Plant Archives Vol. 20, Supplement 1, 2020 pp. 376-383 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

DETERMINATION OF THE IDEAL CONDITIONS FOR THE BIODEGRADATION PROCESS AND LOWEST INHIBITORY CONCENTRATION OF CLORSPAN AND GROUND UP PESTICIDES BY DIFFERENT TYPES OF BACTERIA

Beadaa Abdalqader Mahdii[1] and Ahmed Mohammed Turke[2]

[1]Department of Biology, College of Science, University of Baghdad, Iraq

[2]Department of Biology, College of Science, Anbar University, Iraq

Abstract

Local bacterial isolates isolated from 23 soil samples polluted with different pesticides from Baghdad areas on both sides of Karkh and Rusafa, The ability of bacterial isolates on biodegradation of (Clorspan (Chlorpyrifos) and Ground Up (glyphosate)) pesticides examined, where a preliminary examination of bacterial isolates was performed by using solid and liquid MSM for the purpose of determine the better Bacterial isolate which able to biodegradation of pesticides with a concentration of 100 mg / liter of pesticide with a pH 7 and 37 °C temperature, where the Pseudomonas aeruginosa bacteria proved it efficiency as a bacterial isolate able to analyze Clorspan and Ground up. And for (MIC) experiment the lowest inhibiting concentration of the bacteria growth determined, since the results indicated that Pseudomonas, Aeromonas bacteria continued to grow for all concentrations and both pesticides that start from (50-500) mg/liter. As for Staphylococcus bacteria, the lowest inhibitor concentration was 450 mg/ liter in Clorspan, and for 500 mg/ liter for Ground up. As for the ideal conditions, Pseudomonas aeruginosa proved to be the best isolate bacterium which able to analyze pesticides biologically by registering highest values , it recorded value of (95,53,50, 27) in ideal temperature , in 45 °C for the Clorspan and Ground up pesticides, respectively, and (43.30) during cuddling period for 7 days in clorspan pesticide medium, and (39.15) During cuddling period for 8 days in Ground up medium, And it recorded (27.87) for pH 7 in Clorspan medium. And for Ground up pesticide medium, it recorded a highest values for Staphylococcus bacteria and it was (34.25) at pH 7. The study aimed to determine the best bacterial isolate which able of biodegradation of pesticides and determine MIC (Minimum Inhibition Concentration) for bacterial growth in different pesticide concentrations, study the ideal conditions for biodegradation of pesticides (temperature, PH, Cuddling period **)**.

Keywords: biodegradation process, Clorspan, pesticides, Bacteria

Introduction

Soil is the main warehouse for many environmental pollutants, whether they are pesticides or jungles pesticides , as these pesticides, like any other chemical material, may have some undesirable side effects in the environment and where these pesticides take their path to the soil, whether through immediate treatment after or before germination, or as a result of the fall of part of the pesticide during the treatment of vegetative parts, so the relationship between these pesticides and creation of precise soil (Teng *et al.,* 2010). Pesticides widely studied as environmental pollutants due to their heavy use to control lesion that affect agricultural crops, homes and gardens (Cho *et al.,* 2002). These pollutants have hardly any biodegradability and resistance to treatment with traditional technologies (Bandala *et al.,* 2007). The microorganism are used in biological treatment for environmental pollutants (Ali, 2011; Singh, 2009). The use of Microorganisms is a very effective and natural process when compared with other methods that apply on biological treatment in the polluted site (Sassman *et al.,* 2004, Nawab *et al.,* 2003).

Materials and Methods

Sample collection

Samples collected from January 2019 until the end of February 2019, 23 samples from 6 stations (agricultural nurseries) lies in the city of Baghdad, where it included the following areas (Amiriyah, Sayyidia, Yarmouk, Naffak Al-Shurtah, Palestine Street, Adhamiya) where these areas were polluted with different pesticides as a result of use for agricultural purposes and with different and unknown concentrations, several types of bacteria isolated from these pollute sites where a total of 32 bacterial belonging to the Pseudomonas, Staphylococcus, Aeromonas, family were isolated .These types later diagnosed and their ability of growing in pesticides tested, The samples were collected randomly from the soil surface layer (5-10 cm in depth) using pre-sterilized augar and moved to sterile polyethylene bags, And transplanted immediately after being brought to the laboratory by five Samples for every day.

Table 1 : Shows the number of regions and bacterial isolates for each region.

Locations	Number of samples	Number of isolates
Amiriyah	3	3
Sayyidia	4	6
Yarmouk	3	3
Naffak Al-Shurtah	3	2
Palestine Street	5	8
Adhamiya	5	10
Total	**23**	**32**

Examination of Biologically analyzed bacterial isolates for pesticides:

Primary screening: One gram of a planktonic of soil prepared for each soil sample in 9 ml of pre-sterilized distilled water and kept at room temperature for 24 hours. In the next day, 500 microliter of floating material was spread over a culture medium containing Solid salts MSM, to isolate insecticide-resistant bacteria (Goda *et al.,* 2010).

