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Efficiency Of Pseudomonas Aeruginosa In Bioremediation Of Chlorpyrifos Toxicity

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Abstract:

At present study, extensive varieties of pesticides are being used but the demand for Organophosphorus pesticide 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. It is being transported by circulation far away from the site of application leading to pollution of the environment due to its persistence, it is not only severely detrimental to the target pests but also causes toxicity in non-target organisms including humans. Lately, research activities in this area have demonstrated that microorganisms are potential tool in decaying insecticides into less harmful and non-toxic metabolites through a process known as bioremediation. Bioremediation has now emerged as an innovative technology that is critically important for the clean-up of polluted sites. This study aimed at investigating the efficiency of pseudomonas aeruginosa in bioremediation of chlorpyrifos from contaminated soil in Iraq. HPLC technology was used to estimate the percentage of removal achieved by bacteria, and it is considered one of the advanced modern techniques in measuring ingredient active in solutions. In concluded from the present study the results of the HPLC analysis showed that Pseudomonas aeruginosa is highly efficient in the analysis of chlorpyrifos.

Keywords: Bioremediation, organophosphate, Chlorpyrifos, pseudomonas aeruginosa, Hplc.

1-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 (Rusyniak and Nañagas, 2004). It was first developed by the Germans in the 1930s and first introduced into the marketplace in 1965, has been widely used globally as an insecticide to

control crop pests in agriculture, reduce household pests such as termites, reduce insect damage to turf on lawns, and for mosquito control(Chishti, et al., 2013; Eaton, et al., 2008).

A variety of studies have shown that exposure to CPF elicits acute toxicity through inhibition of acetylcholinesterase (AChE) throughout the central and peripheral nervous systems, leading to acetylcholine accumulation and long-term stimulation of cholinergic receptors(Singh et al.,2009). Chlorpyrifos works basically the same way both in insects and other animals including humans through impairment of nervous system. during development can cause persisting neurobehavioral dysfunction, even with low doses that do not elicit acute toxicity(Koly and Khan,2006).

The fate of chlorpyrifos is affected not only by its own physicochemical properties , but also by characteristics of the soil, management practices and environmental conditions(Muhamad, et al., 2010) 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 the soil(Koly and Khan,2019) and Chlorpyrifos is moderately soluble in water, the half-life of chlorpyrifos in water ranges from 35 to 78 days(Chishti, et al., ,2013).

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 also by microbial degradation that might occur concurrently(Koly and Khan,2019). Chlorpyrifos has been of a great concern due to persistence, toxicity and accumulation in soils and ground waters (Abdul-jabbar., et al2017) and It was observed may be causes altered the structure of the gills and liver of fishes(Deb and Das,2013).

The unsafe misuse of pesticides has caused widespread harm to human health and the environment, because it is one of the most common causes of poisoning around the world (Lu et al,1999) 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 (Helfrich, et al., 2009) When pesticides are dispersed in the environment, they become pollutants, with ecological effects that require remediation(Ortiz-Hernández and Sánchez-Salinas,2011).

It has become important to treat these pollutants, and there are many different methods of treatment, including chemical and physical treatment, which have faced many criticisms because of the main problems they cause such as the production toxic and radioactive acids and bases, in addition to these methods are uneconomic and ineffective and not suitable for large areas (Nyende, et al. 2010).

Lately, research activities in this area have demonstrated that microorganisms are potential tool in decaying insecticides into less harmful and non-toxic metabolites through a process known as bioremediation(de Sousa Fragoeiro,2005) Bioremediation has now emerged as an innovative technology that is critically important for the clean-up of polluted sites.

In summary, due to the pervasive nature throughout Pseudomonas aeruginosa, the potential non-pathogenic effect on fish, and efficiency in CPF metabolism, Pseudomonas species were considered a vital potential agent for testing in bioremediation. The aim of this study was to determine the extent to which Pseudomonas aeruginosa acts as an effective biomarker,

reducing CPF toxicity by breaking down CPF into non-toxic metabolites, and preventing AChE inhibition.

