Bionatura
Latin American Journal of Biotechnology and Life Sciences

Bionatura Issue 1 Vol 8 No 1 2023

Article

The efficiency of *Pseudomonas mendocina* in biodegradation of chlorpyrifos insecticides

Zahraa Khudhair Abbas Al-Khafaji [1,2], Hassanin Sabah Hashim [1,2], Maryam Jabbar Nasser[1,2] and Mohammed A. Dabbi [3]*
[1] Medical Laboratory Technique Department, The Islamic University, Diwaniya, IRAQ.
[2] Research and Studies Department, The Islamic University, Najaf, Iraq.
[3] Nursing Department, Al-mustaqbal University College, Babylon, Iraq.
** Correspondence: edhassen114@gmail.com*
Available from: http://dx.doi.org/10.21931/RB/CSS/2023.08.01.42

ABSTRACT
At present, wide varieties of pesticides are being used, but the demand for Organophosphorus pesticides is increasing globally to control insects. Chlorpyrifos is a broad-spectrum, highly toxic, and chlorinated organophosphate insecticide that is synthetic in origin and is normally ester or thiol derivatives of phosphoric. The mode of action involves inhibiting acetyl-cholinesterase, leading to the accumulation of acetylcholine, causing neurotoxicity. Bacteria capable of degrading the pesticide chlorpyrifos were isolated from soil contaminated with pesticides. This way, three distinct chlorpyrifos degrading strains of *p.mendocina* were isolated and characterized using morphological and biochemical analysis. Strains exhibited the greatest chlorpyrifos degradation rate, reaching 100%, and were consequently selected for further investigation. Degradation of chlorpyrifos by strains was rapid at 20 and 37C. Bacteria species were able to effectively degrade chlorpyrifos in the sterilized medium using high inoculum levels. The maximum degradation rate of chlorpyrifos was calculated as 100% during 6-12 days. Bacteria such as strain PC1 that use chlorpyrifos as a carbon source could be employed for the biodegradation of sites contaminated with pesticides

Keywords: Biodegradation, organophosphate, chlorpyrifos, *Pseudomonas putida*, Hplc.

INTRODUCTION

Chlorpyrifos (O, O-diethyl-O-3,5,6-trichlor- 2-pyridyl phosphorothioate; CPF) is a broad spectrum organophosphate insecticide (OP) that is commercially used to control insects, especially termite[1]. It was first developed by the Germans in the 1930s and introduced into the marketplace in 1965. It has been widely used globally as an insecticide to control crop pests in agriculture, reduce household pests such as termites, reduce insect damage to the turf on lawns, and for mosquito control [2,3]. Various studies have shown that exposure to CPF elicits acute toxicity by inhibiting acetylcholinesterase (AChE) throughout the central

and peripheral nervous systems, leading to acetylcholine accumulation and long-term stimulation of cholinergic receptors [4]. Chlorpyrifos works the same way in insects and other animals, including humans, through impairment of the nervous system. It can cause persisting neurobehavioral dysfunction during development, even with low doses that do not elicit acute toxicity [5].

The fate of chlorpyrifos is affected not only by its physicochemical properties but also by characteristics of the soil, management practices and environmental conditions [6]. Its persistence increases with decreased temperature decreased pH, and decreased light. The half-life of chlorpyrifos showed that it remained stable even after 12 months in soil [5], and chlorpyrifos is moderately soluble in water; the half-life of chlorpyrifos in water ranges from 35 to 78 days [2]. It has also been reported to have short to moderate persistence in the environment as a result of several non-biological methods, including volatilization, photolysis, abiotic hydrolysis, and microbial degradation that might occur concurrently [5]

Chlorpyrifos has been of great concern due to persistence, toxicity and accumulation in soils and groundwaters [7]. The altered structure of the gills and liver of fish

8 may cause the unsafe misuse of pesticides, which has caused widespread harm to human health and the environment because it is one of the most common causes of poisoning around the world [9], as well as the appearance of its residues in crops and soil, and has proven many reports, including the environmental protection organization, that pesticides cause many types of cancer [10]. When pesticides are dispersed in the environment, they become pollutants, with ecological effects that require degradation [11]. It has become important to treat these pollutants. Many different treatment methods, including chemical and physical treatment, have faced many criticisms because of the main problems they cause, such as producing toxic and radioactive acids and bases. In addition, these methods are uneconomic ineffective and unsuitable for large areas [12]. Lately, research activities in this area have demonstrated that microorganisms are potential tools for decaying insecticides into less harmful and non-toxic metabolites through a process known as bioremediation [13]. Biodegradation has emerged as an innovative technology critically important for cleaning polluted sites.

