المجلة العراقية
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# Study the Growth Kinetics of *Pseudomonas aeruginosa* Degrading Some Pesticides which Isolated from Cultivated Soll

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## Abstract

**The ability of the bacterium *Pseudomonas aeruginosa* that isolated from cultivated soil to degrade some pesticides like malathion, cypermethrin and carbofuran was studied by investigate the growth kinetics of bacteria in pesticides enriched media at different concentrations.**

**It was found that *P. aeruginosa* was more resistant to malathion than other pesticides. Growth curves of bacteria showed it ablility to grow in mineral salts medium contain malathion 0.1-1%, cypermethrin 0.1- 0.5% and carbofuran pesticide 0. 1- 0.7%. The optimum concentration which support normal bacterial growth during 24 h was found to be 0.7% malathion, 0.3% cypermethrin and 0.5% carbofuran. When compared with the control test, a significant increased in bacterial population was noted at low concentration of each pesticide. The lowest generation time of *P. aeruginosa* was 75 min. when they grown in mineral salts medium which containing malathion at concentration 0.5%. The results showed that the local isolate *P. aeruginosa* had the ability to degrade the malathion even at high concentration, and we concluded that bacterium can be used as a microorganism for the bioremediation of pesticides contaminated soil and water.**

**Key words: *Pseudomonas aeruginosa*, malathion pesticide, pesticides resistance bioremediation.**

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# دراسة حركيات النمو لبكتريا *Pseudomonas aeruginosa* المحللة لبعض المبيدات والمعزولة من تربة مزروعة

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## الخلاصة

درست قابلية بكتريا *Pseudomonas aeruginosa* المعزولة من تربة مزروعة في تحليل بعض المبيدات مثل مالاثيون وسايبيرميثرين وكاربوفيوران من خلال بحث حركيات النمو للبكتريا في وسط غني بالمبيدات وبتراكيز مختلفة، وقد وجد ان بكتريا *P. aeruginosa* كانت اكثر مقاومة لمبيد المالاثيون من بقية المبيدات وأظهرت منحنيات النمو ان البكتريا كانت قادرة على النمو في وسط الاملاح المعدنية الحاوي على المالاثيون بتركيز 0.1- 1% والسايبيرميثرين بتركيز 0.1- 0.5% والكاربافيوران بتركيز 0.1- 0.7%. بلغ التركيز الامثل لدعم النمو البكتيري الطبيعي خلال 24 ساعة 0.7% مالاثيون و0.3% سايبيرميثرين و0.5% كاربوفيوران وعند المقارنة مع اختبار السيطرة لوحظ وجود زيادة واضحة في اعداد البكتريا عند التراكيز الواطئة من كل مبيد وكان اقل زمن جيل للبكتريا 75 دقيقة عند النمو في وسط الاملاح المعدنية الحاوي على مبيد المالاثيون عند التركيز 0.5%، واظهرت النتائج ان عزلة *P. aeruginosa* المحلية تمتلك القابلية لتحليل المالاثيون حتى عند تراكيز عالية وعليه نستنتج ان البكتريا يمكن ان تستخدم في المعالجة الحيوية للتربة والماء الملوثة بذلك المبيد.

الكلمات المفتاحية : *Pseudomonas aeruginosa*، مبيد المالاثيون، مقاومة المبيدات، المعالجة الحيوية.

## Introduction

Pesticides had made great impact on human health, production and preservation of foods. More than 55% of the land used for agricultural production in developing countries used about 26% of the total pesticides produced in the world (8). Soil microorganisms that are repeatedly exposed to pesticide may develop new capabilities to degrade such chemicals, research studies have revealed that microbial degradation process to detoxify pesticides contaminates can be effectively used to overcome the pollution problems (2). Soil bacteria with the ability to degrade several pesticides have been isolated from soil showing enhanced biodegradation such as *Pseudomonas* and *Bacillus* (14). Biodegradation, ideally the target pesticides will be able to serve as the carbon source and energy for the microorganisms, including the synthesis appropriate enzymes if need (16). The survival bacteria under pesticides stress can provide an efficient, cheaper and eco-friendly solution for bioremediation of the pesticides contaminated soil (17). So that research was aim was to isolated and identify *Pesuedomonas aeruginosa* from the cultivated soil, and investigate there's biodegradation potential in media containing different concentrations of malathion, cypermethrin and carbofuran pesticides by study growth kinetics of bacterium.

