Bioremediation of Chlorpyrifos Insecticide by using *Aeromonas Hydrophila* Bacteria

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Abstract

Several bacteria species may have the ability to degrade and digest soundly the residuals of certain insecticide with causing any deteriorated consequences. This work was designed to examine the ability of *Aeromonas hydrophila* bacteria for biodegrading of chlorpyrifos insecticide from contaminated soil. However, soil sample was collected from home garden at depth of 10 cm while the chlorpyrifos insecticide was obtained from the local market. Meanwhile *Aeromonas hydrophila* bacteria were isolated from the garden soil. The bacterial cultural media were prepared to consist of $(NH_4)_2SO_4$ (1g), NaCl (6g), K_2HPO_4 (0.5g), yeast extract (1g), $MgSO_4$ (0.1g) and CMC (5g). This work has examined the efficiency of *A. hydrophila* bacteria to remove insecticide from the soil under various growing conditions such as five different pH values, five termperature degrees and four insecticide concentrations. The obtained results showed that the highest bacterial efficiency (98.0 ± 7.8%) was recorded as pH value of 6 and it was 91.1 ± 6.0% at temperature of 40 ° C while it was 99.1 ± 6.8 % at insecticide concentration of 25 ppm.

Key words: *chlorpyrifos, Aeromonas hydrophila, pH, temperature, insecticide concentrations.*

Introduction

Significant quantity of works hase examined thoroughly the various chemical synthetic insecticides in terms of agricultural. [(1)] Health and environment Chlorpyrifos insecticide belongs to organophosphate class and its' chemical formula is and also known as lorsban with an average molar mass of 350.59 g/mol and a density of 1.4 g/cm3. [(2)] However, it is intensively used worldwide on various plant crops such as citrus, corn, soybean, cotton, nuts, almonds and other crops. [(3)] However, various works have examined the possible toxic effects of Chlorpyrifos insecticide and reported elevated contents of the insecticide residues in plants [.(4)] On the other hand, various techniques were used for the removal of the pesticide residues such as conventional method [(4)] Phytoremedation is a unique technique for the removal of various environmental pollutants including the pesticide residues being environmentally sound method, almost costless and very accissable and it was intensively and widely used in the treatment of various environmental contaminants. However, this technique consists of using certain plant species [(5)] Several bacterial species are well known being capable of controlling soil contamination with hydrocarbon and heavy metal [(6)] However, these bacterial species are used successfully to face the different environmental challinges. So, the current work was designed to use *A. hydrophilla* bacteria to clean up the soils from the residues of applied chlorpyrifos insecticide[.(7)]

Materials and Methods

Soil samples were collected from home garden at depth of 10. The collected soil sample was air dried under room temperature and sieved via 0.8 mm stainless steel sieve[.(8)] A 100 ml of chlorpyrifos insecticide of 50 ppm was obtained from the local market. *Aeromonas hydrophila* bacteria was isolated from the garden soil by using bacterial cultural media consisting of 1 g ammonium sulfate [$(NH_4)_2SO_4$], 6g of sodium chloride [NaCl], 0.5 g dipotassium phosphate [K_2HPO_4], 1 g yeast extract, 0.1 g magnesium sulfate [$MgSO_4$] and 5 g CMC. [(9)] This synthetic media was placed in volumetric flask containing 1 liter distilled water. A 100 growing mineral salt media with toleum of 125 g /l were placed in 250 ml sterilized volumetric flask. Each of these flasks has received 1 g sieved soil and placed in shaker incubator for one week under pH of 7 and temperature of 28 °C at RPM of 120. (10) The bacteria was isolated in nutrient agar petri dishes and after the purification, each isolated bacteria was planted in small glass vials by slant method and kept for subsequent tests. However, three sets of experiments were carried out during the current study where all of them were related to the factors affecting bacteria effiency for the removal of insecticide from the soil. (11) The first factor was growing pH with five values (4,6,7,8 and 10) were examined with fixing insecticide concentration at 50 ppm and temperature of 28 °C. (12) Saline culture media was placed in volumetric flask with adjusted five pH values where each volumetric flask received 50 ppm and the isolated *A. hydrophila* bacteria was planted on each of these petri dishes. The experiment was replicated 3 times giving a total of 15 volumetric flask and incubated for two weeks and the bacterial effiency for the removal the insecticide was calculated. (13) The second factor was growing temperature which were 5, 10, 20, 30, and 40 °C. The test was done with fixing growing pH at 7 and insecticide concentration of 50 ppm. Again similar saline culture media explained above were used and the same testing procedure was followed and the experimental unites were incubated for one week where the bacterial effiency for the removal the insecticide was again calculated. Finally, the third testing variable was the insecticide concentration and four levels (10, 25, 50 and 100 ppm) were examined using similar growing media described above and following the same method in case of testing both growing pH and temperature. But, these two variables were fixed at 7 for pH and 28 °C for temperature. (14) The experiment was incubated for one week and the bacterial effiency for the removal the insecticide was recorded.(15)

Results and Discussion

Table 1 displays the mean value ± standard deviation of bacterial effiency for the removal of chlorpyrifos insecticide from the contaminated soil under various values of pH, temperature and insecticide concentration. It seems clearly that insecticide removal effiency of *A. hydrophila* bacteria from the contaminated soil is affected significantly (P≤ 0.05) by the examined variables. In general, highest value of the insecticide removal effiency was recorded in bacteria under different growing conditions such as soil pH, incubation temperature and insecticide concentration.