Pseudomonas aeruginosa, Bacillus cereus, Klebsiella sp., and Serratiamar scecens obtained from consortia showed 84, 84, 81, and 80% degradation of chlorpyrifos (50 mg/L) in liquid medium after 20 days and 92, 60, 56, and 37% degradation of chlorpyrifos (50 mg/L) in 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 chlorpyrifosco-metabolically, and

Enterobacter strain B-14, Alcaligenes faecalis, and Klebsiella sp., which degrade and utilize chlorpyrifos as sole carbon source(Jaiswal, et al., 2017) Bacillus sp. And Micrococcus sp. possess potential to degrade chlorpyrifos(Chishti, et al.,2013)

2-Methodology

2-1 sample collection

The soil samples used for the isolation of 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 local pesticide shop . Stock solution was prepared and stored in refrigerator for further use. The samples were collected using sterile petri dish and plain tube and 5 grams of soil samples were taken and added Normal line to it, for the purpose of purification and isolation of bacteria.

2-2 The instruments that used in determining removal of chlorpyrifos and detection of bacteria

1- VITEK 2 (Biomerieux, France)

2- High Performance Liquid Chromatography (Hplc) (Knuaer , Germany).

2-3 Preparation of stock pesticide

Commercial grade chlorpyrifos (48%) pesticide was procured from local pesticide shop. Stock solution was prepared and stored in refrigerator for further use. To estimate the amount of residual chlorpyrifos concentrations after removal by bacterial isolates. Standard was prepared for the active ingredient chlorpyrifos 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 (Mauldin et al.,2006)

2-4 Isolation and identification of bacteria

Prepare the soil suspensions by taking 1 gram of soil, and dissolve in 10 ml of normal saline, then mix by the vortex for 10 minutes and centrifuge for 5mine 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 sulphate 10.00 g / L, Magnesium Chloride 1.4 g / L, Agar 11.00 g / L, and sterilized by autoclaving at 15lbs (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).

2-5 Treating the medium with the pesticide

Nutrient broth is prepared for the purpose of treatment with chlorpyrifos and consists of peptone 5 g / liter, sodium chloride 5 g / liter, meat extract B 1.5 g / liter, yeast extract 1.5 g / liter. Addion 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 then cool to 45° C.

2-6 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 200µl of chlorpyrifos, and mix well and add the last mixture to the liquid medium in order 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 were pour in cup urin 50 ml and inoculated with pseudomonas aeruginosa and incubated at 37°C. Samples were taken at periodic intervals and the residual pesticide concentration was determined using HPLC. Un-inoculated cup served as controls.

3-Result and discussion

The results showed that the pseudomonas spp. is 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 bioremediation of pollutants. P.aeruginosa strain removal 99% of CP with at 6 days, the residual chlorpyrifos concentration was 1.10 to 0.00 µg /mL, (table.2) while P.putida (fig.3) and P.fluorescence (fig.4) degraded CP within 6 days from 0.08µg/mL to 0.002µg/mL and 0.26µg/mL to 0.118µg/mL. The P.aeruginosa could rapidly degrade chlorpyrifos campare with other species during the logarithmic phase of growth. The percentage degradation in first 6 day

was higher and rapidly increased thereafter to 100% within 12 day compare with control (Fig. 2). 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 (Ortiz-Hernández and Sánchez-Salinas,2010; Chishti, et al., 2013) The strain could utilize CP as the sole source of carbon for growth. In HPLC analysis of sample uninoculated (without bacteria) with 200 µl CP, two peaks appeared in HPLC separation (Fig.1), which were further identified as CP by comparing with their authentic standard. HPLC analysis showed that CP could be degraded by P. aeruginosa to TCP and diethylthiophosphoric acid.