## Materials and methods

**Collection of soil samples:**

The soil samples were collected from cultivated fields in Almahmodia city. These fields had been already sprayed with many pesticides for past few years. Soil samples were collected at different sites of the fields by using sterile scalpel and these soil samples were transferred to sterile polythene bag and used for analysis.

**Pesticides used:**

Commercial grade insecticides (malathion, cypermethrin and carbofuran) were purchased from agricultural chemical dealers.

**Isolation and maintenance of bacterial colonies:**

The bacterial culture capable of degrading pesticides was isolated from agricultural soil using enrichment technique, with varying concentration of pesticide in the medium. The soil sample 5g from an agricultural site was inoculated into 250 ml of nutrient broth medium in 500 ml Erlenmeyer flask. The flasks were incubated in a shaking water bath operating at 240 cycles/ minute for five days at room temperature ranged from 20-28 $^{O}$C. At daily intervals on nutrient agar plates supplemented with pesticide 0.1-1% and incubated at 35 $^{O}$C for 48 hr. Individual colonies were sub cultured into nutrient agar plates containing same concentration of pesticide until pure culture was isolated. The isolated strain was maintained at 4 $^{O}$C and sub cultured every three months.

**Identification and characterization of resistant *P. aeruginosa:***

The pesticides impregnated plates showed morphologically dissimilar colonies and the purity of the colonies was isolated in a nutrient agar plates. Then the pure bacterial isolates were used for identification. The identification and characterization of the isolates was performed using morphological, cultural and biochemical tests (5).

**Enumeration of pesticides utilizing bacteria:**

In the enumeration of pesticides utilizing bacteria Mineral Salt Medium (MSM) was used in which pesticides as carbon source. The microbial strains of pesticides resistant bacteria were streaked in triplicated on the mineral salt media containg pesticides at different concentration (0.1%- 1%). After incubation the pesticides utilizing colonies were isolated (15).

**Growth kinetic studies of pesticides resistant bacterium:**

Growth of the isolates was determined by viable cell enumeration immediately after inoculation and at 2, 4, 6 and 24 h later. Sample of bacterial culture 1ml was drawn at regular intervals and serial dilutions $10^{-5}$- $10^{-8}$ of bacterial culture with and with out addition of pesticides (control) was performed using 9 ml sterile saline blank (0.85% NaCl: pH=7). Appropriate dilutions of bacterial samples were plated in triplicate on nutrient agar medium. After incubation the total viable colonies were counted (11).

**Results and discussion:**

In the present investigation, out of a total 9 isolates 4 were found *P.aeruginosa* by using nutrient agar medium enriched with pesticides (malathion, cypermethrin and carbofuran), One of the largest most rapidly growing isolate was selected for growth kinetic study (19). These insecticides are part of a group of bioresistant compounds, which are not biodegradable by the environment or by conventional treatment in water and plant (3). On the basic of morphological, cultural and biochemical characteristics, the bacterial isolates were identified as a member of the species *P.aeruginosa* (4). The ability of isolated bacteria to utilize and degrade some pesticides was evaluated in this study.

Pesticides resistance pattern of *P.aeruginosa* was recorded in (table, 1). The isolated native bacterial colonies of *P.aeruginosa* exhibited remarkable resistance to the pesticides used, were the isolate was more resistant to malathion than other pesticides, *P.aeruginosa* had the ability to resist malathion at different concentrations ranging from 0.1- 1%, while it was found no growing of bacterium at the concentration 0.8% carbofuran and 0.6% cypermethrin. Previous studies indicated that bacteria belonging to the species *P.aeruginosa* were highly oxidative and able to degrade aromatic hydrocarbons, oil, petroleum products and pesticides (1; 12).

The dominant pesticide resistant bacteria, *Staphlococcus aureus, Enterococcus faecalis and P. aeruginosa* had bored plasmids, and the resistant trait observed was found to be plasmid borne (18).

