European Scholar Journal (ESJ)
Available Online at: https://www.scholarzest.com
Vol. 4 No.10, October 2023
ISSN: 2660-5562

EFFECT OF DIFFERENT LEVELS OF SOME CHEMICAL PESTICIDES ON THE NUMBER OF NITROGEN-FIXING AND PHOSPHORUS-SOLUBILIZING BACTERIA

[1]Srour H. Ali; [2]Faris M. Suhail
[1,2]College of Agriculture, Diyala University, Iraq
hsrwr824@gmail.com

Article history:

Received: 20th August 2023
Accepted: 20th September 2023
Published: 23rd October 2023

Abstract:

The study included a field survey to detect pesticide residues in soil and plants. This was done by collecting samples of soil from the rhizosphere of three different fields planted with plants (cabbage, orange, cauliflower) from different locations in Diyala Governorate (Abu Sayda, Al-Muqdadiya, Diyala University), treated with a fungicide, insecticide, weedicide, respectively. The samples were collected from the fields at four stages (before adding the pesticide, one month after addition, flowering stage, harvest stage), with three replicates. Pseudomonas, Bacillus, Azospirillium bacteria were isolated and diagnosed, and the residues of the three pesticides were detected in soil and plants. In addition, a laboratory experiment was conducted to study the effect of four different levels of pesticides on the number of two isolates of each bacterium (an isolate isolated from the pre-treatment stage and an isolate treated with the pesticide) in solid industrial media.

Keywords: Chemical pesticides; nitrogen-fixing; phosphorus-solubilizing; bacteria.

INTERACTION

Chemical pesticides are mixed with other substances or used alone to kill or reduce the damage caused by the target pest. The use of pesticides is an important factor in protecting crops when they are threatened by residents or introduced pests. About 800 pesticides are used to control agricultural pests in the agricultural sector each year. Therefore, the World Health Organization (WHO) pointed out in 2018 that most agricultural products on the market contain varying percentages of pesticides used during the season, which may or may not be within the allowable concentrations (MRL). These concentrations are determined by organizations and laboratories that are fully accredited based on the type of pesticide and the effect of pesticide movement within the plant. The minimum and maximum limits for any pesticide have been indicated by one of the institutions of the Food and Agriculture Organization of the United Nations in a publication called Codex Alimentarius. Therefore, the use of pesticides has become a major concern in agriculture, as pesticide residues are found in the consumed crop, which poses a risk to human health (Jallow et al., 2017). Living organisms affect the decomposition of pesticides into compounds with different properties than their original properties.

For example, Pseudomonas bacteria can decompose pesticides and use them as a source of carbon and energy (Al-Mashhadany, 2022). In addition, Bacillus bacteria have the efficiency to decompose compounds, whether natural or synthetic and break down chemical pesticides (Patel et al., 2020). In addition, Azosprillium bacteria can tolerate high concentrations of insecticidal pesticides (GOMEZ et al., 1998). Azosprillium and Bacillus bacteria can decompose pesticides to use them as a source of carbon and phosphorus (Romeh and Hendawi, 2014).

MATERIAL AND METHODS

1-1- Isolation and identification of Pseudomonas sp

1-1-1-Isolation of Pseudomonas sp

After collecting samples from the rhizosphere of the three fields, dilutions were prepared for each soil sample for the pre-addition and post-addition stages. This was done by adding 10 g of soil to 90 ml of sterile distilled water in a 250 ml Erlenmeyer flask. The mixture was then shaken well to obtain a 10-1 dilution. Then, 1 ml of the soil suspension was transferred to test tubes containing 9 ml of sterile distilled water for each soil sample to obtain a 10-2 dilution. The process was repeated until a 10-6 dilution was obtained.

The King B medium (Appendix 2) was used according to the method described by King et al. (1954), with three replicates per dilution. The plates were incubated at 37 °C for 48 hours. After the appearance of pure colonies, the plates were checked and counted. The count was then multiplied by the reciprocal of the dilution.

A swab was taken from the growing colonies of each isolate and streaked onto the same medium again. The plates were then checked to confirm the purity of the isolates. The isolates were stored at 4 °C on the King A activation medium (Appendix 5) until diagnostic tests were performed.

1-1-2- Diagnosis of Pseudomonas sp.

1. Cultural properties:

The morphological characteristics of the colonies growing on King B medium (Appendix 2) were recorded as King et al. (1954) described, including their shape, size, and growth density.

2. Microscopic properties:

A portion of a colony was transferred to a sterile glass slide using a sterile loop for fixation, staining, and reaction with Gram stain. The shapes, aggregation patterns, and staining reactions were observed (Atlas, 1995).

3. Motility test:

The motility test for Pseudomonas sp. was performed after their growth on King B liquid medium (Appendix 2) as described by King et al. (1954) using the hanging drop method (Black, 1965a).

4- Biochemical tests.

1. Production of pigments test:

Plates of King B medium (Appendix 2) and King A medium (Appendix 5) were inoculated with young colonies of the different bacterial isolates using the streaking method. The plates were incubated at 37 °C for 24-72 hours, and the ability of the isolates to produce pigments was observed as described by Cruickshank et al. (1975).

2. Oxidase production test:

The plates containing N.B nutrient medium (Appendix 1) were inoculated with the different isolates and incubated at 32 °C for 24 hours. Then, drops of oxidase reagent were added to the growing colonies. The appearance of a purple color is considered a positive result for this test (Smibert and Krieg, 1981).

3. Catalase test:

A drop of each isolate was placed on a sterile glass slide, and a drop of 3% hydrogen peroxide solution was added. The appearance of air bubbles on the colony's surface is a positive result (Baron and Finegold, 1990).

4. Growth at 42 °C and 4 °C:

King B medium (Appendix 2) was inoculated with the bacterial isolates and incubated at 42 °C and 4 °C. The appearance of colonies is considered a positive result for the tolerance of high and low temperatures.

5. Growth in Simmon citrate medium:

Plates containing Simmon citrate medium (Appendix 6) were inoculated with the different bacterial isolates and incubated at 121 °C for 48-72 hours. The change of the medium color from green to blue is considered a positive result for the consumption of citrate as the sole source of carbon (Brown and Smith, 2017).