Table 2 : Shows the components of MSM medium.

Component	weight g / l
KH_2PO_4	1 g.
K_2HPO_4	1 g.
NH_4NO_3	1 g.
$(NH_4)_2SO_4$	1 g.
$MgSO_4.7H_2O$	0.2 g.
NaCl	0.5 g.
$FeSO_4.7H_2O$	0.05 g.

Bacterial Diagnosis: Bacteria diagnosed by external shape and electron microscope, and Biochemical tests, bacterial isolation detection by the VITEK2 device system (Pincus, 2013).

Table 3 : Shows how to diagnose bacterial isolates using biochemical and phenotypic examinations.

Microorganism / Characteristics	*Staphylococcus sp.*	*Pseudomonas* sp.	*Aeromonas* sp.
Colony shape	Circular	Smooth convex	Mucoid convex
Colony color	Creamy	green	Creamy
Cell shape	Cocci	rod	Rod
Gram stain	+	-	-
Catalase test	+	+	+
Oxidase test	+	+	+
Growth at 42ºC	-	+	+
Haemolysis on blood agar	-	+	+
Gelatin	-	+	+
Growth at EMB	-	-	-
Urease production test	+	-	-
Indole production test	-	-	+
Motility test	-	+	+
Lipase production test	-	+	+
Lactose fermentation test	+	-	+

Secondary screening of bacterial isolates :

Bacterial isolates also resulted sifting in liquid media, 50 ml of MSM taken and left in 250 ml in Erlenmeyer flasks (conical flasks) containing clorspan and other containing Ground up at 100 mg/liter concentration, then the flasks inoculated with 2% of the chosen bacterial isolates, It was incubated in a rocking incubator at 30 °C, 120 rpm, for 7 days, after that the absorbance (OD)measured by Spectrophotometer device (Zhongli *et al.,* 2001), Through that the most efficient bacterial isolation able of biodegradation of the pesticide were chosen.

Results and Discussion

Primary screening

Thirty-two pure bacterial isolates examined to test their ability of growth in solid mineral salts (MSM) treated with both Clorspan and Ground-up pesticides separately. 7 bacterial isolates that were resistant to the Clorspan and Ground up pesticides isolated as it shown in Tables (4) and (5).

Primary examination of bacterial isolates occurred by using solid and supplemented MSM medium with clorspan and Ground up pesticides. The medium enriched with insecticide at 100 mg /liter concentration. Various colonies were obtained 7 days after cuddling at a temperature of 37 °C.

Table 4 : Shows the growth of bacterial isolates in the culture dishes containing MSM medium and the clorpanic insecticide at a concentration (100 mg / L).

Bacterial isolate	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	-	-	-	-	+	++	++
2	-	-	+	+	++	++	++
3	-	-	+	+	++	++	++
4	-	-	-	-	-	-	-
5	-	-	-	-	+	+	+
6	-	-	-	-	-	+	+
7	-	-	-	-	-	-	-
8	-	-	-	-	-	-	+
9	-	-	-	-	-	-	+

Table 5 : Shows the growth of bacterial isolates in the culture dishes containing MSM medium and Ground Up herbicide at a concentration (100 mg / L).

Bacterial isolate	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	-	-	-	-	+	++	++
2	-	-	-	+	+	+	++
3	-	-	-	+	++	++	++
4	-	-	-	-	-	-	-
5	-	-	-	-	+	+	+
6	-	-	-	-	-	+	+
7	-	-	-	-	-	-	-
8	-	-	-	-	-	-	+
9	-	-	-	-	-	-	+

Where the symbol (-) indicates that there is no growth, the symbol (+) indicates good growth, and the symbol (++) indicates very good growth.

The results showed that the bacterial isolates (3,2,1) grew very well in both clorspan and Ground up after seven days cuddling in the solid MSM medium where bacteria of the pesticides (Clorspan and Ground up) used as the only source of carbon and energy, while the isolates (6, 5) showed Good growth in the solid MSM medium, while the two isolates (9,8) showed weak growth in the solid MSM medium (Abboud *et al.,* 2016) as they indicated an increase in bacterial isolates growth after the third day of the cuddling , As for the two bacterial isolates (7,4), they showed their inability of growing in the solid MSM medium containing the selected pesticides. As for the bacterial growth of the clorspan pesticide, it appeared from the third day, while the bacterial growth of the seven isolates appeared on the fourth day for the ground up pesticide, As it shown in the Tables (4) and (5) above, this may be due to the fact that bacterial colonies that do not grow in this medium because they do not have the ability to break these compounds as a result of the absence of a specialized enzymatic system within the bacterial cell, Or because this bacterial isolates have no metabolic ability and couldn't Normalize and grow on the agriculture medium that containing pesticides, another reason could be a decrease in the solubility of these compounds, which reduces the availability of compounds for microorganisms and then the food becomes not enough for their growth, and finally the bacteria may consume oxidation products that have the ability to act as antioxidants for these compounds (Mollea *et al.,* 2005).

