorescens* produce siderophores compounds which can inhibit fungi growth (Picture, 1). This is in agreement with many workers in this subject [20,15,2,3] found that 20 isolates belonging to *Pseudomonas fluorescens* and 4 isolates belonging to *Pseudomonas putida* showed the ability to produce siderophores compounds.

More over, most of *Pseudomonas putida* and *Pseudomonas fluorescen*s and other species can produce phenozine antibiotic compound which inhibit growth of plant pathogenic fungi [18]. For example, *Pseudomonas chlororaphis* pcl 1391 inhibited successfully growth of *Fusarium oxysporium* which caused root rot for tomato crop [6].

The variation among the isolated bacteria in their ability to inhibit fungi growth may related to the quantity and quality of antifungal compounds which produced by bacteria against fungi growth. These results are in agreement with [2,3] and with Becker & Cook [5] who found that B324 (*P. putida and fluorescens*) inhibited *Pythium* growth on KB media. This agree with James & Gutterson [12] who used *Pseudomonas fluorescens* to inhibit *Pythium ultimum* fungi which caused damping of on cotton crop. This may be related to role of siderophores in suppression of *Pythium* species growth.

Experiment 2:

The results are similar to that discussed in Experiment 1. Table (3) shows a clear variation in the ability of the five isolated bacteria in their inhibition to fungi growth under incubator conditions cultured in KB media (Picture 1).

P. putida2 and P. fluorescens3 gave a high significant inhibition in all growth of fungi compared with control and other treatments.

Table 1: The effects of five isolate of Pseudomonas *sp*. Bacteria on diameter mean (mm) of fungi growth: Pythium sp., Alternaria sp. and Fusarium sp., which cultured in KB media under incubator conditions at 25 C^0 for six days.

Isolates	Pythium	Alternaria	Fusarium	Mean
P. putida 1	12.6	11.6	15.8	13.3
P. putida 2	4.6	5.6	6.6	5.6
P. fluorescens 3	4.8	6.6	4.8	5.4
P. fluorescens 4	9.4	12.8	14.0	12.4
P. chlororaphis	21.0	20.0	13.0	18.0
Control	50.0	55.0	43.0	49.3
LSD at 5%	1.53	2.41	1.71	-

Table 2: The effects of five isolate Pseudomonas *sp*. Bacteria on diameter mean (mm) of fungi growth: Pythium sp., Alternaria sp. and Fusarium sp., which cultured in PDA media under incubator conditions at 25 C^0 for six days.

Isolates	Pythium	Alternaria	Fusarium	Mean
P. putida 1	13	10.5	15.0	13.3
P. putida 2	5	5.7	6.0	5.6
P. fluorescens 3	6.0	6.0	4.0	5.4
P. fluorescens 4	10.0	11.8	13.0	12.4
P. chlororaphis	22.0	21.0	14.0	18.0
Control	43.0	42.0	40.0	41.6
LSD at 5%	3.08	1.13	2.91	

Table 3: The effects of five isolate of *Pseudomonas sp.* Bacteria on diameter mean (mm) of fungi growth: *Pythium sp., Alternaria sp. and Fusarium sp*., which cultured in KB media under incubator conditions at 28 C^0 for seven days.

Isolates	Pythium	Alternaria	Fusarium	Mean
P. putida 1	30.0	25.0	22.0	25.6
P. putida 2	5.0	4.2	4.9	4.7
P. fluorescens 3	4.5	5.5	5.0	5.0
P. fluorescens 4	11.0	21.0	22.0	18.0
P. chlororaphis	20.0	24.0	16.0	20.0
Control	77.0	85.0	87.0	83.0
LSD at 5%	4.6	4.7	3.2	-

Table 4: Comparison between the Biological activity of *Pseudomonas sp.* Bacteria and Dithen fungicide on diameter mean (mm) of fungi growth: *Pythium sp., Alternaria sp. and Fusarium sp*., which cultured in KB media under incubator conditions at 28 C^0 for seven days.

Isolates	Pythium	Alternaria	Fusarium	Mean
P. putida 2	4.6	5.2	4.4	4.7
P. fluorescens 3	4.2	4.8	4.9	4.6
Dithen Fungicide	10.6	12.0	17.0	13.2
Control	77.0	82.0	88.0	82.3
LSD at 5%	4.04	3.0	93.1	-

Picture 1: Shows the effects of Pseudomonas sp. vaccine on some fungi growth under incubator conditions.
Top side:
Right: Pythium fungi treatment (control).
Left: Fusarium fungi treatment (control).
Bottomed side:
Right: The effects of Pseudomonas fluorescens3 on Pythium fungi growth.
Left: The effects of Pseudomonas putida2 on Fusarium fungi growth.

Experiment 3:

Results presented in Table 4, showed a clear effect for *P. putida2* and *P. fluorescens3* efficiency on fungi growth compared with Dithen fungicide treatment. In spite of the high activity of Dithen fungicide against plant pathogenic fungi, but *P. putida2 and P. fluorescens3* in this experiment were more activity and efficiency in inhibition growth of *Pythium, Alternaria and Fusarium* fungi. This, may be related to the siderophores and other metabolites compounds which produced by *Pseudomonas sp* isolates [9,8]. *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases [11].

Indeed, *Pseudomonas putida* and *Pseudomonas fluorescence* produce siderophores compounds which can inhibit fungi growth. This is in agreement with many workers in this subject [20,15,2].

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