6. Growth in Cetrimide Agar:

Plates containing Cetrimide Agar (Appendix 7) were inoculated with the different isolates and incubated at 35 °C for 24-48 hours. The appearance of colonies in blue or bluish-green color is considered a positive result for this test (Brown and Smith, 2017).

7. Mannitol salt agar test:

Mannitol salt agar (Appendix 8) was prepared by adding 111 g of the ready-made medium to 1 liter of sterile distilled water in a pressure cooker at 121 °C for 15 minutes. Then, it was left to cool, poured into plates, inoculated with the different isolates, and incubated at 35 °C for 24-48 hours. Changing the medium color from red to yellow is a positive result (Hassan et al., 2017).

1-2- Isolation and diagnosis of Bacillus spp.

1-2-1-Isolation of Bacillus SPP

After collecting samples from the rhizosphere of the three fields, dilutions were prepared for each soil sample for the pre-addition and post-addition stages. This was done by adding 10 g of soil to 90 ml of sterile distilled water in a 250 ml Erlenmeyer flask. The mixture was then shaken well. A series of dilutions (10-1-10-6) was then performed by transferring 1 ml of the soil suspension to test tubes containing 9 ml of sterile distilled water. For each soil sample, the Pikovskaya agar media solid medium (Appendix 3) was used to inoculate the soil dilutions described by Donca et al. (2005), with three replicates per dilution. The plates were incubated at 37 °C for 48 hours. After the appearance of pure colonies, the plates were counted and multiplied by the reciprocal of the dilution. A swab was taken from the growing colonies of each isolate and streaked onto the same medium again. The plates were then checked to confirm the purity of the isolates. The isolates were stored on the Pikovskaya agar slant medium (Appendix 9) at 4 °C until diagnostic tests were performed.

1-2-2- Diagnosis of Bacillus bacteria

1. Cultural properties:

The morphological characteristics of the colonies growing on Pikovskaya agar media solid (Appendix 3) were recorded as Donca et al. (2005) described, including their shape and growth density.

2. Microscopic properties:

A portion of a colony was transferred to a sterile glass slide using a sterile loop for fixation and staining with Gram stain. The shapes, sizes, aggregation patterns, capsule formation, and staining reactions of the cells were observed as described by Black (1965a).

3. Motility test:
The motility test was performed after the growth of Bacillus sp bacteria on the Pikovskaya agar slant medium (Appendix 9) using the hanging drop technique described by Black (1965a).
4. Biochemical tests:
1. Starch hydrolysis test:
Plates containing starch hydrolysis medium (10 g starch, 3 g Beef extract, 12 g agar, 1000 ml distilled water, pH 7) were inoculated with the bacterial isolates and incubated at 32 °C for 48 hours. The hydrolysis of starch was observed after adding an iodine solution to cover the growing colony. The plates were left for 30 seconds, the iodine solution was poured off, and the plates were left to dry for 2-3 minutes. The appearance of a clear halo around the colony is a positive result for the test (Murray et al., 2007).
A dilute iodine solution was prepared for the starch hydrolysis test by dissolving 2 g of potassium iodide in 300 ml of distilled water.
2. Simmon citrate medium test:
Plates containing Simmon citrate medium (Appendix 6) were inoculated with the bacterial isolates and incubated at 32 °C for 48 hours. The plates were then examined. The change of the medium color from green to blue is a positive result for the consumption of citrate as the sole source of carbon (Brown and Smith, 2017).
3. Gelatin liquefaction medium test:
12% gelatin was added to nutrient broth medium N.B (Appendix 1), and the pH was adjusted to 7.2. The medium was then distributed into test tubes (5 ml per tube) and inoculated with the different isolates by stab inoculation. The tubes were incubated at 32 °C for 7 days. After incubation, the tubes were placed in the refrigerator for one hour and examined. The liquefaction of the gelatin is a sign of the production of the gelatinase enzyme by the bacteria and the breakdown of gelatin (Harrigan and McCance, 1976).
4. Oxidase test:
Plates containing Pickovski medium (Appendix 3) were inoculated with the different isolates and incubated at 32 °C for 24 hours. Then, drops of oxidase reagent were added to the growing colonies. The appearance of a purple color in the colonies is a positive result for this test (Smibert and Krieg, 1981).
5. Catalase test:
A drop of each of the isolates was placed on a sterile glass slide, and a drop of 3% hydrogen peroxide was added. The appearance of air bubbles on the colony's surface is a positive result for this test (Baron and Finegold, 1990).
6. Growth in 7% NaCl solution test:
7% sodium chloride was added to the Pickovski solid medium (Appendix 3), and the pH was adjusted to 7. Then, the isolates were inoculated and incubated at 32 °C for 48 hours. The appearance of colonies is a positive result for the tolerance of the salt level.
7. Growth in different pH tests:
N.B liquid medium (Appendix 1) with pH values of 5, 7, and 10 was used and distributed into plates. The isolates were inoculated and incubated at 32 °C for 48 hours. The appearance of colonies is a positive result.
8. Growth at 55 °C test:
The nutrient broth medium N.B (Appendix 1) was inoculated with the different bacterial isolates and incubated at 32 °C for 48 hours. The appearance of pellicles near the surface of the medium is a positive result of the ability of the isolates to grow at high temperatures.

1-3- Isolation and diagnosis of Azosprillium bacteria

1-3-1-Isolation of Azosprillium bacteria

After collecting samples from the rhizosphere of the three fields for the pre-addition and post-addition stages, dilutions were prepared for each soil sample by adding 10 g of soil to 90 ml of sterile distilled water in a 250 ml Erlenmeyer flask. The mixture was then shaken well. A series of dilutions (10-6-10-1) was then performed by transferring 1 ml of the soil suspension to test tubes containing 9 ml of sterile distilled water. For each soil sample, the nitrogen-free semi-solid culture medium (NF) (Appendix 4) was used and sterilized by autoclaving at 121 °C for 15 minutes and 15 pounds/square inch, with three replicates per dilution. The plates were incubated in an incubator at 30 °C for 28 hours according to the method of Kaur (2014). The plates were then examined after the appearance of colonies. The colonies were counted and multiplied by the reciprocal of the dilution. A swab of the growing colonies was then taken and streaked onto the same medium again. The plates were then incubated to confirm the purity of the isolates. The isolates were then stored on a nutrient agar slant medium (Appendix 1) at 4 °C until diagnostic tests were performed.