Determination of MIC for bacterial isolates

This experiment performed to determine the lowest Minimum Inhibitory Concentration (MIC) for bacterial isolates that able of biodegradation of pesticides using solid MSM after pesticides added in different concentrations, The growth delay of the bacterial isolates coincided with the different pesticide concentrations, In this study, three bacterial isolates that resistant to clorspan and Ground up pesticides selected from different areas of Baghdad (Al-Saydiya, Al-Adamiyah, Palestine Street), which found growing on concentrations (50, 100, 150, 200, 250, 300, 350, 400, 500 mg/liter). The lowest inhibitory concentration was 450 mg/liter for the first bacterial isolate (Staphylococcus) that grow in medium contains clorspan pesticide, and 500 mg/liter of ground up pesticide, While bacterial isolates (Pseudomonas, Aeromonas) continued to grow in all concentrations as shown in the table (6) and (7). During the MIC experiment, it noticed that in the presence of high concentrations of the selected pesticides, the bacteria were very stressful and as a result their growth slowed down, and also it found that adding an external carbon source such as dextrose increases the efficiency of the bacteria in biodegradation and also increases the growth of the bacteria in the presence of the pesticide Pests (Mathew, 2009). And according to Table (6) and (7), it observed that there were significant differences between the concentrations of pesticides as well as between the bacterial isolates selected at the level of P <0.05.

Table 6 : Shows the mean and standard error (SE) for the lowest concentration inhibitor (MIC) of the bacterial isolates growing in a container medium on the clorspan insecticide.

LSD Value	-Concentration Clorspan										Bacterial Type
	500%	450%	400%	350%	300%	250%	200%	150%	100%	50%	
24.26 *	0.00 ±0.00	0.00 ±0.00	25.00 ±5.00	74.00 ±4.00	96.00 ±1.00	111.00 ±1.00	150.00 ±20.00	195.00 ±5.00	205.00 ±5.00	260.00 ±10.00	S
26.61 *	17.50 ±2.50	74.00 ±4.00	126.00 ±4.00	185.00 ±15.00	210.00 ±0.00	280.00 ±10.00	305.00 ±5.00	360.00 ±10.00	400.00 ±0.00	465.00 ±15.00	P
29.38 *	11.00 ±1.00	72.50 ±2.50	121.00 ±1.00	173.00 ±3.00	200.00 ±0.00	225.00 ±15.00	278.00 ±1.50	300.00 ±0.00	360.00 ±20.00	465.00 ±15.00	A
---	**6.99 ***	**12.25 ***	**16.84 ***	**41.08 ***	**2.59 ***	**46.91 ***	**53.71 ***	**29.05 ***	**53.56 ***	**60.93 ***	**LSD Value**
(*P<0.05)											

Table 7 : Shows the mean and standard error (SE) for the lowest concentration inhibitor (MIC) of the bacterial isolates growing in a container medium on the Ground Up.

LSD Value	-Concentration Clorspan										Bacterial Type
	500%	450%	400%	350%	300%	250%	200%	150%	100%	50%	
24.26 *	0.00 ±0.00	0.00 ±0.00	25.00 ±5.00	74.00 ±4.00	96.00 ±1.00	111.00 ±1.00	150.00 ±20.00	195.00 ±5.00	205.00 ±5.00	260.00 ±10.00	S
26.61 *	17.50 ±2.50	74.00 ±4.00	126.00 ±4.00	185.00 ±15.00	210.00 ±0.00	280.00 ±10.00	305.00 ±5.00	360.00 ±10.00	400.00 ±0.00	465.00 ±15.00	P
29.38 *	11.00 ±1.00	72.50 ±2.50	121.00 ±1.00	173.00 ±3.00	200.00 ±0.00	225.00 ±15.00	278.00 ±1.50	300.00 ±0.00	360.00 ±20.00	465.00 ±15.00	A
---	**6.99 ***	**12.25 ***	**16.84 ***	**41.08 ***	**2.59 ***	**46.91 ***	**53.71 ***	**29.05 ***	**53.56 ***	**60.93 ***	**LSD Value**
(*P<0.05)											

The reduction or absence of bacterial numbers at high concentrations of pesticides at the cuddling period (7) days may be happened due to the permeation of the pesticide at that time of cuddling and then the permeation of the nutrients resulting from the pesticide decomposition and the accumulation of toxic products which leads to a lack of bacterial numbers, or it is believed that the bacteria was able to transfer the pesticide into toxic products for the same type of organism that affects the original compound (Alexander, 1982) .The decrease in growth and numbers of bacteria in

solid industrial environments by increasing levels of pesticides as mentioned (Milosevic *et al.,* 2000), As the bacteria growth in the environment with a high content of pesticides may lead to an increase in the absorption of pesticides by the bacteria or link it to the effective sites of the cell surface so that they stop the work of enzymes, which leads to stop growth and metabolic processes (Nastaija *et al.,* 2002).