In summary, due to the pervasive nature throughout Pseudomonas mendocina, the potential non-pathogenic effect on fish, and efficiency in CPF metabolism, Pseudomonas species were considered a vital potential agent for testing in bioremediation. This study aimed to determine the extent to which Pseudomonas mendocina acts as an effective biomarker, reducing CPF toxicity by breaking CPF into non-toxic metabolites and preventing AChE inhibition. Pseudomonas aeruginosa, Bacillus cereus, Klebsiella sp., and Serratiamarscecens obtained from consortia showed 84, 84, 81, and 80% degradation of chlorpyrifos (50 mg/L) in the liquid medium after 20 days and 92, 60, 56, and 37% degradation of chlorpyrifos (50 mg/L) in the soil after 30 days. Some recent reports indicate bacterial degradation of chlorpyrifos by Flavobacteriumsp. ATCC 27551 and Arthrobacter sp., isolated from contaminated sources, which degrade chlorpyrifos-metabolically, and Enterobacter strain B-14, Alcaligenes faecalis, and Klebsiella sp., which degrade and utilize chlorpyrifos as sole carbon source [14]. Bacillus sp. And Micrococcus sp. possesses the potential to degrade chlorpyrifos[2].

MATERIALS AND METHODS

Sample collection

The soil samples used to isolate pesticide-degrading bacteria were collected from agricultural fields, residential buildings, and Garden yards from the Iraq-Al Diwaniyah, where chlorpyrifos pesticide is used extensively. This study included a total of 50 samples were collected at a depth of 5cm to 10cm from different regions. Commercial chlorpyrifos (48%) pesticide was procured from a local pesticide shop. The stock solution was prepared and stored in the refrigerator for further use. The samples were collected using a sterile petri dish and plain tube, and 5 grams of soil samples were taken. A Normal line was added to it for purification and isolation of bacteria.

The instruments used in determining the removal of chlorpyrifos and detection of bacteria

1. VITEK 2 (Biomerieux, France)
2. High-Performance Liquid Chromatography (HPLC) (Knuaer, Germany).

Preparation of stock pesticide

Commercial grade chlorpyrifos (48%) pesticide was procured from a local pesticide shop. The stock solution was prepared and stored in the refrigerator for further use. To estimate the amount of residual chlorpyrifos concentrations after removal by bacterial isolates. Standard was prepared for the active ingredient CP by selecting more than one manufacturer of the pesticide containing the active ingredient chlorpyrifos at a concentration of 48%, then 5 mg was taken from each package and analyzed by high-performance chromatography according to [15].

Isolation and identification of bacteria

Prepare the soil suspensions by taking 1 gram of soil and dissolving it in 10 ml of normal saline, then mix by the vortex for 10 minutes and centrifuge for 5 at, and then filter with a 0.4 volume filter and use pseudomonas agar medium from (Himedia Company, India) to isolate the bacteria that analysis the pesticides. The culture medium consisting of Gelatin peptone 16.00 g / L, Tryptone 10,000 g / L, Potassium sulfate 10.00 g / L, Magnesium Chloride 1.4 g / L, Agar 11.00 g / L, and sterilized by autoclaving at 15Ibs (121° C) for 15 min, cool to 45-50 ° C and aseptically add sterile rehydrated contents of one vial of either celrinix supplement (FD029) or CFC supplement (FD036) as desired. The pH was set to 7.1 at 25 ° C. mix well and pour into sterile petri dish plates for selective isolation of pseudomonas species.