1-3-2- Diagnosis of Azospirillum sp

1. Cultural properties:
RC medium (Appendix 10) was prepared, and the isolates were streaked onto it. The plates were incubated at 35 °C for 96 hours. The growing colonies on the plates were examined, and their morphological characteristics were recorded, including their shape, growth, color, and reaction (Khammas et al., 1989).
2. Microscopic properties:

A portion of the colony was transferred to a sterile glass slide using a loop for fixation and staining with Gram stain. The slide was examined under a light microscope using a 100x oil immersion lens. The cells' aggregation and reaction with the stain were recorded (Black, 1965a).

3. Motility test:

The motility test was performed for Azospirillum bacteria after their growth on nutrient broth medium (Appendix 1) by stabbing (Black, 1965a).

4. Biochemical tests:

1. Growth in 3% sodium chloride:

The semi-solid nitrogen-free medium (Nfb) (Appendix 11) containing 3% sodium chloride was inoculated with 1 ml of the different isolates. The tubes were incubated at 30 °C for 4 days. The appearance of bacterial colonies is a positive result for this test (Holt et al., 1994).

2. Growth at pH (5, 6, 7):

The semi-solid nitrogen-free medium (Nfb) (Appendix 11) was prepared, and its pH was adjusted to 5, 6, or 7 using 1 N NaOH. The medium was then distributed into test tubes (10 ml per tube) and inoculated with the different isolates using a loop. The tubes were incubated at 30 °C for 48 hours. The appearance of bacterial colonies is a positive result for this test (Holt et al., 1994).

3. Oxidase test:

The plates containing nutrient broth medium (NB) (Appendix 1) were inoculated with the different isolates and incubated at 30 °C for 24 hours. Then, drops of oxidase reagent were added to the growing colonies. The appearance of a purple color is a positive result for this test, while the appearance of a white color is a negative result (Holt et al., 1994).

4. Catalase test:

A drop of each of the isolates was placed on a sterile glass slide, and a drop of 3% hydrogen peroxide was added. The appearance of air bubbles on the colony's surface is a positive result (Baron and Finegold, 1990).

5. Urease test:

The plates containing nutrient broth medium (NB) (Appendix 1) were inoculated with the different isolates and incubated at 30 °C for 24 hours. Then, drops of urease reagent were added to the growing colonies. The appearance of a red color is a positive result for this test (Smibert and Krieg, 1981).

6. Biotin requirement test:

This test was used to distinguish between the species of Azospirillum based on their requirement for or lack of biotin. The medium (Appendix 12) was prepared, and 5 ml was added to the test tubes. The tubes were divided into two groups, one with biotin and the other without biotin. All tubes were inoculated with 0.1 ml of the different isolates and incubated at 35 °C for 48 hours. The absence of a clear turbidity in the absence of biotin is a positive result for this test. The presence of a clear turbidity in the absence of biotin is a negative result for this test (Smibert and Krieg, 1981).

7. Carbohydrate fermentation tests:

The semi-solid nitrogen-free medium (Nfb) (Appendix 11) containing 1 g of ammonium sulfate and lacking bromothymol blue dye and malic acid was inoculated with the different isolates. 5 g of each carbohydrate was dissolved in 100 ml of sterile distilled water: D-mannitol, D-glucose, maltose, sucrose, lactose, and pectin. 1 ml of each carbohydrate solution was added to 9 ml of the inoculated medium. The tubes were incubated at 30 °C for 72 hours. The appearance of bacterial growth is a positive result (Baron and Finegold, 1990).

8. Nitrogen fixation ability test:

The nitrogen-free liquid medium (Nfb) (Appendix 1) was prepared, and 50 ml was placed in 250 ml flasks. The different isolates were inoculated by adding 1 ml of the liquid cultures. The flasks were incubated in a shaking incubator (Chaker) for three weeks at 30 °C. The amount of ammonia produced in the Nfb environment was estimated by taking 2 ml and estimating it using a Microkildahl device (Brenner et al., 2004).

The laboratory experiment: effect of different levels of pesticides on the number of bacterial isolates Azosprillium, Bacillus, and Pseudomonas in solid industrial media

A factorial experiment was conducted for each bacterium using the (RCD) design to study the effect of different levels of pesticides on the number of bacterial isolates. The experiment included (8) treatments resulting from the interaction of two factors: the first was the bacterial isolates for the two stages before and after the addition (two isolates), and the second was the levels of pesticide addition (C0 control, C1 recommended concentration, C2 1.5 times concentration, C3 2 times concentration). Each treatment was repeated thrice to become 24 experimental units for each bacterium and pesticide (fungicide, insecticide, weedicide). The media (Kig B, Pikovskaya agar media, NF) were prepared for each Pseudomonas, Bacillus, and Azosprillium bacteria (Appendix 2, 3, 1), respectively. A 250 ml beaker was prepared to add media specific to the bacteria and the presence of the pesticide (four concentrations). The medium was prepared and sterilized in an autoclave for 20 minutes at 121 °C. Then, the medium was poured into Petri dishes and inoculated with the bacterial isolates (Pseudomonas, Bacillus, Azosprillium). The plates were incubated for two days, and the bacteria were counted.

RESULTS AND DISCUSSION

1-1- Isolation and estimating the numbers of Pseudomonas bacteria when treating and not treating fields with pesticides.

Six isolates of Pseudomonas bacteria were isolated from treated and untreated agricultural fields with fungicide, insecticide, and herbicide (Table 1). The bacteria were isolated using the Kig B nutrient medium, suitable for this type of bacteria.

The table showed that the bacteria were present in all samples with different growth densities. There were no significant differences in the number of bacteria for both the fungicide and insecticide fields, but there were significant differences in the number of bacteria for the herbicide field. The number of bacteria in the soil samples of the herbicide-treated field was the lowest, at 1.8 × 106 cfu g-1 dry soil, with significant differences in their number in the fungicide- and insecticide-treated fields, which reached (4.05, 3.65) × 106 cfu g-1 dry soil, respectively.

