Isolation and Identification of Some Lactobacillus Spp. Bacteria and Evaluation Their Efficacy in The Management of Damping off Disease on Peas

Duha Faisal Ajaj and Abdullah Abdulkarim Hassan

1,2College of Agriculture, Tikrit University, Iraq.

Email: darabdullah.has67@tu.edu.iq

Abstract

Twenty-eight isolates of Lactobacillus bacteria were isolated from the rhizosphere of pea plants grown in the fields of five districts in Salah al-Din, which included: Tikrit, Al-Alam, Al-Sharqat, Samarra and Bajji, diagnosed according to phenotypic and biochemical tests. Results showed the effect of L. paralimentarius 1081 on vegetative growth characteristics. Treatment of (bacterial filtrate + Ridomil in the presence of pathogenic fungi) was recording the highest values in dry weight of the vegetative and root systems, was 3.91 and 1.23 g respectively in the local cultivar, compared with the lowest values was 2.60 g and 0.76 g respectively in the Syrian cultivar. All treatments inducing plant resistance compared with healthy plants, and the highest activity of the Peroxidase and Polyphenol oxides in the treatment of (Bacterial filtrate + ridomil in the presence of pathogenic fungi), were 1.08 and 1.33 units/ml in the local cultivar, compared to the Syrian cultivar were 0.015 and 0.013, respectively. Results showed a significant decrease in the severity of infection for all treatments compared to the pathogenic fungus treatment, and the lowest infection severity of the vegetative and root systems was recorded in the treatment of (Bacterial filtrate + ridomil in the presence of pathogenic fungi) which was 14.11 and 12.47% in the local cultivar. There was a significant superiority of all treatments in productivity parameters of pea compared to the treatment of pathogenic fungi only, the highest of those parameters including weight of pods and grains weight/plant were recorded in the treatment (Bacterial filtrate + ridomil in the presence of pathogenic fungi) for the local cultivar was 18.07 g and 14.04 g compared to 10.43 g and 8.20 g in the treatment of the Syrian cultivar with pathogenic fungi only.

Keywords: P.aphanidermatum, L.paralimentarius 1081, Pea, ISR.

1. Introduction

Peas are one of the important crops belonging to the Fabaceae family, which is cultivated in the northern and central regions of Iraq. Peas are the second most important leguminous crop in the world after beans, as they contain a high percentage of protein, amino acids, minerals and vitamins [1,2]. The global production of peas was amounted 14.2 million tons in 2020, harvested from about 7.2 million hectares [3]. Peas crop are infected many bacterial, viral, fungal and nematode diseases, and the most common fungal diseases that affect them are Aschochyta blight caused by the fungus Mycosphaerella pinodes [4] and Powdery Mildew disease caused by Erissiphe Pisi. It spreads with air and is distributed all over the world and is active in dry, warm environments with cold nights [6], and rust disease caused by the fungus Uromyces viciae-fabea is the main agent and cause of pea rust in tropical and subtropical regions where warm humid weather [5], as well as many pathogens that cause wilt, root rot and damping off, which is a wide serious disease spread in most countries of the world, causing losses of up to 80% of the yield of most vegetable crops [6]. Lactobacillus bacteria have many anti-pathogenic properties, especially some pathogenic microorganisms and food contaminants from bacteria and fungi they have some mechanisms in inhibiting the growth of these pathogens, such as production of organic acids, hydrogen peroxide, diacetyl, enzymes, inhibitors of some enzymes, bacteriocins , reuterin, fatty acids and phenolic compounds [7]. And this explains the role of these effective antimicrobial bacteria , their use as a food preservation agent and an alternative to chemical preservatives [8] reported that although the efficacy of LAB antifungal bacteria has been demonstrated in vitro, there is limited information on the relationship of LAB with plant pathogens.

Research focused to reduce the use of pesticides and chemicals that are harmful to plants, the environment and humans, using biological factors, this study aimed to:

1. Isolation and identification of Lactobacillus spp. by some biochemical methods.

2. Evaluation of the efficiency of this bacteria to control damping off disease caused by the fungus Pythium aphanidermatum on three cultivars of peas.
3. Evaluating the efficiency of these bacteria in enhancing the vegetative and productive parameters of these cultivars.

2. Materials and Methods

2.1 Phenotypic and biochemical isolation and diagnosis of isolated Lactobacillus species

*Lactobacillus* bacteria were isolated from five regions within belong to Salah El-Din Governorate using the dilution method by taking 1 g of the Rhizosphere soil of pea, adding to 9 ml of distilled water to get a dilution of $10^{-1}$ then 1 ml was taken from it and added to 9 ml of distilled water to obtain a $10^{-2}$ dilution. MRS Selective medium was inoculated with 1 ml of the last diluted solution then incubated at 30°C for 24-72 hours. After completion of the incubation process, the grown colonies were identified depending on the size, texture, color and edges of the colonies, in addition to bacterial growth at a temperature of 10-50°C, gram stain, and the enzymes test for catalase, oxidase, urease and gelatinase. And indole test, esters consumption test, motility test, sugars fermentation test and hydrogen sulfide (H2S) production according to the methods provided by [9].