The cultivated bacterial isolates were incubated on the nutrient medium for 24 hours at a temperature of 37 ° C, and the bacteria were diagnosed by a modern device called VITEK2 SYSTEM from the company (Biomerieux, France). VITEK2 SYSTEM was examined against three bacteria, as shown in Table 1.

Treating the medium with the pesticide

The nutrient broth is prepared for treatment with chlorpyrifos and consists of peptone 5 g / liter, sodium chloride 5 g / liter, meat extract B 1.5 g / liter, and yeast extract 1.5 g / liter. Add 800 ml of distilled water and dissolve 10.2 grams from the medium, then heat the mixture by microwave and sterilize with an autoclave at 15lbs (121° C) for 15min and cool to 45° C.

Biodegradation of chlorpyrifos

After that, add 3 ml of water and use a 0.4 volume filter to sterilize the water with a syringe; add 200 chlorpyrifos, mix well, and add the last mixture to the liquid medium to inoculate the medium with the isolated and diagnosed bacteria. The residual concentrations were assessed and measured after removal with HPLC, and un-inoculated flasks served as controls. Biodegradation experiments were carried out in 250 mL Erlenmeyer flasks containing 60 mL of medium with 200 µL of CP. The mixture was poured into a cup of 50 ml, inoculated with pseudomonas mendocina, and incubated at 37°C. Samples were taken at periodic intervals, and the residual pesticide concentration was determined using HPLC. Un-inoculated cups served as controls.

RESULTS

The results showed that three distinct chlorpyrifos degrading strains of P.mendocina were highly efficient in the biological removal of organic phosphorous pesticides (chlorpyrifos) at a concentration of 120 µg/l, thus the possibility of using it in the biodegradation of pollutants. PC1 strain removal 97% of CP with at 6 days, the residual chlorpyrifos concentration was 0.37µg/ml to 0.130µg/ml, while PC2 and PC3 degraded CP within 6 days from 0.36µg/ml to 0.137µg/ml and 0.44µg/ml to 0.077µg/ml table 2.

The PC1 could rapidly degrade chlorpyrifos compared with other species during the logarithmic phase of growth. The percentage degradation in the first 6 days was higher and rapidly increased to 100% within 12 days compared with control Figure 2,3,4. These results are consistent with many studies and research that have confirmed the ability of microorganisms (bacteria and fungi) to consume a wide range of pesticides and, in most cases, their ability to consume one or more compounds as a source of carbon and energy [2,11]. The strain could utilize CP as the sole source of carbon for growth. In HPLC analysis of a sample uninoculated (without bacteria) with 200 µl CP, two peaks appeared in HPLC separation Figure 1, which were further identified as CP by comparing with their authentic standard. HPLC analysis showed that PC1 could degrade CP to TCP and diethylthiophosphoric acid.

NO.	Bactria	Probability%
1.	*P.mendocina C1*	98%
2.	*P.mendocina C2*	96%
3.	*P.mendocina C3*	99%

Table 1. The probability of diagnostic bacterial isolates by Vitek2compact system

Bacterial sample	Concentrate Of chlorpyrifos	Residual Concentrate After 6 days	Residual Concentrate after 12 days	control
PC1	120 µg/ml	0.37µg/ml	0.130µg/ml	63.40 µg/ml
PC2		0.36µg/ml	0.137µg/ml	
PC3		0.44µg/ml	0.077µg/ml	

Table 2. shows the percentages of residual concentrations after treatment for 6 to 12 days for chlorpyrifos in two experiments.