Table [1] : The probability of diagnostic bacterial isolates by Vitek2compact system

NO.	Bactria	Probability%
1.	P.aeruginosa	99%
2.	P. putida	99%
3.	P. fluorescence	94%

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

Bacterial sample	Concentrate Of chlorpyrifos	Residual Concentrate After 6 days	Residual Concentrate after 12 days	control
P.aeruginosa	120 µg/ml	1.10 µg/ml	0.000 µg/ml	63.40 µg/ml
p.putida		0.08 µg/ml	0.002 µg/ml	
p.fluorescence		0.26 µg/ml	0.118 µg/ml	

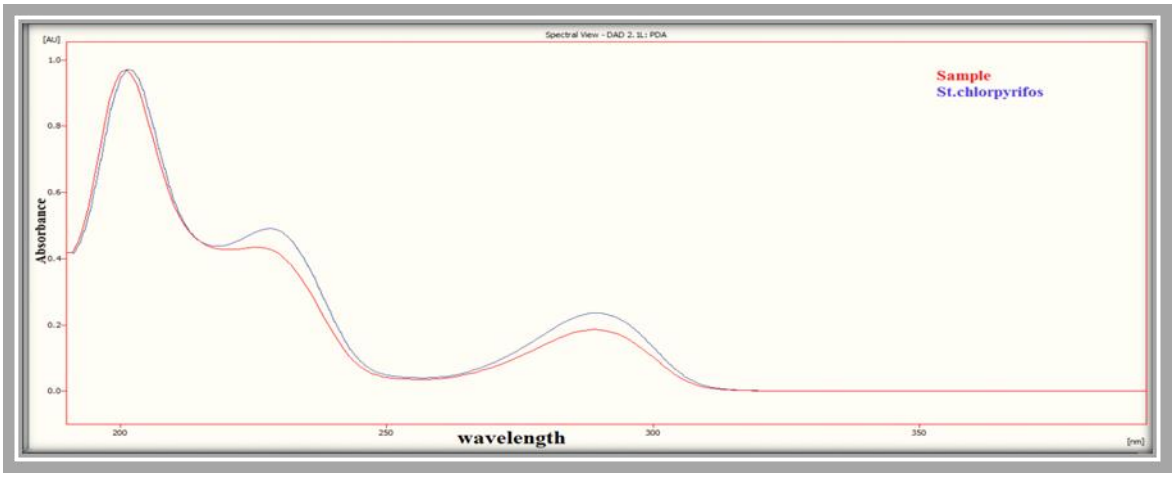

Figure (1): shows the absorbance and spectrum of St.chlorpyrifos and the control (sample uninoculated) treated with the pesticide without bacteria.