2.2 Preparation of bacterial cell suspensions and their filtrates

Bacterial isolates were grown separately in NB nutrient broth media by transferring a swab of the newly grown colony (24 hours age) into beakers containing 100 ml of sterile NB medium, then incubating the flasks at 30°C for 48 hours with continuous stirring, then assay. The number of bacteria in 1 ml of the bacterial suspension was $6.62 \times 10^8$ cells/ml, and this suspension was used in subsequent experiments. As for the preparation of bacterial filtrate, it was prepared in the same way except for collecting the filtrate after passing it through filter paper and then centrifuged it for 10 minutes in a centrifuge (5000 rpm) and using the filtrate in subsequent experiments.

2.3 Pathogenicity test

Potato dextrose agar medium (PDA) was used without adding any antibiotic, 0.5 cm diameter of colony piece of pathogenic fungus *P.aphanidermatum* (obtained from Plant Protection Department / University of Tikrit) was placed, age 5 days, by means of a cork piercing in the center of each dish. Then the center was plotted with three bacterial lines 3 cm in length and 3 cm away from the fungus. It was planned from three directions using the inoculation loop from the cell suspension prepared above, while in the fourth direction was a line from the middle of NB sterile liquid not cultured with bacteria with the same dimension as the three lines, which represents the control line. When the growth of the fungal colony reached the control line, the inhibition area was measured using a digital ruler from the edge of the pathogenic fungus colony to the bacterial lines, then according to the rate of the inhibition area [10].

3. Field experiment

The field experiment was carried out in the fields of the College of Agriculture / Tikrit University for 2020-2021 season. The soil was sterilized using a 5% formaldehyde solution, then it was covered with a tight nylon cover, and after sterilization for a week, it was ventilated for three days, then the plastic pots were filled with 5 kg of soil per pot.

3.1 Soil inoculation with the pathogenic *P.aphanidermatum*

The soil was moistened with water one day before inoculation with pathogenic fungus, 5 pieces of pathogenic fungus, the size of the piece 1 cm² were placed five days before planting and the fungus pieces were covered with the soil of the same pot by 3-5 cm, then the pots were covered with pieces of nylon to obtain the appropriate moisture for fungus growth.

3.2 Peas Varieties

Three varieties of peas were used in carrying out field experiment, Dutch, Syrian and local variety, obtained from local markets.

3.3 Experimental treatments

The field experiment included eight treatments as follows:

- Control/healthy plant (without any addition).
- *P.aphanidermatum* (P.a).
- (P.a) + *L. paralimentarius* (L.p) suspension.
- (P.a) + bacterial filtrate L.p.
(P.a) + ridomil.
(P.a) + Suspended + Bacterial filtrate (L.p)
(P.a) + Bacterial filtrate (L.p) + ridomil.
(P.a) + suspended bacteria (L.p) + ridomil.

Suspended bacteria cells were added at the rate of 100 ml/pot, and bacterial filtrate was added at a concentration of 20% with the same volume (100 ml), and Ridomil was added at a concentration of 2 mg/ml with the same volume. As for the double treatments, (50 ml + 50 ml) were added equally to each pot. In order to show the effect of bacteria (suspended bacteria cells or their filtrates), only pathogenic fungi were treated with a volume of 100 ml of sterile NB medium that was not cultured with bacteria. Pots were planted on 15/11/2020 with 7 seeds per pot, according to the above treatments, for the 3 varieties with 5 replications (pots) for each treatment, with a total of 120 experimental units.

4. Studied parameters

4.1 Estimation of root and vegetative dry weights

The vegetative system was separated from the root system from the crown area, the roots were washed well with distilled water, then the root and vegetative parts were dried at a temperature of 50 °C using the electric oven until the weight was stable. The average vegetative and dry weights in grams were extracted using a sensitive scale.

4.2 Estimation of indicators of induction of resistance of pea plants under conditions of infection with P. aphanidermatium

4.2.1 Preparation of the enzyme filtrate

The enzyme filtrate was prepared according to the method mentioned in [11].

4.2.2 Determination of the enzyme polyphenol oxidase

The activity of polyphenol oxidase was estimated according to [12] by taking 2.5 ml of catechol solution and adding 0.1 ml of the enzyme filtrate to each treatment, then the absorption of the reaction mixture was measured using a spectrophotometer with a wavelength of 470 nm, the enzymatic unit was estimated by the change in absorbance 0.01 per minute.

4.2.3 Peroxidase enzyme determination

The activity of the peroxidase enzyme was estimated as indicated by [13] by mixing 2.5 ml of “guaicol” solution with hydrogen peroxide with 0.1 ml of the enzyme filtrate for each treatment. Then the absorbance of the reaction mixture was measured using a spectrophotometer with a wavelength of 470 nm. Enzymatic unit was estimated in the same way as the enzyme polyphenol oxidase.