**Table (1):** Pesticides resistance pattern of *P. aeruginosa*.

| Malathion Conc.% | result | Cypermethrin Conc% | result | Carbofuran Conc.% | result |
|---|---|---|---|---|---|
| 0.1 | + | 0.1 | + | 0.1 | + |
| 0.2 | + | 0.2 | + | 0.2 | + |
| 0.3 | + | 0.3 | + | 0.3 | + |
| 0.4 | + | 0.4 | + | 0.4 | + |
| 0.5 | + | 0.5 | + | 0.5 | + |
| 0.6 | + | 0.6 | - | 0.6 | + |
| 0.7 | + | 0.7 | - | 0.7 | + |
| 0.8 | + | 0.8 | - | 0.8 | - |
| 0.9 | + | 0.9 | - | 0.9 | - |
| 1.0 | + | 1.0 | - | 1.0 | - |

**+ =Presence of growth.**
**- = Absence of growth.**

A series of growth curves was performed with specific doses of pesticides (malathion, cypermethrin and carbofuran) in order to determine the viable count of *P. aeruginosa* and to verify whether they could utilize these compounds for their growth. The optimum concentration of each pesticide, which supports bacterial growth, was also evaluated. The growth curve of *P. aeruginosa* in the present of different concentrations of malathion 0.1 to 1% had been shown in (figure, 1). The stimulatory and inhibitory responses of *P. eruginosa* were observed when compared with the control test, the growth pattern of *P. aeruginosa* in the medium containing 0.7 and 0.5% malathion was similar to control. The results indicated that malathion concentration 1% showed a marked reduction in bacterial count at 24 h, this indicated that bacterial enzymes at high concentration suppressed and the growth rate thus decreased. The lag phase of growth at concentration 0.1% and 1% (6 h) was longer than control (4h) and the concentration1% may be toxic to the cells of bacteria while the optimum of growth was at 0.7%. The generation time was calculated to be 75 min. at 0.5% concentration of malthion in contrast with control 52.17 min. (table, 2). Detoxification of several organophosphates (malathion and parathion insecticides) in the environment was carried out by carboxy esterase, and these enzymes are found in many bacteria such as *P. aeruginosa* (9).

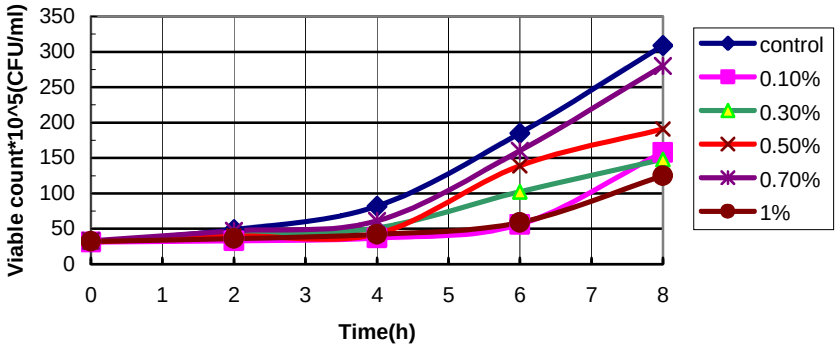


**Figure 1. Growth of *P.aeruginosa* in the presence of different concentrations of malathion.**

Growth curves experiment with different concentrations of carbofuran pesticides (figure, 2) was indicated that carbofuran in concentration of 0.1 to 0.5% provoked an increase in bacterial growth when compared with the control. It was found a marked reduction in viable cell count at concentration 0.7% and 1%, where their was no bacterial growth at 6 h with concentration 1% of carbofuran. These results indicated that the bacterial isolate of *P. aeruginosa* was unable to utilize carbofuran at high concentration to support their growth because of toxicity. It was obvious the difficulty that the bacteria had to adapt to this type of pesticides.

**Table (2):** Generation time in minutes of pesticides resistant *P. aeruginosa* at concentration 0.5%.

| medium | Generation time (min.) |
|---|---|
| M SM (control) | 52.17 |
| MSM + 0.5% malathion | 75 |
| MSM + 0.5% cypermthrin | 200 |
| MSM + 0.5% carbofuran | 120 |

**MSM= Mineral Salts Medium**

The generation time at concentration 0.5% of carbofuran was 120 min. and this concentration was the optimum for the higher number of bacterial count at 24 h. Many bacteria which able to degrade carbofuran pesticide had been isolated from soil around the world (7). *P. putida* was able to degrade carbofuran to carbofuranphenol and the result was degraded to 2- hydroxy- 3- phenol (6).