The presence of bacteria in all treated and untreated samples may be because Pseudomonas bacteria are widely distributed microorganisms in the soil that live in colonies around the roots of many plants (Al-Akaabi, 2021). They are abundant in different regions due to their ability to colonize different environments. They have low nutritional requirements, which reflects their ability to adapt and grow in most environments. They also can utilize more than one carbon source for energy. They also can grow rapidly (Hasan et al., 2017). Pesticides may also affect bacteria. Pesticides have a toxic effect on the environment, the structure of the organisms in the soil, and their function within the soil associated with the roots (Mohanty et al., 2019).

The stages of isolating the bacteria significantly affected the number of bacteria. The highest numbers were recorded at the pre-treatment stage, reaching (4.03) × 106 cfu g-1 dry soil, then decreased significantly at the post-treatment stage.

The interaction between the isolation stages and pesticides significantly affected the number of Pseudomonas bacteria. At the pre-treatment stage, the number of isolates P3, isolated from the herbicide-untreated field, which was 1.6 × 106 g-1 dry soil, decreased significantly from the number of isolates P1 and P2, isolated from the herbicide-untreated field, which recorded (5.6, 4.8) × 106 cfu g-1 dry soil, respectively. At the post-treatment stage, no significant differences existed between isolates P4, P5, and P6, isolated from the fields treated with fungicide, insecticide, and herbicide, respectively. The number of isolates P4 and P5, isolated from the fungicide- and insecticide-treated fields, respectively, which reached (2.5) × 106 cfu g-1 dry soil for each, decreased significantly from the number of isolates P1 and P2, isolated from the herbicide-untreated field, which reached (5.6, 4.8) × 106 cfu g-1 dry soil. However, isolates P3 and P6, isolated from the herbicide-untreated and herbicide-treated fields, respectively, did not have significant differences in their numbers. This is because some pesticides do not affect microorganisms in the soil, such as herbicides, where the number of organisms decreases slightly when pesticides are added at low concentrations. However, when added at the recommended concentration, the number of soil organisms decreases significantly, such as Pseudomonas bacteria (Al-Tamimi and Al-Sammara'ee, 2018).

Table 1: Sample numbers, source of collection, and number of pseudomonas bacteria cells (*106 cfu g-1 dry soil) when treated with chemical pesticides

Sample number	Pesticides	Isolation stages				Average
		Before treatment		**After treatment**		
		Symbol of isolation	**Bacteria numbers**	**Symbol of isolation**	**Bacteria numbers**	
1	**fungicide**	**P1**	**5.6 a**	**P4**	**2.5 dc**	**4.05 A**
2	**insecticide**	**P2**	**4.8 ab**	**P5**	**2.5 dc**	**3.65 A**
3	**weedicide**	**P3**	**1.6 d**	**P6**	**2.0 dc**	**1.8 B**
	Average		**4.03 A**		**2.33 C**	

* The values of each group of averages are compared with each other. The values in one group with similar letters do not differ significantly according to the Tuky test at the probability level 0.05.

1-2- Isolation and estimation of the numbers of Bacillus bacteria when treating and not treating fields with pesticides

Six isolates of Bacillus bacteria were obtained from treated and untreated agricultural fields with fungicide, insecticide, and herbicide (Table 2). The bacteria were isolated using the Pikovskaya agar media solid medium, which is a suitable medium for the growth of this type of bacteria.

The table showed that the bacteria were present in all samples with different growth densities. There were significant differences in the number of bacteria for both the fungicide and insecticide fields and the herbicide field. The number of bacteria in the soil samples of the herbicide-treated field was higher than their numbers in the fungicide- and insecticide-treated fields, reaching (3.0, 1.5, 2.2) × 106 cfu g-1 dry soil, respectively. This may be attributed to the fact that herbicide pesticides are not toxic to microorganisms, so they do not cause a significant decrease in them, as they do not target non-target organisms. Herbicide pesticides are a new and effective agricultural strategy for

managing resistance to weeds, and they have been widely used to control the growth and removal of weeds in fields (Mohanty et al., 2019).

The isolating stages had a non-significant effect on the number of bacteria. The highest numbers were recorded at the pre-treatment stage, reaching (2.47) × 106 cfu g-1 dry soil, compared to the post-treatment stage (1.97) × 106 cfu g-1 dry soil.

The interaction between the isolation stages and pesticides had a non-significant effect on the number of Bacillus bacteria, except for isolate B3, which was isolated from the field and not treated with herbicide. This isolate had significantly higher numbers than all other isolates, reaching (3.7) × 106 cfu g-1 dry soil. All bacterial isolates decreased non-significantly at the post-treatment stage compared to the pre-treatment stage, except for isolate B6, which was isolated from the soil treated with herbicide. This isolate had significantly lower numbers than isolate B3, isolated from the soil not treated with herbicide. This may be because Bacillus bacteria have physiological capabilities to compete with other living organisms (Gordon, 1974).

Table 2: Sample numbers, collection source, and number of Bacillus bacteria cells (×cfu 106 gm-1 dry soil) when treated with chemical pesticides.

Sample number	Pesticides	Isolation stages				Average
		Before treatment		**After treatment**		
		Symbol of isolation	**Bacteria numbers**	**Symbol of isolation**	**Bacteria numbers**	
1	**fungicide**	**B1**	**1.6 bc**	**B4**	**1.4 bc**	**1.5 C**
2	**insecticide**	**B2**	**2.1 bc**	**B5**	**2.3 bc**	**2.2 B**
3	**weedicide**	**B3**	**3.7 a**	**B6**	**2.3 bc**	**3.0 A**
	Average		**2.47 A**		**1.97 A**	

* The values of each group of averages are compared with each other. The values in one group with similar letters do not differ significantly according to the Tuky test at the probability level 0.05.

1-3- Isolation and estimation of the numbers of Azosprillium bacteria when treating and not treating fields with pesticides

Six isolates of Azospirillum bacteria were obtained from treated and untreated agricultural fields with fungicide, insecticide, and herbicide (Table 3). The bacteria were isolated using the NF nutrient medium, which is suitable for the growth of this type of bacteria.

The table showed that the bacteria were present in all samples with different growth densities. There were non-significant differences in the number of bacteria for both the fungicide and insecticide fields and the herbicide field. The most bacteria were found in the herbicide-treated field, reaching (2.05, 1.95, 1.8) × 106 cfu g-1 dry soil, respectively.