Estimate the severity of the infection (%)
The severity of the infection was estimated according to the scale followed by [14] as follows:
0 = no infection (plant healthy), 1 = main root discoloration, 2 = secondary root discoloration, 3 = primary and secondary root discoloration, 4 = complete root rot.
The severity of infection for all treatments was estimated according to Mckinney, 1923 equation as follows:

\[
\text{the severity of the infection} = \frac{\text{sum (the number of infested plants at degree 0-6) + (the number of infested plants at degree 4-6) + 100}}{\text{total number of plants} + \text{the highest score in the disease index}}
\]

4.3 Estimation of some productivity parameters of pea plants under conditions of infection with P. aphanidermatium

4.3.1 Legumes weight/plant

Three Legumes were weighed from each replicate using a sensitive scale and the final rate of the treatment was extracted.

4.3.2 Grain weight/plant

Grains of 3 plants from each replicate were weighed using the sensitive scale and then the final rate of the treatment was extracted.
4.3.3 Statistical analysis

The study experiments were carried out using the Completely Randomized Design and the analysis of variance was conducted using the program (SPSS). Means were compared according to the Least Significant Deference (LSD) test at a level of significance of 0.05 [15].

5. Results and Discussion

5.1 Isolation, phenotypic and biochemical diagnosis of isolated Lactobacillus species

Twenty-eight isolates of Lactobacillus bacteria were obtain from soil samples from the rhizosphere of pea plants (planted from last season) from five sites of Salah al-Din governorate, including Tikrit, Al-Alam, Al-Sharqat, Samarra and Baiji, and they were diagnosed based on phenotypic and biochemical characteristics into five species, with 7 isolates belonging to the species L. fermentum, 3 isolates of L. casei and 9 isolates of L. plantarum, 4 isolates of L. herbarum and 5 isolates of L. paralimentarius. Table (1) shows the phenotypic and biochemical characteristics of the species and isolates of Lactobacillus bacteria isolated in this study. These species varied with some characteristics. The colony color of L. casei, L. plantarum, L. herbarum, and L. paralimentarius ranged from white to cream, while the colony of L. fermentum was white in color and in terms of colony strength both L. herbarum and L. plantarum showed coarse texture, while it was smooth and shiny for species L. casei, L. fermentum, L. paralimentarius, while all species of colonies for the five isolates were circular in terms of shape and Gram positive, immobile and non-forming endospores. In terms of microscopy, results showed that the rod form is dominant for L. casei, L. fermentum, and L. plantarum, while it was rod to ovate for L. herbarum, L. paralimentarius, and the isolate on MRS medium was positive for all isolates, and this indicates the relevance of this bacteria to Lactobacillus bacteria, which can grow successfully on this medium. The enzyme tests of catalase, oxidase, galatiniase, urea and citrate consumption were negative for all isolates, while the indole test was positive for L. fermentum bacteria and it was negative for the rest of the isolates as well. Some differences were recorded in terms of the fermentation of sugars, the isolates of the five species isolated in this study showed a positive test for glucose, lactose, sucrose and fructose, while there was a difference in the fermentation and consumption of mannitol sugar, as the isolates of L. casei and L. herbarum did not ferment (except for isolate 1985). L. herbarum bacteria. In terms of growth at different temperatures, all isolates grew at temperatures of 20° C, 30° C and 40° C, while isolates of L. casei, L. plantarum and L. herbarum could not grow at temperatures 10°C and 50°C. Results also showed that all bacterial isolates are negative for gelatin decomposition and produce catalase and do not produce hydrogen sulfide gas (H₂S) and do not produce indole except for isolates 1970 and 1990 of L. fermentum bacteria. There were some differences between the species as a result of the fact that these species are isolated from different geographic areas and the different environments affect the genetic factors of these isolates and this effect is reflected on the color and texture of the colony and the ability of cells to ferment sugars and others. These results are in agreement with the study of [16], in which 54 isolates of Lactobacillus were isolated from the soil, and those isolates were divided into eight species: L. casei, L. fermentum, L. plantarum, and L. paralimentarius, according to the phenotypic diagnosis in terms of the shape of the bacterial cell, its ability to move, its positivity to gram staining, its absence of spores or the presence of spores, and the colony’s shape, texture, color and greasy, and according to the biochemical tests, which are the test for esters, glucose, lactose, sucrose. Phenotypic and biochemical tests are one of the important methods for diagnosing bacteria at the species level, and this was mentioned.

Sixteen isolates of LAB bacteria were used in the study, and they were classified into Lactobacillus plantarum (7 isolates) and Lactobacillus fermentum (9 isolates). All were positive for gram stain, cylindrical in shape, negative for catalase and esters test, and positive for sucrose, glucose, mannitol and trehalose.