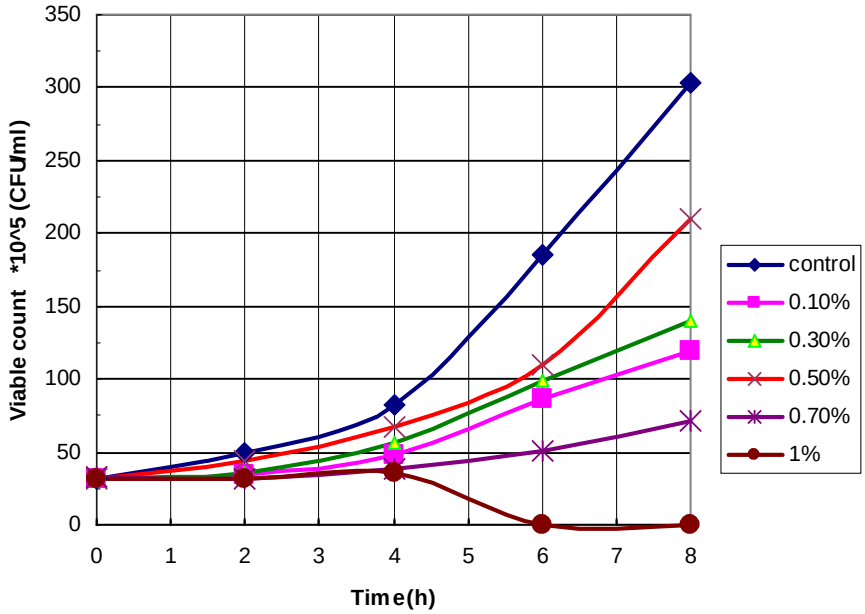


**Figure 2. Growth of *P.aeruginosa* in the presence of different concentrations of carbofuran**

A bacterial isolate of *P. aeruginosa* was also tested to determine its potential for cypermethrin degradation. Mineral salts medium was inoculated with inoculum of isolate .The extent of growth during 24 h of incubation was recorded in (figure, 3). The growth curve showed that the growth of bacteria was inhibited at 0.7% concentration and significant die at 6h. The isolate could survive at 0.1 to 0.5% concentrations but the viable count significantly decreased at 24 h at 0.5 and 0.1%, while the growth at 0.3% concentration was similar to control. Also the generation time at 0.5% was more than other pesticides (200 min.) (table, 2.). Our results disagree with the study of Muragesan *et al*. 2010 (13) who found that *P.aeruginosa* was able to grow in the presence of cypermethrin at 0.1 to 1% concentration. *P. aeruginosa* was responsible for cypermethrin degradation and the increasing of cypermethrin concentration had a marked effect on biodegradation performance of *P. aeruginosa* with increasing in the duration of lag phase (10).

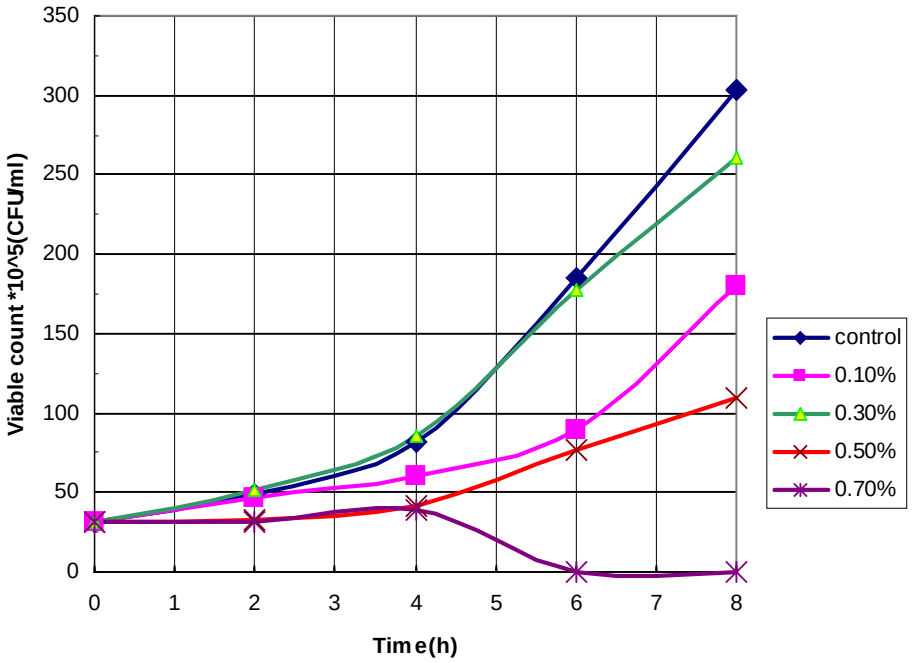


**Figure 3. Growth of P.aeruginosa in the presence of different concentrations of cypermethrin.**

From the findings it could be concluded that the bacterial isolate could be useful for the treatment of pesticides contaminants in industrial effluent and can be detoxify agriculture waste. The use of microorganisms in the degradation and detoxification of many toxic xenobiotics, especially pesticides, is an efficient tool for the decontamination of polluted sites in the environment.