Conditions affecting bacterial growth and biodegradation of pesticides :

(a) Effect of temperature on bacterial growth:

Table (8) indicates the Averages values and standard e mistake of bacterial growth of bacterial isolates growing in liquid MSM medium with clorespan pesticide 100 mg / liter concentration, the table (9) indicates averages values and standard mistake of bacterial growth to the bacterial isolates that growing in liquid MSM medium with Ground up pesticide, growing at (50, 45, 40, 35, 30, 25) temperatures and for 7 days cuddling period .

The results indicated that the highest averages bacterial growth in the clorspan pesticide recorded by Pseudomonas bacteria (53.95 ± 0.75) at 40 °C temperature and the lowest average bacterial growth recorded by Staphylococcus bacteria and reached (13.00 ± 0.30) at 50 °C temperature, Bacterial growth averages were measured at a wavelength of 600 nm and the results of statistical analysis (LSD) indicated that there are significant differences between the bacterial growth averages values for each type of bacterial isolates and the presence of significant differences of averages values of different temperatures at the level P <0.05.

As for Ground up pesticide, the highest averages of bacterial growth recorded by Pseudomonas (27.50 ± 2.50) and Aeromonas bacteria (27.45 ± 2.85) 40 °C temperature, and Aeromonas bacteria recorded lowest averages of bacterial growth reached (8.85 ± 1.15) in 50 °C temperature, bacterial growth averages were measured at a wavelength of 600nm, Also, the results of the statistical analysis (LSD) indicated that there are significant differences between the averages values of bacterial growth for each type of bacterial isolates at the level of P <0.05, while the most averages values of different temperatures for bacterial isolates most of them did not notice the presence of significant differences except for the two (50, 25 °C) temperature, there were significant differences between them at the P <0.05 level.

Table 8 : Shows the mean values and standard error of SE for the growth of bacterial isolates at different temperatures present in the medium of the clorspan after 7 days of brood and measured at 600nm.

Mean ± standard error				Temperature
LSD Value	**A**	**P**	**S**	
5.891 *	16.20 ±0.00	22.50 ±1.50	15.00 ±1.70	°C 25
1.708 *	23.10 ±0.00	34.10 ±0.10	20.65 ±0.65	°C 30
5.997 *	30.75 ±1.55	51.45 ±1.05	27.35 ±1.35	°C 35
9.809 *	26.90 ±0.00	53.95 ±0.75	25.00 ±3.70	°C 40
8.731 *	30.80 ±0.00	28.20 ±2.70	21.30 ±2.00	°C 45
7.481 *	15.00 ±1.20	21.60 ±2.60	13.00 ±0.30	°C 50
---	**2.769 ***	**5.989 ***	**6.762 ***	**LSD Value**
(*P<0.05)				

Table 9 : Shows the mean values and standard error of SE for the growth of bacterial isolates at different temperatures present in the medium of the Ground Up after 7 days of brood and measured at 600nm.

Mean ± standard error				Temperature
LSD Value	**A**	**P**	**S**	
2.131 *	16.17 ±0.02	17.50 ±0.50	14.65 ±0.65	**°C 25**
10.24 NS	17.70 ±2.30	22.50 ±2.50	20.00 ±2.00	**°C 30**
7.875 NS	21.05 ±0.25	23.00 ±3.00	22.35 ±0.35	**°C 35**
9.994 NS	27.45 ±2.85	27.50 ±2.50	15.35 ±0.65	**°C 40**
8.368 NS	11.15 ±0.35	19.50 ±2.50	13.30 ±2.00	**°C 45**
4.167 *	8.85 ±1.15	16.50 ±0.50	9.70 ±1.00	**°C 50**
---	**5.457 ***	**7.508 ***	**4.460 ***	**LSD Value**
(*P<0.05)				

The effect of temperature on the microbial activity is stimulated by increasing the temperature and some environmental groups tend to control certain ranges of temperatures because these ranges help them to grow and effect on the microbial activity in the soil (Zhu *et al.,* 2004), generally the tropical and Sub-tropical regions showed more biological diversity of bacteria more than mild and cold regions (Zabalgogeazcoa, 2008).