5.2 The effect of isolates of Lactobacillus bacteria on inhibiting the pathogenic P.aphanidermatum

Figure (1) shows the inhibition effect of Lactobacillus isolates on the growth of P.aphanidermatum, as all isolates showed inhibition against the pathogenic fungus. Results showed that the highest inhibition was recorded by L. paralimentarius (isolate 1981), where the area of the fungus growth inhibition was 2.7 mm (Fig. 2) followed by L. plantarum (isolate 1982), L. herbarum (isolate 1980), L. fermentum (isolate 1979) and L. casei (isolate 1978) with inhibition distances of 2.5, 2, 1.8 and 1.5 mm, respectively, while the lowest inhibition was recorded by L. fermentum (Isolate 1990), and it was 0.5 mm. According to these results, isolate 1981 of L. paralimentarius was selected in the field experiment.
Table 1. Phenotypic and biochemical characteristics of isolates per species of lactic acid bacteria isolated in this study.

| Traits                          | *L. fermentum* | *L. casei* | *L. plantarum* | *L. herbarum* | *L. paralimentarius* |
|---------------------------------|----------------|------------|----------------|---------------|----------------------|
|                                 | 1973, 1979,    | 1976, 1977,  | 1993, 1994, 1982, 1983, 1984, 1987, 1986, 1988, 1989 | 1980, 1985, 1998, 1999 | 1981, 2000, 2001, 2003, 2005 |
|                                 | 1969, 1970,    | 1977, 1978  | 1982, 1983, 1984, 1987, 1986, 1988, 1989 |               |                      |
|                                 | 1971, 1990,    |            |                |               |                      |
|                                 | 1991           |            |                |               |                      |
| Colonial color                  | White          | White - off white | White - off white | White - off white | White - off white |
| Colony strength                 | smooth glossy  | smooth glossy | Rough          | Rough          | smooth glossy |
| Colonial shape                  | Circular       | circular     | Circular       | Circular       | Circular |
| Gram dye reaction               | gram positive  | gram positive | gram positive  | gram positive  | gram positive |
| The Motility                    | immobile       | immobile     | immobile       | immobile       | immobile |
| The formation of endospores     | -              | -            | -              | -              | -          |
| Cell shape                      | Bacillus       | Bacillus     | Bacillus       | Bacillary Oval | Bacillary Oval |
| Growth in MRS medium            | +              | +            | +              | +              | +         |
| Catalase enzyme                 | -              | -            | -              | -              | -          |
| Oxidase enzyme                  | -              | -            | -              | -              | -          |
| Citrate consumption             | -              | -            | -              | -              | -          |
| Gelatinase enzyme               | -              | -            | -              | -              | -          |
| Urease enzyme                   | -              | -            | -              | -              | -          |
| Indole production               | ±              | -            | -              | -              | -          |
| Fermentation of sugars          |                |              |                |                |            |
| Glucose                         | +              | +            | +              | +              | +         |
| Galactose                       | +              | +            | +              | +              | +         |
| Sucrose                         | +              | +            | +              | +              | +         |
| Mannitol                        | +              | -            | +              | ±              | +         |
| Fructose                        | +              | +            | +              | +              | +         |
| Growth Temperature (°C)         |                |              |                |                |            |
| 10°C                            | +              | -            | -              | -              | +         |
| 20°C                            | +              | +            | +              | +              | +         |
| 30°C                            | +              | +            | +              | +              | +         |
| 40°C                            | +              | +            | +              | +              | +         |
| 50°C                            | -              | -            | +              | -              | +         |


Figure 1. Inhibition of *Lactobacillus* isolates to the growth of *P. aphanidermatum*.

5.3 Effect of *L. paralimentarius* and Radomil on vegetative parameters of pea plants under *P. aphanidermatum* infection conditions.

5.3.1 Vegetative system dry weight

Table (2) shows the effect of filtrate, *L. paralimentarius* cell suspension and Radomil on vegetative system dry weight (g) of three pea cultivars under *P. aphanidermatum* infection conditions. The highest vegetative system dry weight was recorded in the treatment (pathogenic fungus + bacterial filtrate + The pesticide) as it reached 3.60 g, and the lowest weight of the dry vegetative system was recorded in the treatment of pathogenic fungus only, as it reached 1.5 g. As for the cultivars, the local variety showed the highest weight of the dry vegetative system, reaching 2.87 g, followed by the Dutch variety, where the dry vegetative weight reached 2.54 g, while the lowest weight was in the Syrian variety, which amounted to 2.30 g. The level of interaction between cultivars and treatments, the treatment of the local variety with (pathogenic fungus + bacterial filter + pesticide) showed the highest weight of the dry vegetative system, which amounted 3.91 g. The results also showed that the lowest weight of the dry vegetative system was recorded in the treatment of pathogenic fungus only for all studied varieties, as it reached the lowest weight of the dry vegetative system in the Syrian variety, which amounted 1.03 g. The increase in the dry vegetative weight of plants treated with *Lactobacillus* compared with the control treatment was occurred due to the production of growth stimulating factors that prevent or reduce the effect of growth inhibitory factors [20] mentioned that *L. plantarum* -1050 increased the dry vegetative weight of tomato, as it reached the highest dry vegetative weight in the treatment of (pathogenic fungus Polycopersici + *L. plantarum*-1050) reaching 188.04 g compared with the treatment of pathogenic fungus only, which reached 188.04 g. 91.40 g.  