The isolating stages had a non-significant effect on the number of bacteria. The number of bacteria increased non-significantly at the treatment stage, reaching (2.17) × 106 cfu g-1 dry soil, compared to the pre-treatment stage, which was (1.71) × 106 cfu g-1 dry soil.

The interaction between the isolation stages and pesticides had a non-significant effect on the number of Azospirillum bacteria. The isolate A6 from the herbicide-treated field had the highest number, reaching (2.7) × 106 cfu g-1 dry soil.

Table 3: Sample numbers, source of collection, and numbers of Azosprillium bacteria cells (×cfu 106 gm-1 dry soil) when treated with chemical pesticides.

Sample number	Pesticides	Isolation stages				Average
		Before treatment		**After treatment**		
		Symbol of isolation	**Bacteria numbers**	**Symbol of isolation**	**Bacteria numbers**	
1	**fungicide**	**A1**	**1.6 a**	**A4**	**2.3 a**	**1.95 A**
2	**insecticide**	**A2**	**2.1 a**	**A5**	**1.5 a**	**1.8 A**
3	**weedicide**	**A3**	**1.4 a**	**A6**	**2.7 a**	**2.05 A**
	Average		**1.71 AB**		**2.17 A**	

* The values of each group of averages are compared with each other. The values in one group with similar letters do not differ significantly according to the Tuky test at the probability level 0.05.

1-4- Estimating the numbers of Pseudomonas bacteria

The results of Table 4 indicate significant differences in the numbers of bacteria isolated from the untreated soil sample P1 and the soil treated with the fungicide P4. The numbers of bacteria isolated from the soil treated with the fungicide P4 were higher than those isolated from the untreated soil P1, reaching (18.92, 12.58) × 106 cfu ml-1, respectively. This increase in P4 isolates may indicate that the bacteria are more tolerant to the fungicide than the P1 isolate isolated from the untreated soil. This would make isolate P1 more tolerant of fungicides (Albrusci et al., 2011).

The increase in the levels of the fungicide had a non-significant effect on the number of P. aeruginosa bacteria. The numbers increased slightly with increasing levels (C1, C2, C3) compared to level C0.

The interaction between bacterial isolates and levels of the fungicide had a non-significant effect on the number of bacteria. The levels of the fungicide led to a slight increase in the numbers for both isolates P1 and P4.

The table's results also indicate significant differences in the numbers of bacteria isolated from the untreated soil sample P2 and the numbers of the isolate (P5) isolated from the soil treated with the insecticide. The numbers of isolate P5 were higher than those of isolate P2, reaching (27.91, 15.16) × 106 cfu ml-1, respectively. This increase is evidence of the bacteria's tolerance to the insecticide and their ability to use it as a source of metabolism, as stated by (Albrusci et al., 2011). The bacteria's tolerance to these pesticides is due to their high solubility in water and relatively low stability in the environment compared to other pesticides (Ahmad et al., 2022).

The increase in the levels of the insecticide had a significant effect on the number of bacteria. The numbers increased significantly with levels C1, C2, and C3 compared to level C0. The highest numbers were recorded at the recommended level C1, then decreased significantly at levels C2 and C3 compared to level C0. Adding pesticides at the recommended concentration may increase the number of bacteria.

The interaction between bacterial isolates and levels of the insecticide had a significant effect on the number of bacteria. The numbers of both isolates P2 and P5 increased significantly with increasing levels of the pesticide (C1, C2, C3) compared to level C0. The highest numbers were recorded at level C1 for both isolates P2 and P5, reaching (22.00, 36.00) × 106 cfu ml-1, respectively. The numbers of isolate P2 decreased slightly at levels C2, C3 compared to level C1, while the numbers of isolate P5 decreased slightly from level C2 and significantly at level C3. Isolate P5 isolated from the soil treated with the insecticide had the highest numbers compared to isolate P2 isolated from the untreated soil for all levels and with significant differences. This increase is evidence of the bacteria's tolerance to the insecticide and their ability to use it as a source of metabolism, as stated by (Albrusci et al., 2011).

The table's results also indicate significant differences in the numbers of bacteria isolated from the untreated soil sample P3 and the numbers of the isolate (P6) isolated from the soil treated with the herbicide. The numbers of isolate P6 were higher than those of isolate P3, reaching (23.66, 15.91) × 106 cfu ml-1. This may be attributed to the fact that most soil microorganisms can secrete enzymes and have effective enzymatic systems in the degradation of pesticides, even if their number is very small, by chemo-physical processes such as oxidation and reduction (Liu et al., 2012).

The increase in the levels of the herbicide had a significant effect on the number of bacteria. The increase in the levels of the herbicide led to a significant increase at level C2, reaching 26.33 × 106 cfu ml-1, and was non-significant at levels (C1 and C3), reaching (22.83, 16.83) × 106 cfu ml-1, compared to level C0, which was (13.16 × 106 cfu ml-1). Pseudomonas bacteria can be bioremediate herbicides by oxidizing the original compounds of herbicides, thereby providing nutrients and increasing the number of bacteria (Mendoza et al., 2011).

The interaction between bacterial isolates and levels of the herbicide had a non-significant effect on the number of bacteria. The increase in the levels of the herbicide led to a non-significant increase in the numbers of bacteria for both isolates, and there were no significant differences between the isolates at all levels.

Table 4: Effect of different levels of chemical pesticides on the population of Pseudomonas bacteria Isolated from soil treated and untreated with pesticides (×106 ml-1).

Isolation type	**Fungicide levels**				Average
	C0	C1	C2	C3	
P1	9.33 a	11.66 a	12.66 a	16.66 a	12.58 B
P4	12.66 a	22.00 a	21.00 a	20.00 a	18.91 A
Average	11.00 A	16.83 A	16.83 A	18.33 A	
Isolation type	**Insecticide** levels				Average
	C0	**C1**	**C2**	**C3**	
P2	6.66 e	22.00 dc	16.00 d	16.00 d	15.16 B
P5	16.00 d	36.00 a	33.66 ab	26.00 bc	27.91 A
Average	11.33 C	29.00 A	24.83 AB	21.00 B	

Isolation type	Weedicide levels				Average
	C0	C1	C2	C3	
P3	8.66 b	17.33 ab	22.66 ab	15.00 ab	15.91 B
P6	17.66 ab	28.33 a	30.00 a	18.66 ab	23.66 A
Average	13.16 B	22.83 AB	26.33 A	16.83 AB	

* The values of each group of averages are compared with each other. The values in one group with similar letters do not differ significantly according to the Tuky test at the probability level 0.05.