It becomes clear that bacterial systems are able to do biodegradation process of pesticides in high speed at temperatures with temperature ranges between 15-35 ºC, bacterial ability to biodegradation of organic phosphates has been verified in standard laboratory temperatures through many studies (Singh *et al.,* 2006). The results of the current study were agreed with (Essa *et al.,* 2019), as it found that the activity and resistance of bacteria to antibiotics Increase in high temperatures (44 °C).

(Feitkenhauer *et al.,* 2012) noticed that the average of biodegradation is very slow at lower temperatures and it is believed that the reason is the decreased of enzymatic activity, and it increases when temperatures increase, and their height enhances the rates of hydrocarbon metabolism, and it has been found that the dissolution of naphthalene increases ten times when the temperature increase from 20 °C to 30 °C.

(b) Effect of incubation period on bacterial growth:

Tables (10) and (11) show the averages values of bacterial growth during different cuddling periods ranged from (0-10) days which bacterial growth measured during them with a wavelength of 600 nm, and the standard mistake measured too, as bacteria were cultivated in the agriculture medium of liquid MSM with the (Clorspan and Ground Up) pesticides separately at 100 mg/liter concentration and at 37 °C ± 3 °C temperature.

The results indicated that the highest bacterial growth averages in clorspan pesticide recorded by Pseudomonas bacteria (43.30 ± 2.50) during 7 days of cuddling and Aeromonas (40.15 ± 5.05) during 8 days of cuddling , and the lowest average values of bacterial growth recorded by Aeromonas bacteria reached to (8.00 ± 0.30) during day 2 cuddling and bacterial growth averages measured at 600nm wavelength.

The results of the statistical analysis (LSD) indicated the presence of significant differences between the averages values of bacterial growth for each type of bacterial isolates at the level of P <0.05, while most of the averages values of the different cuddling periods for bacterial isolates recorded presence of significant differences except for cuddling periods for the (9, 8, 5, 4) days, No significant differences were recorded among them at the P <0.05 level.

For Ground up pesticide, the results indicated that the highest rates of bacterial growth recorded by Pseudomonas bacteria and it was (39.15 ± 1.65) during 8 days of cuddling and Staphylococcus bacteria reached to (35.20 ± 0.30) during 8 days of cuddling also, and the lowest averages values for bacterial growth recorded by Aeromonas bacteria and reached to (8.00 ± 0.90) during the first three cuddling days, bacterial growth averages measured at 600 nm wavelength.

The results of statistical analysis (LSD) indicated the presence of significant differences between the averages values of bacterial growth for each type of bacterial isolates at the level of P <0.05, while the averages values of the different cuddling periods of most bacterial isolates of which recorded the presence of significant differences at the level of P <0.05, except for Cuddling days (10, 7, 6, 5, 4), no significant differences were recorded among them.

Table 10 : Shows the mean values and the standard error (SE) for the growth of bacterial isolates during different time periods of brood present in the medium of Clorspan and measured at 600nm.

mean ± standard error				Incubation period (days)
Value LSD	**A**	**P**	**S**	
1.299 *	8.30 ±0.00	11.10 ±0.00	10.00 ±0.50	**0**
0.779 *	8.30 ±0.00	11.10 ±0.00	10.20 ±0.30	**1**
1.860 *	8.00 ±0.30	10.50 ±0.00	8.55 ±0.65	**2**
4.131 *	30.05 ±0.85	23.15 ±0.95	26.65 ±0.95	**3**
8.572 NS	34.50 ±2.40	26.25 ±1.55	27.95 ±1.65	**4**
9.806 NS	34.50 ±2.40	33.30 ±2.40	29.25 ±1.65	**5**
7.079 *	35.40 ±2.10	38.50 ±1.70	29.95 ±0.35	**6**
10.414 *	37.35 ±2.85	43.30 ±2.50	29.60 ±1.30	**7**
13.408 NS	40.15 ±5.05	34.85 ±0.35	29.90 ±1.00	**8**
18.777 NS	37.50 ±7.10	29.30 ±0.90	21.40 ±1.00	**9**
3.271 *	29.20 ±0.60	28.85 ±1.05	20.05 ±0.35	**10**
---	**9.438 ***	**4.222 ***	**3.119 ***	**LSD Value**
(*P<0.05)				

Table 11 : Shows the mean values and the standard error (SE) for the growth of bacterial isolates during different time periods of brood present in the medium of Ground Up and measured at 600nm.