**Table 2.** Effect of filtrate, *L. paralimentarius* cell suspension and radomel on dry vegetable weight (gm) of three cultivars of peas under *P. aphanidermatum* infection conditions.

| Treatment’s rate | Cultivars   | Treatments                                      |
|------------------|------------|-------------------------------------------------|
| 2.71             | Syrian     | control/healthy plant                           |
| 1.5              | Dutch      | Pathogenic fungus *P. aphanidermatum* (P.a)     |
| 1.92             | local      | Pathogenic fungus (P.a) + *L. Paralimentarius* suspension (L.p-s) |
| 2.15             | control    | Pathogenic fungus (P.a) + *L. Paralimentarius* filtrate (L.p-f) |
| 2.67             | Dutch      | Pathogenic fungus (P.a) + Radomil              |
| 2.76             | Syrian     | Pathogenic fungus (P.a) + (L.p –s) + (L.p –f)   |
| 3.60             | local      | Pathogenic fungus (P.a) + (L.p -f)+ Radomil     |
| 3.23             | control    | Pathogenic fungus (P.a) + (L.p –s)+ Radomil     |
| 2.30             | Dutch      | cultivars rate                                  |

Varieties: 0.10 Treatments: 0.12

The least significant difference at the 0.05 level
5.3.2 Dry weight of root system

The results shown in Table (3) indicate the effect of filtrate, *L. paralimentarius* cell suspension, and Radomil on the dry weight of root system (g) of three cultivars of peas under *P. aphanidermatum* infection conditions. The highest weight of dry root system was recorded in the (pathogenic fungus + bacterial filtrate) treatment, as it reached 1.31 g, and the lowest weight was in the control treatment, which amounted to 0.9 g. At the level of cultivars, the local variety showed the highest weight of the dry root system, which amounted to 0.99 g, followed by the Dutch variety, which reached 0.81 g, while the lowest weight of the dry root system 0.69g was recorded in the Syrian variety.

As for the interaction between the three cultivars and the treatments, the treatment (pathogenic fungus + bacterial filtrate + pesticide) showed the highest weight of the dry root system in the local variety, as it reached 1.23 g. The lowest dry root system weight was recorded in the Dutch variety, as it reached 0.76 and 0.82 g, respectively. indicated that *L. plantarum*-1050 increased the dry root system weight, and the highest dry root system weight was recorded in the treatment of (pathogenic fungus *Folycopersici* + *L. plantarum*-1050) as it reached 51.16 g compared with the treatment of pathogenic fungus only, which amounted51.16 g. 47.15 g.

The reason for the increase in the dry root and vegetative weight of plants treated with *Lactobacillus* bacteria compared to the control treatment is due to the production of encouraging and stimulating factors for plant growth that prevent or reduce the effect of growth-inhibiting factors [21,22] and secretion of IAA, which increases cell division and size and enhances plant biomass such as root length and its area and helps relieve stress on the plant and increase the growth of lateral roots [23].

**Table 3.** Effect of filtrate and suspended *L. paralimentarius* and radomil on dry root system weight (gm) of three varieties of peas under conditions of infection with *P. aphanidermatum*.

| Treatments’ rate | Cultivars | Treatments |
|-----------------|-----------|------------|
| Syrian          |           |            |
| 0.9             | 0.76      | control/healthy plant |
| 0.53            | 0.41      | *P. aphanidermatum* (P.a) |
| 0.70            | 0.58      | Pathogenic fungus *P. aphanidermatum* + *L. Paralimentarius* suspension (L.p-s) |
| 1.31            | 0.65      | Pathogenic fungus (P.a) + (L.p-f) |
| 0.85            | 0.72      | Pathogenic fungus (P.a) + Radomil |
| 0.90            | 0.74      | Pathogenic fungus (P.a) + (L.p-s) + (L.p-f) |
| 1.05            | 0.86      | Pathogenic fungus (P.a) + (L.p-s) + Radomil |
| 0.97            | 0.80      | Pathogenic fungus (P.a) + (L.p-s) + Radomil |
| Varieties: 0.06 | Treatments: 0.08 |          |
| Varieties × Treatments: 0.21 | The least significant difference at the 0.05 level |

5.4 Effect of *L. paralimentarius* and Radomil on induction of pea plant resistance under *P. aphanidermatum* infection conditions.

5.4.1 The efficacy of polyphenol oxidase

Table (4) shows the effect of *L. paralimentarius* filtrate and the pesticide Radomil on the activity of the polyphenol oxidase enzyme for three varieties of peas under conditions of infection with the fungus *P. aphanidermatum*. The highest percentage of polyphenol oxidase was reached in the treatment of (pathogenic fungi + bacterial filters + bacterial suspensions reached 0.78, and the lowest percentage of the enzyme was in the treatment of (pathogenic fungi + bacterial filter + pesticide), which reached 0.9. At the cultivar level, the local cultivar had the highest enzyme percentage, reaching 0.603, followed by the Dutch variety, which amounted to 0.535, while the lowest percentage of enzyme was in the Syrian cultivar, reaching 0.446. For the local variety, it was the highest, reaching 1.08, and the control treatment for the Syrian variety was the lowest, reaching 0.015.
Table 4. Effect of filtrate, L. paralimentarius cell suspension and radomel on polyphenol oxidase enzyme activity of three pea cultivars under conditions of P. aphanidermatum infection.