1-5- Estimating the number of Bacillus bacteria

Table 5 shows significant differences in the numbers of Bacillus bacteria isolated from untreated soil (B1) and treated soil (B4). The number of bacteria isolated from treated soil (B4) was higher than the number of bacteria isolated from untreated soil (B1), reaching (25.33, 16.83) × 106 cfu ml-1. This may be due to the bacteria's tolerance to the pesticide, as it was isolated from treated soil. The bacteria may also use the pesticide as an energy source (Albrusci et al., 2011).

The increase in the levels of the fungicide had a non-significant effect on the number of bacteria.

The interaction results show that the numbers of bacteria isolated from untreated soil (B1) decreased significantly at levels C2 and C3 compared to levels C0 and C1. The number of bacteria isolated from treated soil (B4) decreased significantly at level C3. This suggests that level C2 was inhibitory to isolate B1, while level C3 was inhibitory to isolate B4. This indicates that isolate B4 is more tolerant than isolate B1. Isolate B4 had the highest numbers at level C2, reaching (31.66) × 106 cfu ml-1, while isolate B1 had the highest numbers at level C1, reaching 23.66 × 106. Isolate B4 had higher numbers than isolate B1 at all levels of the pesticide, with non-significant differences except for level C2, where isolate B4 had significantly higher numbers.

The table's results also show significant differences in the numbers of bacteria isolated from untreated soil (B2) and treated soil (B5). The number of bacteria isolated from treated soil (B5) was higher than the number of bacteria isolated from untreated soil (B2), reaching (38.08, 22.66) × 106 cfu ml-1. This may be due to the bacteria's tolerance to the pesticide, as it was isolated from treated soil (Bacmaga et al., 2021).

The increase in the levels of the insecticide had a significant effect on the number of bacteria. The numbers of bacteria increased significantly with increasing levels of the insecticide compared to level C0, reaching (39.83) × 106 cfu ml-1 at level C1, then decreased significantly at levels C2 and C3, reaching (36.00, 27.66) × 106 cfu ml-1 at levels C2 and C3, respectively.

The interaction between bacterial isolates and levels of the insecticide had a significant effect on the number of bacteria. The numbers of both isolates (B2 and B5) increased significantly with increasing levels of the insecticide compared to level C0, except for isolate B2, which increased significantly at level C3 compared to level C0. The highest numbers were recorded for both isolates at level C1, reaching (31.00, 48.66) × 106 cfu ml-1 for isolates B2 and B5, respectively. The number of bacteria decreased significantly at levels C2 and C3 compared to level C0. Isolate B5 had higher numbers than isolate B2 at all levels of the pesticide, with significant differences. This may be because high concentrations of pesticides can enhance the bioremediation efficiency of the bacterial strain.

The table's results also show significant differences in the numbers of bacteria isolated from untreated soil (B3) and treated soil (B6). The number of bacteria isolated from treated soil (B6) was higher than the number of bacteria isolated from untreated soil (B3), reaching (34.75, 20.66) × 106 cfu ml-1. This may be because the herbicide may have led to a significant increase in microbial communities, while at the same time, it affected soil microbes significantly due to herbicide stress (Ma et al., 2023).

The increase in the levels of the herbicide had a significant effect on the number of bacteria. The numbers of bacteria increased significantly at levels C1 and C2 compared to level C0 and decreased significantly at level C3. The highest numbers were recorded at level C2, reaching (33.38) × 106 cfu ml-1, and the lowest numbers were recorded at level C3, reaching (16.38) × 106 cfu ml-1.

The interaction between bacterial isolates and levels of herbicide had a non-significant effect on the number of bacteria. The number of isolates B3 increased non-significantly at level C2 and decreased at level C3. The number of isolates B6 also increased non-significantly at levels C1 and C2 and decreased at levels C3. Isolate B6, isolated from treated soil, had higher numbers than isolate B2, isolated from untreated soil, at all levels of the herbicide, with non-significant differences except for level C1, where the numbers increased significantly.

This is likely due to the fact that the herbicide is a broad-spectrum herbicide, and exposure to high concentrations can cause serious oxidative damage. This damage is manifested by increased lipid peroxides, protein oxidation levels, and oxidase activity (Hamed et al., 2022). The herbicide is also systemic and affects the soil and the microbes present in the soil environment. It affects the enzymes present in the soil and their properties. Due to their accumulation, the residues of this herbicide cause changes in the physical and chemical properties of the soil, such as pH and organic matter content (Machado et al., 2018).

The increase in the number of bacteria at level C1 maybe because this level is below the threshold of toxicity for the bacteria. The increase in the numbers of bacteria at levels C2 and C3 may be because the bacteria can metabolize the herbicide and use it as a source of nutrients.

Table 5: Effect of different levels of pesticides on the numbers of Bacillus bacteria isolated from soil Untreated and treated with pesticides (×106 ml-1).

Isolation type	**Fungicide levels**				Average
	C0	C1	C2	C3	
B1	22.00 b	23.66 ab	10.00 d	11.66 dc	16.83 B
B4	23.66 ab	25.00 ab	31.66 a	21.00 bc	25.33 A
Average	22.83 A	24.33 A	20.83 AB	16.33 A	
Isolation type	**Insecticide** levels				Average
	C0	**C1**	**C2**	**C3**	
B2	13.00 f	31.00 bc	28.00 cd	18.66 ef	22.66 B
B5	23.00 ed	48.66 a	44.00 a	36.66 b	38.08 A
Average	18.00 D	39.83 A	36.00 B	27.66 C	
Isolation type	**Weedicide levels**				Average
	C0	**C1**	**C2**	**C3**	
B3	22.66 edc	18.66 ed	29.66 bcd	11.66 e	20.66 B
B6	33.00 abc	46.00 a	38.00 ab	22.00 cde	34.75 A
Average	27.83 A	32.33 A	33.83 A	16.83 B	

* The values of each group of averages are compared with each other. The values in one group with similar letters do not differ significantly according to the Tuky test at the probability level 0.05.