mean ± standard error				Incubation period (days)
LSD Value	**A**	**P**	**S**	
2.884 *	8.00 ±0.90	11.10 ±0.60	11.45 ±0.25	**0**
2.884 *	8.00 ±0.90	11.10 ±0.60	11.45 ±0.25	**1**
2.627 *	8.00 ±0.90	10.80 ±0.30	9.55 ±0.35	**2**
2.070 *	11.60 ±0.30	12.95 ±0.65	8.25 ±0.35	**3**
6.111 NS	20.50 ±0.90	21.35 ±0.85	20.40 ±2.00	**4**
9.256 NS	27.40 ±1.20	28.70 ±1.50	30.60 ±3.00	**5**
11.936 NS	28.90 ±1.50	31.50 ±0.60	33.20 ±4.30	**6**
4.794 NS	30.65 ±0.85	35.20 ±1.60	34.55 ±0.35	**7**
5.358 *	32.70 ±1.20	39.15 ±1.65	35.20 ±0.30	**8**
7.269 *	26.55 ±2.05	32.45 ±0.95	19.45 ±1.65	**9**
10.961 NS	24.70 ±2.70	24.95 ±2.55	18.40 ±2.00	**10**
---	**4.267 ***	**3.898 ***	**5.845 ***	**LSD Value**
(*P<0.05)				

As shown in tables (10) and (11) for the bacterial growth averages of the growing bacterial isolates in both pesticides, the bacterial pseudomonas were the most adaptive bacteria to growth in the liquid MSM medium containing both pesticides separately, except that Aeromonas bacteria were also highly efficient in growing and adaptiving, during

the different cuddling periods, it observed that the bacteria of the three species, Staphylococcus, Pseudomonas, and Aeromonas, could not grow and adapt during the cuddling days (2, 1, 0), Later, these three types of bacteria used pesticides as a food medium (i.e. a source of carbon, energy, and phosphorus) so with that it agrees with the results of (Al-Jawhari, 2013) on studying the effect of the Vydate (Oxamyl) pesticide on Pseudomonas aeruginosa and E. coli bacteria Laboratory , and also agreed of the current study results with (Abboud *et al.,* 2016), where he observed through his study on nitrification bacteria, that bacteria increase in numbers by increasing the cuddling period.

Many researches have indicated that it is possible to isolate and diagnose different types of bacteria, which have the ability to represent different types of compounds (hydrocarbons), most of them belong to the negative bacteria, such as Pseudomonas aeruginosa.

The results of the current study w agreed with (Hammadi, 2014) in terms of the efficiency and ability of bacterial isolates, especially Pseudomonas bacteria to dissolve the clorspan and Ground up pesticide and use them as a food medium containing carbon, energy, and phosphorus sources.

(c) Effect of PH on bacterial growth:

Tables (12) and (13) show averages values of bacterial growth at different pH degrees during a 7-day cuddling period, where pH levels include concentrations (9, 8, 7, 7, 6, 5, 4), During that bacterial growth measured with a wavelength of 600nm for clorspan and Ground up pesticides, as well as a standard mistake, as bacteria were cultivated in liquid agricultural MSM medium with each (Clorspan and Ground up) separately with a 100 mg /liter and in 37 °C ± 3 °C temperature.

As the results indicated that the highest average of bacterial growth in the clorspan pesticide recorded by Pseudomonas bacteria and it was (27.87 ± 1.87) that were growing in pH 7, Staphylococcus bacteria were also not less efficient than their growth levels of Pseudomonas aeruginosa bacteria where they recorded averages values reached to (26.63 ± 0.25) At pH 7 and for the lowest averages values of bacterial growth recorded by both Pseudomonas and Staphylococcus (6.88 ± 0.00) and (6.56 ± 0.31), respectively in pH4, bacterial growth averages measured at 600nm wavelength.

The results of the statistical analysis (LSD) indicated the presence of significant differences between the averages values of bacterial growth for each type of bacterial isolates at the level of P <0.05, and the absence of significant differences for the mean values of different pH degrees except the mean values of the pH 8. Lt has been noticed the presence of significant differences between bacterial isolates At the P level <0.05. And for the Ground up pesticide , the results indicated that the highest averages of bacterial growth recorded by Staphylococcus bacteria and it was (34.25 ± 4.87) which were growing in pH7 , Pseudomonas and Aeromonas bacteria also had no lack in growth efficiency less than Staphylococcus where they recorded averages values reached to (29.69 ± 1.56) in pH (8,7), and (28.53 ± 0.88) in pH7, respectively, either for the lowest averages values of bacterial growth recorded by Aeromonas and was (7.94 ± 0.29) in pH 4, for Pseudomonas and Staphylococcus, they recorded similar and low averages values reached to (8.44 ± 0.31) in pH4, and they Bacterial growth averages were measured at a wavelength of 600nm. The results of the statistical analysis (LSD) indicated that there were significant differences between the averages values of bacterial growth for each type of bacterial isolates at the level of P <0.05, and the absence of significant differences for the averages values of different pH degrees except the averages values of the pH 9. It has been noticed significant differences between bacterial isolates at the level p<0.05.