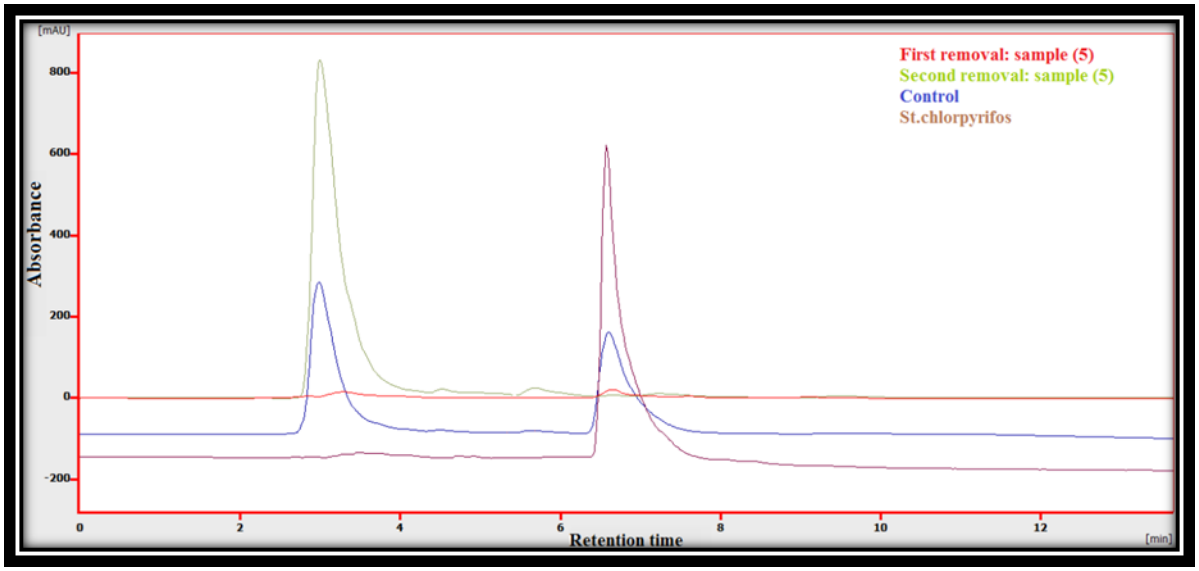

Figure (2): Showed growth kinetic for P. aeruginosa on broth media with chlorpyrifos as carbon source and energy.

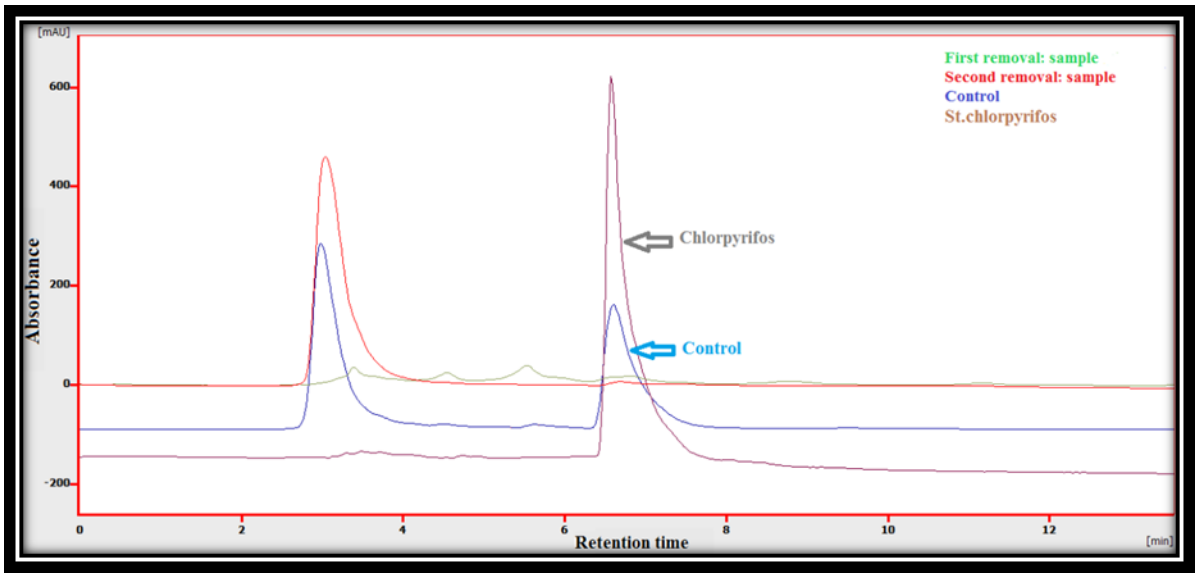

Figure (3): Illustrates peaks Chlorpyrifos (CP) degradation by Pseudomonas putida strain. 1) Residual CP in inoculated sample after 6 day, 2) Residual CP in inoculated sample after 12 day,. 3) CP degradation in un-inoculated control, and 4) standard of chlorpyrifos 48%.