## References

**1.** Ajaz, M.; Noor, N.; Rasool, S. A. and Khan, S. A. (2004). Phenol resistance bacteria from soil: Identification– characterization and genetic studies. Pak J. Bot. 36(2): 412-424.

**2.** Bhandhade, B. J.; Satnaik, S. S. and Kanetar, P. P. (2002). Bioremediation of an industrial effluent containing monocrotophos. Curr. Microbiol. 45(5): 346- 349.

**3.** Bending, G. D. and Cruz, M. S. R. (2007). Microbial aspects of the interaction between solid depth and biodegradation of the herbicides isoproturon. Chemosphere. 66: 664-671.

**4.** Bergey, H. W. R. (1994). Manual of Determinative Bacteriology, Edited By, Williams and Willkins, Baltimore. 122-135.

**5.** Cappuccino, J. G. and Sherman N. (1999). Microbial Laboratary Manual. 4th ed., Addison-Wilsey, California. 470-477.

**6.** Chainka, E.; Geoorgidon, D.; Souref, E. and Tzortzakaki, E. A. (2011). Isolation of soil bacteria able to hydrolyze both Orgaophosphate and Carbomate Pesticides. Bioresource Technology. 102: 3184-3192

**7.** Desaint, S.; Hartman, H. A.; Parekh, N. R. and Fournier, J. C. (2004). Genetic diversity of Carbofuran– degrading soil bacteria. FEMS Microbiology Ecology 34: 173-180.

**8.** Dollacker, A. (1991). Pesticides in third world. Pflank. Nuthr. Bayer 44(1): 84-99.

**9.** Gilbert, E. S.; Walker, A. W. and Kengling J. D. (2003). A constructed microbial consortium for biodegradation of the Organophosphorus insecticides. App. Microbiol. Biotechnol. 61: 77- 81.

**10.** Jilani, S. and Altat Khan, M. (2006). Biodegradation of Cypermthrin By *Pseudomonas* in a batch activated sludge process. Int. J. Environ. Sci. Tech. 3(4): 371-380.

**11.** Kamanavalli, C. M and Ninnekar, H. Z. (2000). Biodegradation of propoxur by *Pseudomonas* species. World Journal of Microbiology and Biotechnology .16: 329-331.

**12.** Martin, M.; Mengs, G.; Plaza, E., and Feerer, E. (2000). Propachlor removal By *Pseudomonas* strain GCHI in an Immobilized cell system. App. Microbiol. 66: 1190-1194.

**13.** Murgesan, A. G.; Jeyasanthi, T. and Maheswari, S. (2010). Isolation and Characterization of Cypermethrin Utilizing Bacteria.

From Brinjal cultivated soil. African Journal of Microbiology Research. 4(1): 10-13.

**14.** Ralebits, T. K.; Senior, T. and Van versevell, H. W. (2002). Microbial Aspects of Atrazine Degradation in Natural Environments. Biodegradation. 13:11-14.

**15.** Sarkar, S.; Sathesn, A. and Premlumar, R. (2009). Biodegradation at Dicofl by *Pseudomonas* strain isolated from tea rhizosphere microflora. International Journal of Interactive Biology. 5(3): 164-167.

**16.** Shelton, D. R.; Khader, S., Karns, J. S. and Pogel, B. H. (1996). Metabolism at twelve herpicides by Streptomycetes. Biodegradation. 22(3): 129-136.

**17.** Trpton, D. K.; Roiston, D. E and Scow, K. M. (2003). Bioremedation and Biodegradation. J. Environment. Qual.( 32: 40-46.

**18.** Umamaheswari, S. and Marali, M. (2010). Prevalence of plasmid merdiated resistant bacterial assemblages in crop fields. Journal of Environmental Biology. 31(6): 957-967.

**19.** Yung Long, Y.; Fengming, S.; Zhong, Z., Hexing, C. and Defang, F. (1997). Isolation and Identification of broad– Spectrum bacterial strain (*Alcaligenes* sp.) Degrading pesticides. J. Zhe. Agri. Uni. 23(2): 111-115.