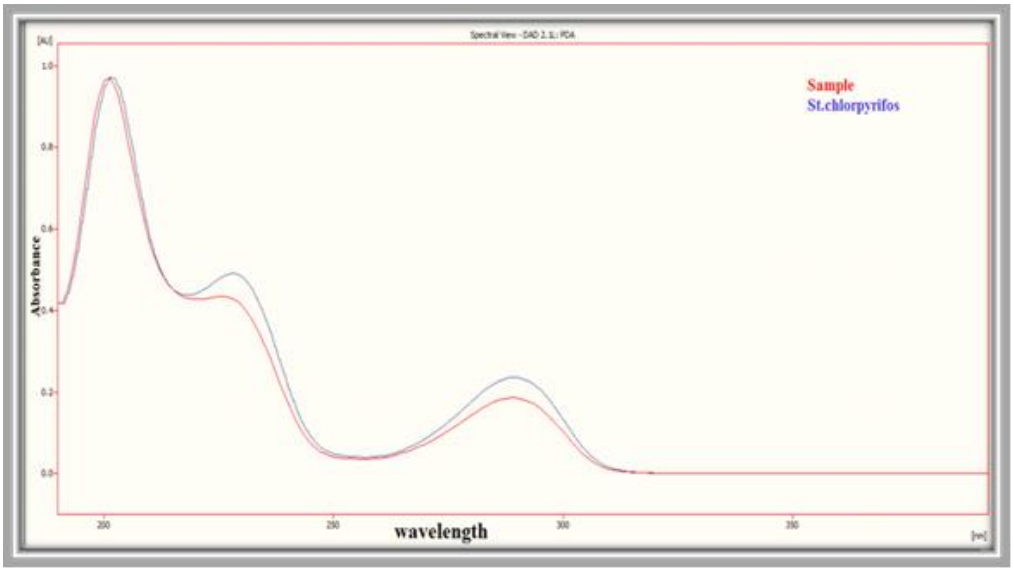

Figure 1. shows the absorbance and spectrum of St.chlorpyrifos and the control (sample uninoculated) treated with the pesticide without bacteria.

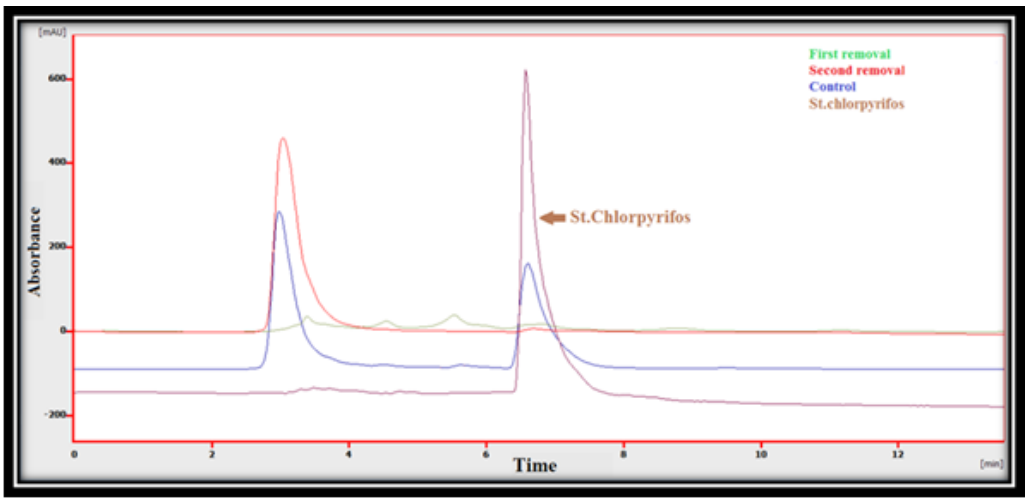

Figure 2. shows the growth kinetic for *p.mendocina C1* on broth media with chlorpyrifos as a carbon source and energy. Illustrates peaks Chlorpyrifos (CP) degradation by *Pseudomonas mendocina C1* strain. 1) Residual CP in the inoculated sample after 6 days, 2) Residual CP in the inoculated sample after 12 days, 3) CP degradation in un-inoculated control, and 4) standard of chlorpyrifos 48%.