1-6- Estimation of the number of Azospirillum bacteria

Table 6 shows significant differences in the numbers of Azospirillum bacteria isolated from untreated soil (A1) and treated soil (A4). The numbers of isolate A4 were higher than that of isolate A1, reaching (28.58, 20.33) × 106 cfu ml-1, respectively.

The increase in the levels of the fungicide had a significant effect on the number of bacteria. The increase in the fungicide C1, C2, and C3 levels led to a non-significant increase at levels C1 and C3 and a significant increase at level C2 compared to level C0. The highest numbers were recorded at level C2, reaching (28.50) × 106 cfu ml-1, with non-significant differences from levels (C1, C3).

The interaction between bacterial isolates and levels of the fungicide had a non-significant effect on the number of bacteria. The increase in the levels of the fungicide led to a non-significant increase in the numbers of bacteria for both isolates and all levels, except for level C2, which led to a significant increase in isolate A4 compared to level C0. Isolate A4 had the highest numbers compared to isolate A1 for all levels, with non-significant differences, except for level C3, which led to a significant increase.

Table 6 also shows significant differences in the numbers of bacteria isolated from untreated soil (A2) and treated soil (A5). The numbers of isolate A5 isolated from treated soil were higher than the numbers of isolate A2 isolated from untreated soil, reaching (28.33, 19.33) × 106 cfu ml-1, respectively.

The increase in the levels of the insecticide had a significant effect on the number of Azospirillum bacteria. The increase in the levels of the insecticide led to a significant increase in the number of bacteria compared to level C0. The highest numbers were recorded at level C2, reaching (29.00) × 106 cfu ml-1.

The interaction between isolates and levels of the insecticide had a significant effect on the number of bacteria. The increase in the levels of the insecticide led to a non-significant increase in the number of isolate A2 isolated from untreated soil and a significant increase in the number of isolate A5 isolated from treated soil compared to level C0. The highest numbers were recorded at level C2 for isolate A5, reaching (36.00) × 106 cfu ml-1, with non-significant differences from levels C1 and C3. Isolate A5 had the highest numbers compared to isolate A2 at levels C1, C2, C3, with non-significant differences. The increase in the number of bacteria isolated from treated soil is evidence of the ability of organisms to tolerate the concentration of pesticides and to metabolize and use them as a source of energy and carbon (Kaliwal et al., 2014).

Table 6 also shows significant differences in the numbers of bacteria isolated from untreated soil (A3) and treated soil (A6). The numbers of isolate A6 were significantly higher than those of isolate A3, reaching (21.25, 13.08) × 106 cfu ml-1, respectively.

The increase in the levels of the weed killer had a non-significant effect on the number of bacteria. There were no significant differences in the numbers of bacteria at all levels.

The interaction between isolates and levels of the weed killer had a non-significant effect on the number of bacteria. The increase in the levels of the weed killer had a non-significant effect on the numbers of both isolates (A3, A6) of A.lipoferum and A. brasilense, respectively.

Table 6: Effect of different levels of pesticides on the number of Azosprillium bacteria cells Isolated from soil treated and untreated with pesticides (×106 ml1)

Isolation type	**Fungicide levels**				Average
	C0	C1	C2	C3	
A1	19.00 bc	26.00 abc	21.66 abc	14.66 c	20.33 B
A4	20.66 bc	27.66 ab	35.33 a	30.66 ab	28.58 A
Average	19.83 B	26.83 AB	28.50 A	22.66 AB	
Isolation type	**Insecticide** levels				Average
	C0	**C1**	**C2**	**C3**	
A2	18.66 bc	18.66 bc	22.00 abc	18.00 bc	19.33 B
A5	12.00 c	31.66 ab	36.00 a	32.66 ab	28.33 A
Average	15.83 B	25.166 A	29.00 A	25.33 A	
Isolation type	**Weedicide levels**				Average
	C0	**C1**	**C2**	**C3**	
A3	17.00 a	18.00 a	8.66 a	8.66 a	13.08 B
A6	23.66 a	22.66 a	23.00 a	15.66 a	21.25 A
Average	20.33 A	20.33 A	15.83 A	12.16 A	

* The values of each group of averages are compared with each other. The values in one group with similar letters do not differ significantly according to the Tuky test at the probability level 0.05.

CONCLUSIONS

1- Bacillus and Azospirillum bacteria were more pesticide-resistant than Pseudomonas bacteria.

2- Bacterial isolates from Pseudomonas, Bacillus, and Azospirillum bacteria isolated from soil treated with fungicide, insecticide, and weed killer in industrial environments were more efficient in degrading pesticides and increasing their numbers than isolates isolated from soil not treated with pesticides in solid industrial environments.

RECOMMENDATIONS

Use a mixture of Pseudomonas, Bacillus, and Azospirillum bacteria with pesticides side by side and at the recommended level to eliminate pesticide residues and their toxic effects.

REFERENCES

1. **Ahmad, S., A. P Pinto; F. I., Hai, M. E. T. I. Badawy; R. R. Vazquez; T. A Naqvi; ... and H. J. Chaudhary, 2022.** Dimethoate residues in Pakistan and mitigation strategies through microbial degradation: a review. Environmental Science and Pollution Research, 29(34), 51367-51383.
2. **Al-Akabi, S. H. A. 2021.** Study of the microbial community of the rhizosphere of bulgur and toadstool plants in some areas of Diyala Governorate, doctoral thesis, College of Education for Pure Sciences. Diyala University.
3. **Albrusci, C. J. L.; T. Pablos; J.Corrales; I. Lopez-Marin; F. Marin and Catalina. 2011.** Biodegradation of photodegraded mulching films on polyethylenes and stearates of calcium and iron as prooxidant-based.
4. **Al-Mashhadani, S. S. K. 2022.** Bioremediation of pesticide residues in soil and barley plants. Master Thesis. Faculty of Agriculture. Diyala University.
5. **Al-Samarrai, I. K. and Al-Tamimi, F. M. S. 2018.** Concepts and applications of soil microbiology. Diyala University Press. Iraq.