Table 12 : Shows the mean values and standard error (SE) for the growth of bacterial isolates at different pH grades present in the medium of Clorspan during 7 days incubation and measured at 600 nm.

Mean ± standard error				PH
LSD Value	**A**	**P**	**S**	
0.818 NS	7.05 ± 0.00	6.88 ± 0.00	6.56 ± 0.31	**4**
18.71 NS	21.47 ±0.29	17.19 ±7.19	9.69 ± 0.31	**5**
4.997 NS	22.05 ±0.29	24.69 ±0.31	24.37 ±1.87	**6**
5.148 NS	24.12 ±0.59	27.87 ±1.87	26.63 ±0.25	**7**
2.970 *	24.11 ±0.58	25.30 ±0.30	21.56 ±0.93	**8**
7.196 NS	20.20 ±2.73	25.30 ±0.30	19.06 ±0.31	**9**
---	**4.080 ***	**10.522 ***	**3.078 ***	**LSD Value**
(*P<0.05)				

Table 13 : Shows the mean values and standard error (SE) for the growth of bacterial isolates at different pH grades present in the medium of Ground Up during 7 days incubation and measured at 600 nm.

Mean ± standard error				PH
Value LSD	**A**	**P**	**S**	
1.373 NS	7.94 ± 0.29	8.44 ± 0.31	8.44 ± 0.31	**4**
7.361 NS	14.41 ±0.29	12.81 ±2.81	10.75 ±0.12	**5**
6.650 NS	21.47 ±1.47	21.87 ±1.87	24.06 ±0.93	**6**
13.495 NS	28.53 ±0.88	29.69 ±1.56	34.25 ±4.87	**7**
5.632 NS	25.85 ±1.17	29.69 ±1.56	28.44 ±0.94	**8**
1.134 *	22.05 ±0.29	28.81 ±0.06	27.81 ±0.31	**9**
---	**3.022 ***	**5.722 ***	**7.166 ***	**LSD Value**
(*P<0.05)				

The degree of pH has two affects, the first effects on the solubility of mineral salts in the medium, where its liberation increases with a decrease in the pH while its availability decreases when the pH rises because it inter in insoluble complexes formation and the second effect affects in the membranous permeability of cells causing an imbalance between the internal and the external concentration, This has an effect on the growth and activity of bacteria (Ahmed, 2007).

Each enzyme has an ideal pH degree (suitable for its action). It is preferable to bind the enzyme to the materials to be analyzed. It is useful to define this pH to achieve a suitable condition for the enzyme reaction (Srividya and Mala, 2011). There are numbers of conditions that increase or decrease the success of the biodegradation process such as PH, temperature, humidity, nutrients, microbial factors, competitiveness, pesticide bioavailability, etc. Soil disinfection has been successfully occurred by using isolated microbes by many researchers (Johnsen *et al.,* 2001).

The results of the current study agreed with (Ilori *et al.,* 2005), that pH affects the production of degradable enzymes for hydrocarbons from microorganisms, as it found that the ideal pH for lipase production was at pH 7, and (Pawar, 2012) indicated that it is better Biological breakdown of phenol by Pseudomonas pictorum was at an optimum pH between 6.8-7.

References

Abboud, H.Y.; Jaber, M.I. and Salman, F.M. (2016). The effect of soil salinity and glayphosphate on the preparation of nitrifying bacteria for different tissues. Al-Furat Journal of Agricultural Sciences, 8(1): 176-181.

Muhsin, A.E.; Bushra, D.H. and Dhuha, J.M. (2019). Effect of Ascorbic acid on the sensitivity of *Salmonella typhi* to antibiotics. Journal of Education and Science, 28(2): 113-121.

Ahmad, M. (2007). The effect of mercury pollution on the spread of soil fungi in the Izabah area. Ph.D. thesis, College of Science, Mentouri University of Constantine. Algeria.

Alexander, M. (1982). Interoduction to soil microbiology. John Wiley and Sons. Inc New York.

Al-Jawhari, I.F.H. (2013). Study of the effect of the insecticide (Vydate) Oxamyl on *Pseudomonas aeruginosa* and *E. coli* in vitro, University of Karbala, the second scientific conference of the College of Education for Pure Sciences.

Ali, A.H. (2011). Performance of adsorption/bio sorption for removal of organic and inorganic pollutants, Ph.D. thesis, University of Baghdad, Environmental Engineering Department, College of Engineering.

Bandala, E.R.; Gelover, S.; Leal, M.T.; Bulnes, C.A.; Jimenez, A.; Estrada, C.A. (2007). Solar photocatalytic degradation of Aldrin. Catal. Today, 76(2-4): 189-199.