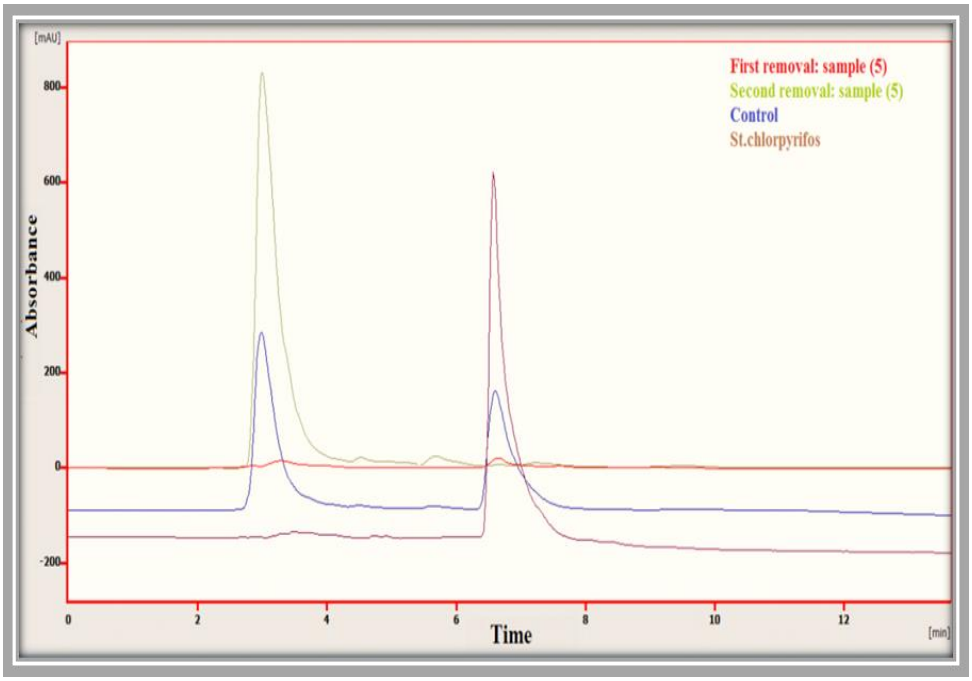

Figure 3. shows the growth kinetic for *p.mendocina C2* on broth media with chlorpyrifos as a carbon source and energy. Illustrates peaks Chlorpyrifos (CP) degradation by *Pseudomonas mendocina C2* strain. 1) Residual CP in the inoculated sample after 6 days, 2) Residual CP in the inoculated sample after 12 days, 3) CP degradation in un-inoculated control, and 4) standard of chlorpyrifos 48%.