6. Atlas, R. M. 1995. PrincipleofMicrobiology.Mosby-Year Book, U.S.A.
7. **Bacmaga, M.; J. Wyszkowska and J. Kucharski. 2021.** Bacterial diversity and enzymatic activity in a soil recently treated with tebuconazole. Ecological Indicators, 1 2 3, 1 0 7 3 7 3.
8. **Baron, E. J. and Finegold, S. M. 1990**. Diagnostic microbiology. 8th. (ed). The C. V. Mosby Company.
9. **Black C.A. 1965.** Methods of soil analysis –Part 2. Chemical and microbiological properties. Am. Soc. Agron. Inc. Madison. Wisconsin USA.
10. **Brenner, D. J., N. R. Krieg and J. T. Staley. 2004.** Bergey's Manual of Systematic Bacteriology. Williams and Wilking. Baltimore. London. PP1136.
11. **Brown, A.E. and, H.R. Smith .2017.** Benson's Microbiological Applications, Laboratory Manual in General Microbiology 14th (Ed). McGraw-Hill Higher Education. New York. 438pp
12. **Cruickshank, R.; B.Maion, and S.Duguid.1975.** Medical; Microbiology: The practice of medical Microbiology. 12th (ed). Churchill Living Stone, Edinburgh, UK. Vol.2.
13. **Gomez, f., v. Salmeron, b. Rodelas, m.v. martinez-toledo and j. Gonzalez-Lopez. 1998.** Response of Azospirillum brasilense to the pesticides bromopropylate and methidathion on chemically denned media and dialysed-soil media. Ecotoxicology 7, 43 -47.
14. **Gordon, R. E., W.C. Haynes, and C.H.N. Pang. 1974.** The genus bacillus (No. 427). Agricultural Research Service, US Department of Agriculture.
15. **Hamed, S. M.; I.J. Al-Nuaemi; S.M. Korany; E.A. Alsherif; H.S. Mohamed; and H. AbdElgawad. 2022.** Hazard assessment and environmental fate of propiconazole degradation by microalgae: Differential tolerance, antioxidant and detoxification pathway. Journal of Environmental Chemical Engineering, 10(4), 108170.
16. **Harrigan, W. F. and McCance, M. E. 1976.** Laboratory Methods in Food and Dairy Microbiology. Academic Press, London.
17. **Hassan, A. A.; Abdul-Karim O. S., and Heba, M. Y. 2017.** Isolation and identification of effective antifungal compounds from Pseudomonas fluorescens isolates from Iraqi soil and evaluation of their efficiency in inhibiting the pathogenic fungus Macrophomina phaseolina growth. Tikrit University Journal of Agricultural Sciences, special issue on the Sixth Scientific Conference for Agricultural Sciences proceedings, March 28-29, 2017, pp. 169-181.
18. **Holt, J., N. R. Krieg., P. H. A. Sneath., J. T. Staley and S. T. Williams. 1994.** Manual Determinative Bacteriiology. 9th, ed. USA.
19. **Jallow. F.A., G. Awadh, S. Albaho, Y. Devi, and Nisar Ahmed. 2017.** Monitoring of pesticide Residues in Commonly used fruits and Vegetables in Kuwait. 14(8):833.
20. **Kaur, I.2014.** Effect of the nitrogen-fixing capacity of Azotobacter and Azospirillum on the growth of rosa polyantha. International J.OF Emerging inSci and Technology.1 (7):1073-1080.
21. **Khammas, K. M.; E.Ageron; B. A. D. Grimont and B. Kaiser. 1989.** Azospirillum irakense sp. Nov., Anitrogen. Fixing bacterium associated with rice roots and rhizosphere soil Res. Microbial. 104: 679-693.
22. **Liu L ao P Lu Ni Y X Li X Yan, and S P Li .2012.** Logan, N. A., and Vos, P. D. 2015. Bacillus. Bergey's manual of systematics of archaea and bacteria, 1-163.
23. **Ma, Q.; Q. Li; J. Wang; R.E. Parales; L. Li, and Z. Ruan, 2023.** Exposure to three herbicide mixtures influenced maize root-associated microbial community structure, function and network complexity. Environmental Pollution, 122393.
24. **Machado De-Melo, A. A., Almeida-Muradian, L. B. D., Sancho, M. T., and Pascual-Maté, A. 2018.** Composition and properties of Apis mellifera honey: A review. Journal of apicultural research, 57(1), 5- 37.
25. **Mendoza, J. C.; Y. Perea and J.A.Salvador. 2011.** Bacterial biodegradation of permethrin and cipermetrina pesticides in a culture assemblage. Avances en Ciencias e Ingenieria, 2(3), 45-55.
26. **Mohanty, S. S.; and H.M. Jena. 2019.** Degradation kinetics and mechanistic study on herbicide bioremediation using hyper butachlor tolerant Pseudomonas putida G3. Process Safety and Environmental Protection, 125, 172-181.
27. **Murray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. L. and Pfaller M. A. 2007.** Manual of Clinical Microbiology. 9th (ed). Washington, DC. American Society for Microbiology, USA.
28. **Patel, PH., and, M.F.2020.** Macrolides. Statpearls [Intrnet}
29. **Romeh, A. A. and M. Y. Hendawi .2014.** Bioremediation of Certain Organophosphorus Pesticides by Two Biofertilizers, Paenibacillus (Bacillus) polymyxa (Prazmowski) and Azospirillum lipoferum (Beijerinck). J. Agr. Sci. Tech. (2014) Vol. 16: 265-276
30. **Smibert,R.M. and Krieg, N.R.1981.**General characterization.In: Sneath P.H. MURRAY, R.G.E., and Costilow, R. N., Nester, E.W., Wood, W. A., Krieg, Philips, G, P.(eds). Manual of Methods for General Bacteriology: 409-443
31. **Smibert,R.M. and N.R. Krieg.1981.**General characterization.In: Sneath P.H. MURRAY, R.G.E., and Costilow, R. N., Nester, E.W., Wood, W. A., Krieg, Philips, G, P.(eds). Manual of Methods for general bacteriology: 409-443.
32. **WHO.2018.** Recommended classification of pesticides by hazard and guidelines to classification 2009 [displayed 6 September 2012].