| Treatment’s rate | Cultivars          | Treatments                      |
|------------------|--------------------|---------------------------------|
| 0.031            | Syrian: 0.015      | control/healthy plant           |
|                  | local: 0.056       |                                 |
|                  | Dutch: 0.022       |                                 |
| 0.47             | Pathogenic fungus  | Pathogenic fungus P. aphanidermatum (P.a) |
| 0.49             | P. aphanidermatum  | Pathogenic fungus P. aphanidermatum (P.a) + L. Paralimentarius suspension (L.p-s) |
| 0.59             | Pathogenic fungus  | Pathogenic fungus P. aphanidermatum (P.a) + L.p-f |
| 0.29             | Pathogenic fungus  | Pathogenic fungus P. aphanidermatum (P.a) + Radomil |
| 0.78             | Pathogenic fungus  | Pathogenic fungus P. aphanidermatum (P.a) + (L.p-s) + (L.p-f) |
| 0.89             | Pathogenic fungus  | Pathogenic fungus P. aphanidermatum (P.a) + (L.p-f) + Radomil |
| 0.68             | Pathogenic fungus  | Pathogenic fungus P. aphanidermatum (P.a) + (L.p-s) + Radomil |
|                  | Varieties: 0.073    | cultivars rate                  |
|                  | Treatments: 0.061   |                                 |

| Varieties × Treatments: 0.31 | The least significant difference at the 0.05 level |

5.4.2 The activity of the peroxidase enzyme

The results listed in Table (5) show the effect of filtrate and suspension of L. paralimentarius cells and Radomil on the activity of peroxidase enzyme for three cultivars of peas under P. aphanidermatum infection conditions. The highest percentage of peroxidase enzyme was recorded in (pathogenic fungi + filtrate bacteria + pesticide) treatment, which amounted 1.23, and the lowest percentage of enzyme was recorded in the control treatment, which amounted 0.025. The Dutch variety percentage was 0.755, while the lowest percentage of peroxidase enzyme was in the Syrian variety, reaching 0.665. As for the interaction between cultivars and treatments, (pathogenic fungus + bacterial filtrate + pesticide) treatment showed the highest percentage of the enzyme, reaching 1.33 for the local variety, and the control treatment for the Syrian variety was the lowest in the percentage of peroxidase enzyme, reaching 0.031. Through the results, it is clear that all bacterial treatments gave a higher enzymatic activity for each of peroxidase and polyphenol oxidase enzymes compared to plants that were not treated with bacteria, and this indicates that the bacteria led to the induction of plant resistance. An increase in the enzymatic activity is observed in pea plants infected by the pathogenic fungus and treated with bacteria, this may be attributed to the induction of plant resistance by bacteria, as well as the plant's response to infection with pathogens, and accordingly, the indicators of resistance induction will rise.

The enzyme elevation in affected plants may be occurred due to bacterial production of several activating compounds for induced systemic plant resistance such as siderophores, salicylic acid, c-LPs and pyocyanins [24]. Therefore, the more enzymes produced, the higher the ISR [25]. Enzymes such as Polyphenol oxides and Peroxidase participate in plant defense mechanisms as they work on the alkalosis of the cell wall and polymerize its proteins, which lead to the formation of a defensive barrier against pathogens, and the activity of the enzyme increases during the interaction between the host and the pathogen [26].

This is consistent with what was demonstrated by [27], as the efficiency of bacterial species in inducing resistance depends on the formation of enzymes such as: peroxidase, lipoxygenase, chitinase and glucanase responsible for inhibiting the growth of pathogens. They indicated that the production of these enzymes led to a significant reduction in effective infection against P. aphanidermatum in cucumber plant. The induction of resistance in plants can be increased through the addition of beneficial bacteria that stimulate the plant's defense capacity against pathogens by improving the mechanical strength of the plant cell wall and the synthesis of defensive chemicals such as total phenols in response to pathogens whose growth is suppressed by these substances [8].

As [28], demonstrated the effectiveness of L. plantarum C10 against the pathogenic fungus Trichothecium roseum in watermelon plant, as it led to a higher percentage of total phenols, reaching the highest value of 0.85 and 2.87 OD325/FW compared to the control treatment, and this led to the induction of plant resistance against the pathogen fungus and curb its growth.
**5.5 Effect of *L. paralimentarius* and Radomil on the severity of pea plant infection under *P. aphanidermatum* conditions**

Table 5 shows the effect of filtrate, *L. paralimentarius* cell suspension and radomil on the severity of root system infection (%) of three pea cultivars under *P. aphanidermatum* infection conditions.