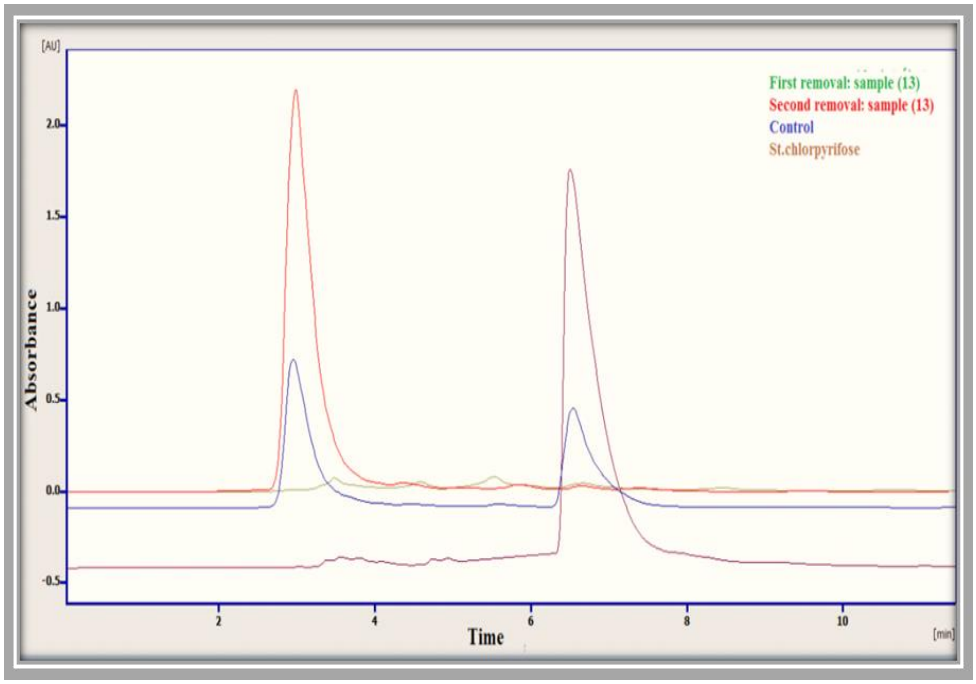

Figure 4. shows the growth kinetic for *p.mendocina C3* on broth media with chlorpyrifos as a carbon source and energy. Illustrates peaks Chlorpyrifos (CP) degradation by *Pseudomonas mendocina C3* strain. 1) Residual CP in the inoculated sample after 6 days, 2) Residual CP in the inoculated sample after 12 days, 3) CP degradation in un-inoculated control, and 4) standard of chlorpyrifos 48%

Only a few organisms capable of degrading chlorpyrifos have been reported to date. The maximum rate of chlorpyrifos removal was observed from 6 to 12 days, which coincided with the period of maximum proliferation. This might indicate that the pseudomonas mendocina utilized CP as an energy source during its growth phase. It was noted that on reaching lower concentrations of residual pesticide in the medium, the degradation rate decreased significantly, as previously observed.
The rapid removal of CP from liquid medium within 6 days by the pB1 strain thus could be one of the fastest reported for effective CP degradation and the first of its kind amongst the Pseudomonads.

To demonstrate the capacity of the recombinant P.mendocina strain to degrade CP, the recombinant strain was inoculated into a nutrient broth medium supplemented with CP. High-performance liquid chromatography (HPLC) analysis indicated that CP was completely degraded within 6 and 12 days, respectively. Moreover, the concentration of 3,5,6- trichloro-2-pyridinol (TCP) in the medium increased gradually with the decrease in CP concentration.

DISCUSSION

The reported minimum incubation time required for completely removing chlorpyrifos was 6 h by Cupriavidus sp. DT-1 in a liquid medium [16]. Most of the common CP-degrading bacteria were reported to degrade the pesticide within 48 h in liquid media [17]. Chlorpyrifos is degraded metabolically in liquid media by bacteria [14]. P.mendocina is the most common Gram-negative bacterium that is aerobic, rod-shaped, and found in soil. Isolates of this bacterium have been found to have the potential to degrade chlorpyrifos [18].

Since Pseudomonas mendocina, capable of degradation of various OPs, have great potential for bioaugmentation of soil [19], these strains of bacteria, with their high degradation potential, fast growth, minimal nutrient requirements and tolerance to high concentrations of pesticide, can play a vital role in the decontamination of polluted soil. CP was degraded quickly in the first 6 days, accounting for 48% of the initially added pesticides, respectively. Maybe a reduction in the degradation rate after 12 days may be due to the accumulation of TCP, which has antimicrobial activity and is toxic to bacterial growth and metabolism [20,21]

CONCLUSION

The present study shows that the use of the p.mendocina culture could effectively degrade chlorpyrifos pesticide. Removal of xenobiotic compounds, a major threat to environmental pollution, is of major concern as they enter the food chain and are the major causes of various diseases. The study shows that the bacterial isolate can use chlorpyrifos as a sole carbon source. Therefore, these bacteria cultures can be used effectively for bioremediation of contaminated sites. Future studies aim to study the biotechnology of pesticide degradation and its significance in field conditions.