Cho, C.M.; Mulchandani, A. and Chen, W. (2002). Bacterial cell surface display of organophosphorus hydrolase for selective screening of improved hydrolysis of organophosphate nerve agent. Appl. Environ. Microbiol. 68: 2026–2030.

Feitkenhauer, H.; Muller, R.; Markl, H. (2012). Degradation of polycyclic aromatic hydrocarbons and long chain alkanes at 60_70°C by Thermusand *Bacillus* spp. Biodegradation 14: 367-372.

Hammadi, I.S.M. (2014). Biodegradiation, ability of bacterial isolates prevailing in contaminated soils with oil derivatives. Annals of Agric. Sci., 52(3): 407–418.

Ilori, M.O.; Amobi, C.J. and Odocha, A.C. (2005). Factors effecting biosurfactant production by oil degrading *Aeromonas* spp. isolated from tropical environment. Chemosphere. 61: 985-992.

Johnsen, K.; Jacobsen, C.; Torsvik, V. and Sorensen, J. (2001). Pesticide effects on bacterial diversity in agricultural soils-a review. Biol. Fertil. Soils. 33: 443-453.

Goda, S.K.; Iman, E.; Elsayed, T.A.; Khodair, W.; El-Sayed, Mohamed, M.E. (2010). Screening for and isolation and identification of malathion-degrading bacteria: cloning and sequencing a gene that potentially encodes the malathion-degrading enzyme, carboxylestrase in soil bacteria, Biodegradation 21(6): 903–913.

Mathew, M. (2009). A comparison study of gravimetric and ultraviolet fluorescence methods for the analysis of total petroleum hydrocarbons in surface water. Civil Engineering Master's Thesis, 61.

Milosevic, N.; Govedarica, M. and Konstan tinovic, B. (2000). Uticaj Herbicide no dulaciju Soje Microbiological Aktivnost Zemlista. Sesti kongres korovima, Zabonik radova, Banja kovilijaca 455-460.

Mollea, C.; Bosco, F. and Ruggeri, B. (2005). Fungal biodegradation of naphthalene: Microcosms studies, Chemosphere, 60: 636–643.

Nastaija, B.A.C. and Milic, M. (2002). Effects of pesticides on Azotobacter chroococcum. Natural Sciences Matica Srpska Novi Sad, No.102: 23-28.

Nawab, A.; Aleem, A. and Malik, A. (2003). Determination of organochlorine pesticides in agricultural soil with special reference to γ-HCH degradation by Pseudomonas strains. Biores. Technol. 88(1): 41-46.

Patel, R.N. and Desai, A.J. (1997). Surface active properties of raminolipids from *Pseudomonas aeroginosa* GS3. J. Basic Microbiol. 32: 518-520.

Pawar, R.M. (2012). The effect of soil pH on degradation of polycyclic aromatichydrocarbons (PAHs). Ph.D. thesis.

Pincus, D.H. (2013). Microbial identification using the biomerieuxVITEK® 2 system. In: Miller, M.J. Encyclopedia of rapid microbiological methods volume 4. DHI Publishing, LL River Grove, IL, USA.

Sassman, S.A.; Lee, L.S.; Bischoff, M. and Turco, R.F. (2004). Assessing N,N'-Dibutylurea (DBU) Formation in Soils after Application of n-Butylisocyanate and Benlate Fungicides. J. Agric. Food Chem. 52(4): 747-754.

Singh, B.K.; Kuhad, R.C.; Singh, A.; Tripathi, K.K. and Ghosh, P.K. (2009). Microbial degradation of the pesticide lindane (hexachlorocyclohexane). Adv. Appl. Microbiol. 47: 269–298.

Srividya, S. and Mala, M. (2011). Influence of process parameters on the production of detergent compatible alkaline protease by a newly isolated Bacillus sp. Y. Turk. J. Biol. 35: 177-182.

Teng, Y.; Luo, Y.M.; Sun, X.H.; Tu, C.; Xu, L.; Liu, W.X.; Li, Z.G. and Christie, P. (2010). Influence of carbuncular mycorrhiza and Rhizobium on Phytoremediation by Alfalfa of an agricultural soil

contaminated with weathered PCBs: A Field Study. Inter. J. Phytoreme. 12(5): 516-533.

Zabalgogeazcoa, I. (2008). Fungal endophytes and their interaction with plant pathogens. Spanish Journal of Agricultural Research, 6: 138-146.

Zhongli, C.; Shunpeng, L. and Guoping, F. (2001). Isolation of methyl parathion-degrading strain M6 and cloning of the methyl parathion hydrolase gene. Appl. Environ. Microbiol. 4922-4925.

Zhu, G.; Wu, H.; Guo, J. and Kimaro, F. (2004). Microbial degradation of fipronil in clay loam soil. Water Air Soil Pollut. 153: 35-44.