**Table 5. Effect of filtrate and suspension of *L. paralimentarius* cells and radomil on the activity of peroxidase enzyme for three cultivars of peas under conditions of infection with the fungus *P. aphanidermatum***

| Treatment’s rate | Cultivars | Treatments |
|-----------------|-----------|------------|
| Syrian | local | Dutch |          |
| 0.025 | 0.013 | 0.041 | 0.021 |  control/healthy plant |
| 0.78 | 0.65 | 0.87 | 0.81 | Pathogenic fungus *P. aphanidermatum* (P.a) |
| 0.78 | 0.67 | 0.86 | 0.81 | Pathogenic Fungus (P.a) + *L. Paralimentarius* suspension (L.p-s) |
| 0.86 | 0.80 | 0.91 | 0.88 | Pathogenic Fungus (P.a) + (L.p-f) |
| 0.34 | 0.27 | 0.44 | 0.32 | Pathogenic Fungus (P.a) + Radomil |
| 1.04 | 0.93 | 1.17 | 1.03 | Pathogenic fungus (P.a) + (L.p-s) + (L.p-f) |
| 1.23 | 1.14 | 1.33 | 1.22 | Pathogenic fungus (P.a) + (L.p-f) + Radomil |
| 0.92 | 0.85 | 0.96 | 0.95 | Pathogenic fungus (P.a) + (L.p-s) + Radomil |
| 0.665 | 0.822 | 0.755 | 0.75 | cultivars rate |

Varieties: 0.07 Treatments: 0.082

The least significant difference at the 0.05 level

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The highest infection severity of the root system was in the treatment of pathogenic fungi only, which amounted 87.08 %, and the lowest severity of infection of the root system was in the treatment of bacterial infiltrate with Radomil fungicide in the presence of pathogenic fungus, which amounted 15.27 %, followed by the treatment of bacterial suspension with Radomil in the presence of pathogenic fungus, which amounted 17.30 %. At the level of cultivars, the Syrian cultivar was the highest in the severity of the root system infection, when it reached 27.41 %, and the local cultivar was the lowest in the severity of the root infection, reaching 21.65 %. At the level of interaction between cultivars and treatments, the treatment of pathogenic fungus on the Syrian variety was the highest, reaching 87.08 %, while the lowest decrease in the severity of infection of the root system was recorded in the treatment of bacterial infiltrate with Radomil in the presence of pathogenic fungus, amounted 12.47 % for the local variety, followed by the treatment of bacterial suspension with Radomil with the presence of pathogenic fungus as it reached 14.10 % for the local variety as well.

The decrease in the rate and severity of infection occurred as a result of *L. Paralimentarius* isolates on pea plants under the conditions of infection with the pathogenic fungus *Pythium aphanidermatum* in the field is one of the most important indicators that confirm the efficiency of LAB bacteria in inhibiting pathogenic fungi and resisting root rot disease and Damping off, due to the active compounds that produced such as: Carboric and valeric acid and organic acids such as: propionic acid, lactic acid, hydroxyphenyllactic acid, phenyllactic acid, fatty acid, benzoic acid, cyclic peptides, reuterin, hydrogen peroxide and other compounds such as acetone, diacetyl and a number of protein compounds with anti-fungal pathogenic effect [22]

These compounds spread in the cell membranes of the pathogenic organism in the form of un-dissolved hydrophobic acids, and this leads to lowering the pH of the cytoplasm and hinders all vital activities [27]. The reason for the decrease in the rate...
and severity of infection may be due to the ability of LAB bacteria to inhibit the production of toxic metabolic substances produced by the pathogenic fungus, and this is consistent with the study of [7], as *L. fermentum* bacteria inhibited the production of aflatoxin and inhibited the growth of pathogenic fungus *A. flavus*. Results showed that the control treatments in healthy and infected plants did not record the rate of infection with pathogenic fungus, because they were not treated with it, so it was free from infection, and it is noted that the infection increased in the treatment of pathogenic fungus and decreased in treatments that contain filtrate or bacterial suspension, perhaps the reason for this is due to the inhibition of the disease in a way mainly due to the abundant secretion of Siderophores, Calicylic acid and HCN, which reduce the incidence of the disease and prevent the development of its symptoms, or because the virulence factors of the pathogen are inhibited or removed by bacteria Muthukumar (et al., 2010). These results are in agreement with the study of Hassan et al., (2020), which proved the efficiency of *L. plantarum* in inhibiting the growth of the pathogenic fungus *Fusarium oxysporum*, by recording the isolate 1052 the lowest percentage and severity of infection, which amounted 18.62 and 15.44%, respectively.

5.6 Effect of *L. paralimentarius* and Radomil on production parameters of pea plants under *P. aphidermatum* infection conditions

5.6.1 Legumes weight

Results of Table (7) indicated that the highest weight of legumes was recorded in the treatment of (pathogenic fungi + bacterial filtrate + pesticide) which amounted 15.86 g, while the lowest weight of legumes was recorded in the treatment of pathogenic fungus only, which amounted 3.69 g. The treatment of (pathogenic fungus, bacterial filter and fungicide) for the local variety was the highest in legumes weight, reaching 18.07 g. The results also showed that the lowest weight of legumes was recorded in the treatment of pathogenic fungus only for all studied varieties, and it reached the lowest weight of legumes in the Syrian variety, followed by the Dutch variety, reaching 3.13 and 3.82 g, respectively.

| Treatment’s rate | Cultivars  | Treatments          |
|------------------|-----------|---------------------|
|                  | Syrian    | local               | Dutch  |
| 13.4             | 10.43     | 16.04               | 13.76  |
| 3.69             | 3.13      | 4.11                | 3.82   |
| 10.63            | 8.0       | 13.87               | 10.02  |
| 11.44            | 8.74      | 14.06               | 11.51  |
| 12.03            | 9.11      | 14.71               | 12.27  |
| 12.72            | 10.17     | 15.55               | 12.44  |
| 15.86            | 13.64     | 18.07               | 15.87  |
| 14.31            | 11.79     | 17.22               | 13.92  |
|                  | 9.38      | 14.20               | 11.70  |