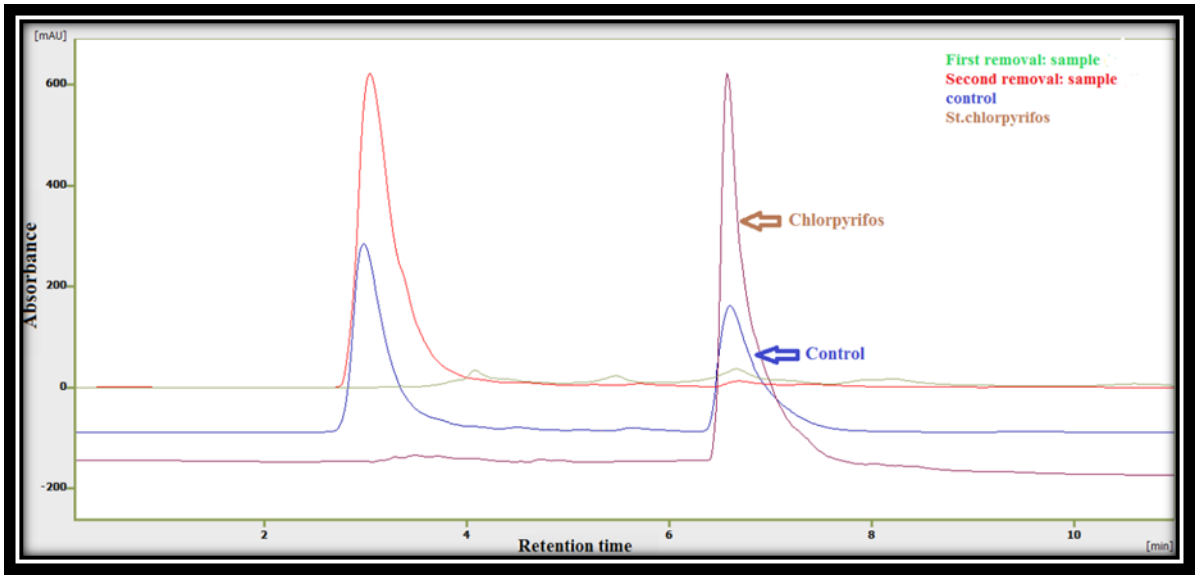

Figure (4): Illustrates peaks Chlorpyrifos (CP) degradation by Pseudomonas fluorescence strain. 1) Residual CP in inoculated sample after 6 day, 2) Residual CP in inoculated sample after 12 day,. 3) CP degradation in un-inoculated control, and 4) standard of chlorpyrifos 48%.

Only a few organisms capable of degrading chlorpyrifos have been reported till 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 aeruginosa utilized CP as a source of energy 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 by.

The reported minimum incubation time required for complete removal of chlorpyrifos was 6 h by Cupriavidus sp. DT-1 in a liquid medium(Lu et al.,2013). Most of the common CP degrading bacteria were reported to degrade the pesticide within 48 h in liquid media(Gómez et al.,2007). Chlorpyrifos has been shown to be degraded cometabolically in liquid media by bacteria (Jaiswal, et al., 2017) Pseudomonas aeruginosa is the most common Gram negative bacterium found in soil. Isolates of this bacterium have been found to have potential to degrade chlorpyrifos(Fulekar and Geetha,2008).

Since Pseudomonas aeruginosa capable of degradation of various OPs have great potential for bioaugmentation of soil(Cycoń et al,2017); these strain of bacteria with its high degradation potential, fast growth, minimal nutrient requirements and tolerance to high concentrations of pesticide can play a vital role in decontamination of polluted soil. The rapid removal of CP from liquid medium within 6 day by the p.aeruginosa strain thus could be one of the fastest reported for effective CP degradation and first of its kind amongst the Pseudomonads. P.aeruginosa is a known plant growth promoter(Trinh et al.,2018), and this combined with the reduced time for decontamination of CP makes it a promising candidate for pesticide removal from agricultural lands and also for treating pesticide industry effluents.

To demonstrate the capacity of the recombinant P. aeruginosa strain to degrade CP, the degradation experiments were performed by inoculating the recombinant strain into a nutrient broth medium supplemented with CP. High-performance liquid chromatography (HPLC) analysis indicated that CP were 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. CP was degraded quickly in the first 6 days, which accounted for 48% of the amount 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 have antimicrobial activity and are toxic to the bacterial growth and metabolism(Singh et al.,2004 ; Nguyen et al.,2014)

4. CONCLUSION

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

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