References

1. Rusyniak, D.E. and K.A. Nañagas. Organophosphate poisoning. in Seminars in neurology. Copyright© 2004 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New. **2004**.
2. Chishti, Z., et al., Microbial degradation of chlorpyrifos in liquid media and soil. *J Environ Manage*, **2013**. *114*: p. 372-80.
3. Eaton, D.L., et al., Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. *Critical reviews in toxicology*, **2008**. *38(sup2)*: p. 1-125.
4. Singh, P., et al., Biosurfactant production by Pseudomonas sp. and its role in aqueous phase partitioning and biodegradation of chlorpyrifos. *Letters in applied microbiology*, **2009**. *49(3)*: p. 378-383.
5. Koly, F.A. and R.A. Khan, Biodegradation of organophosphorous pesticide: chlorpyrifos. *Scientific Review*, **2019**. *5(1)*: p. 8-18.
6. Muhamad, H., et al., Downward movement of chlorpyrifos in the soil of an oil palm plantation in Sepang, Selangor, Malaysia. *Journal of Oil Palm Research*, **2010**. *22(1)*: p. 721-728.
7. Abdul-jabbarAbbas Ali., et al., Treatment of Chlorpyrifos Pesticide in Water Using Nano Scales Zero Valent Iron (nZVI). *Iraqi Journal of Science and Technology*,**2017**.*8(1):* p. 91-97.
8. Deb, N. and S. Das, Chlorpyrifos toxicity in fish: a review. *Current World Environment*, **2013**. *8(1)*: p. 77.
9. Lu, Y.-T., N.G. Love, and C.L. Grady Jr, Microscopic methods for distinguishing among three cell types in TOL plasmid-carrying Pseudomonas putida cultures. *FEMS microbiology letters*, **1999**. *173(1):* p. 195-201.
10. Helfrich, L.A., et al., Pesticides and aquatic animals: a guide to reducing impacts on aquatic systems. **2009**.
11. Ortiz-Hernández, M.L. and E. Sánchez-Salinas, Biodegradation of the organophosphate pesticide tetrachlorvinphos by bacteria isolated from agricultural soils in México. *Revista internacional de contaminación ambiental*, **2010**. *26(1):* p. 27-38.
12. Nyende, A., Et Al. Biodegradation Of Diazinon And Methomyl Pesticides By White Rot Fungi From Selected Horticultural Farms In Rift Valley And Central Provinces, Kenya. In Jkuat Annual Scientific Conference Proceedings. **2010**.
13. de Sousa Fragoeiro, S.I., Use of fungi in bioremediation of pesticides. **2005**, Cranfield University at Silsoe.
14. Jaiswal, S., et al., Bioremediation of Chlorpyrifos Contaminated Soil by Microorganism. *International Journal of Environment, Agriculture and Biotechnology*, **2017**. *2(4)*: p. 238833.
15. Mauldin, R.E., et al., A simple HPLC method for the determination of chlorpyrifos in black oil sunflower seeds. *Journal of liquid chromatography & related technologies*, **2006**. *29(3)*: p. 339-348.
16. Lu, P., et al., biodegradation of chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol by Cupriavidus sp. DT-1. *Bioresource technology*, **2013**. *127*: p. 337-342.
17. Gómez, M.J., et al., The environmental fate of organic pollutants through the global microbial metabolism. *Molecular Systems Biology*, **2007**. *3(1)*: p. 114.
18. Fulekar, M. and M. Geetha, Bioremediation of Chlorpyrifos by Pseudomonas aeruginosa using scale-up technique. *J Appl Biosci*, **2008**. *12*: p. 657-60.
19. Cycoń, M., A. Mrozik, and Z. Piotrowska-Seget, Bioaugmentation as a strategy for the remediation of pesticide-polluted soil: A review. *Chemosphere*, **2017**. *172*: p. 52-71.
20. Singh, B.K., et al., biodegradation of chlorpyrifos by Enterobacter strain B-14 and its use in bioremediation of contaminated soils. Appl. Environ. *Microbiol*., **2004**. *70(8)*: p. 4855-4863.
21. Nguyen, T.P.O., et al., Genetic and metabolic analysis of the carbofuran catabolic pathway in Novosphingobium sp. KN65. 2. *Applied microbiology and biotechnology*, **2014**. *98(19):* p. 8235-8252.

Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023

Citation: Al-Khafaji, Z.K.A.; Hashim, H.S.; Nasser, M.J.; Dabbi, A.A. Efficiency of *Pseudomonas mendocina* in biodegradation of chlorpyrifos insecticides. Revis Bionatura 2023;8 (1) 42. http://dx.doi.org/10.21931/RB/CSS/2023.08.01.42