Varieties: 0.81 Treatments: 0.64 Varieties × Treatments: 1.13

The least significant difference at the 0.05 level

5.6.2 Grain weight

Results of Table (8) showed the effect of filtrate, *L. paralimentarius* cell suspension and Radomil on grain weight (gm) of three cultivars of peas under conditions of infection with *P. aphidermatum*. The highest grain weight was recorded in the treatment of (pathogenic fungi + bacterial filter) reaching 52.24 g, and the lowest grain weight was recorded in the treatment of pathogenic fungus only, which amounted 2.63 g. The Syrian variety was the lowest in grain weight, reaching 7.19 g. at the level of interaction between varieties and treatments, the treatment of (pathogenic fungus + bacterial filter + fungicide) of the local variety was the highest in grain weight, reaching 14.04 g. The results also showed that the lowest grain weight was recorded in the treatment of pathogenic fungus only for all studied varieties, and it reached the lowest grain weight in the Syrian variety, followed by the Dutch variety, as it reached 2.04 and 2.73 g, respectively.
Table 8. Effect of filtrate, L. paralimentarius cell suspension and Radomil on grain weight (g) of three cultivars of peas under P. aphanidermatum infection conditions.

| Treatment’s rate | Cultivars       | Treatments                        |
|------------------|-----------------|-----------------------------------|
|                  | Syrian          | local                             | Dutch                   |
| 9.90             | 8.20            | 12.14                             | 9.35                    |
| 2.63             | 2.04            | 3.11                              | 2.73                    |
| 7.22             | 5.87            | 8.93                              | 6.86                    |
| 52.24            | 7.13            | 10.55                             | 8.44                    |
| 9.44             | 7.76            | 11.56                             | 9.0                     |
| 10.16            | 8.10            | 12.75                             | 9.62                    |
| 11.58            | 9.58            | 14.04                             | 11.12                   |
| 10.95            | 8.86            | 13.60                             | 10.38                   |
| 7.19             | 10.83           | 8.44                              |                         |
|                  |                 | Pathogenic fungus P. aphanidermatum (P.a) |
|                  | Pathogenic fungus (P.a) + L. Paralimentarius suspension (L-p-s) |
|                  | Pathogenic fungus (P.a) + Radomil |
|                  | Pathogenic fungus (P.a) + (L-p-s) + (L-p-f) |
|                  | Pathogenic fungus (P.a) + (L-p-f) + Radomil |
|                  | Pathogenic fungus (P.a) + (L-p-s) + Radomil |
|                  | Cultivars rate  |

Varieties: 0.87 Treatments: 0.95 Varieties × Treatments: 1.33

The least significant difference at the 0.05 level

This is consistent with what was demonstrated by Hassan et al., (2020) in the efficiency of L. plantarum bacteria, as it increased the productivity of tomato plant in the presence of the pathogenic fungus F. oxysporum, reaching 4981.26 g/plant\(^1\) compared to the lowest productivity in the treatment of pathogenic fungus only, which amounted to 716.56 g/plant. The fungus, Pythum aphanidermatum, attacks the root zone and produces toxins and enzymes that weaken the plant, and this is reflected in the vegetative standards, as it causes yellowing of the leaves and heavy losses in production parameters. Lactobacillus bacteria are used as a biological control agent because of their activity against harmful microorganisms in general and fungi in particular, as they produce bacteriocin, which suppresses them, and organic acids such as acetic acid and lactic acid, which works to reduce acidity and prevent the growth of harmful organisms, hydrogen peroxide, fatty acids, digestive enzymes that break down the cell walls of harmful microorganisms and the compound rotrin, which stops the action of the enzyme Ribonuclease, which contributes to the synthesis of DNA in fungi (Singh, 2018) and other compounds such as: cyclic peptides that break down mycotoxins and inhibit the growth of fungi. And that the treatment with bacteria + pathogenic fungus gave results of an increase in vegetative and production parameters and has the ability to increase the systemic resistance of the plant by producing defensive chemicals and improving the mechanical strength of the cell wall by increasing the production of peroxidase enzyme and polyphenol oxidase and this increase is a clear evidence of the ability of bacteria to induce systemic resistance in plants [21]. In addition to the production of total phenols, flavonoids and plant-related proteins and their encouraging and stimulating properties for plant growth, as they produce hydrogen cyanide and plant hormones and have the ability to chelate iron and their ability to dissolve phosphates and work to increase soil fertility. They are among the root bacteria that stimulate plant growth PGPR [8]. As mentioned previously, all these factors were positively reflected in increasing plant productivity and inhibiting the growth of pathogenic fungi.

**Conclusion**

As a conclusion, lactic acid bacteria are among the biological control agents, that have the ability to inhibit pathogenic fungus Pythum aphanidermatum in the laboratory and in the field, as well as its role in inducing systemic plant resistance and stimulating the growth of vegetative plants and increasing its productivity.

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