Table 1 The mean value ± sd of bacterial effiency for the removal of chlorpyrifos insecticide from the contaminated soil under various values of pH, temperature and insecticide concentration

Examined Variable	Values	Mean Effiency ± SD
pH	4	0.9 ±0.05
	6	98.0 ± 7.8
	7	90.9 5.5
	8	90.0 ± 4.8
	10	0.6 ± 0.03

Cont... **Table 1 The mean value ± sd of bacterial effiency for the removal of chlorpyrifos insecticide from the contaminated soil under various values of pH, temperature and insecticide concentration**

Temperature	5	25.3 ± 2.6
	10	36.3 ± 4.2
	20	86.0 ± 5.2
	30	90.0 ± 5.9
	40	91.1 ± 6.0
Insecticide concentration	10	90.3 ± 7.2
	25	99.1 ± 6.8
	50	50.8 ± 4.5
	100	9.0 ± 1.7

The effects of various pH values (4,6,7,8,10) on the bacterial insecticide removal efficiency were found being the best removal efficiency was 98.0 ± 7.8 % recorded at pH of 6 followed by 90.9 ± 5.5 % at pH 7 and 90.0 ± 4.8 at pH 8 while the lowest efficiency percentage of 0.6 ± 0.3 % was recorded at pH of 10 followed by 0.9 ± 0.05 % detected at pH 4 (Table 1; Figure 1).

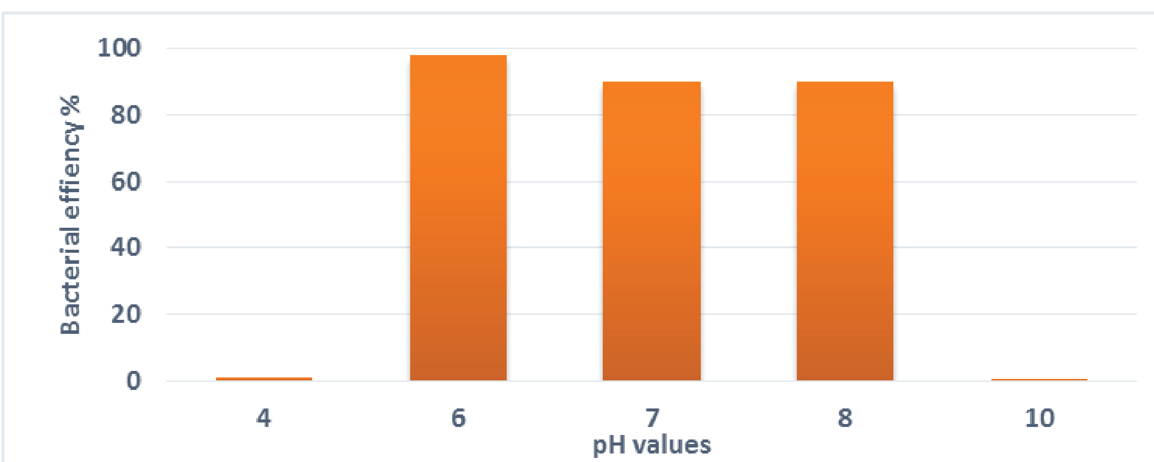

Figure 1: The effect of differenr pH values on the bacterial efficiency forf the removal of insecticide from the soil.

In case of the temperature, the obtained results showed that the highest mean value (91.1 ± 6.0 %) of insecticide removal effiency by examined bacteria was recorded at temperature of 40 °C followed by that detected at 30°C which was 90.0 ± 5.9 while the lowest mean value (25.3 ± 2.6 %) was found at ° C followed by those recorded at 10 °C which was 36.3 ± 4.2 % and at 20 ° C, it was 86.0 ± 5.2 % (Table 1; Figure 2)

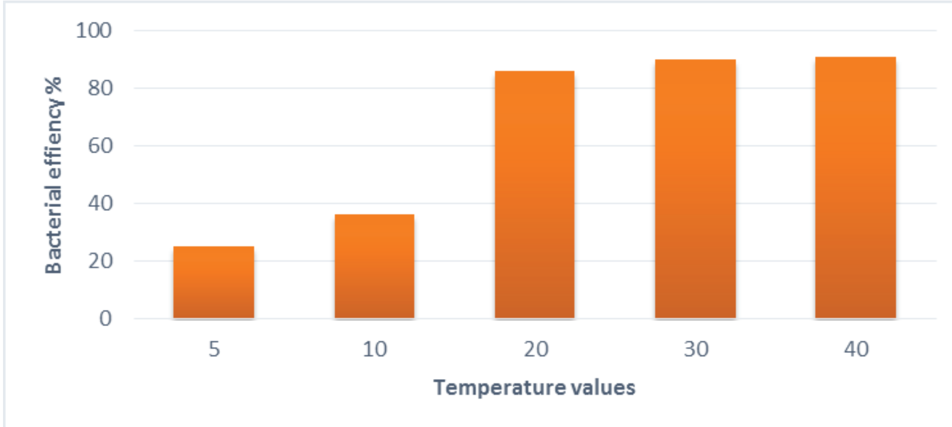

Figure 2: The effect of different temperature values on the bacterial efficiency forf the removal of insecticide from the soil.

Regarding the insecticide concentration, this work has found that the highest mean value of bacterial effiency for the removal of insecticide residues from the soil was 99.1 ± 6.8 % which recorded at 25 ppm followed by that detected at 10 ppm which was 90.3 ± 7.2 % while the lowest mean value (9.0 ± 1.7 %) was found at 100 ppm and at 50 ppm, the mean value was 50.8 ± 4.5 % (Table 1; Figure 3).

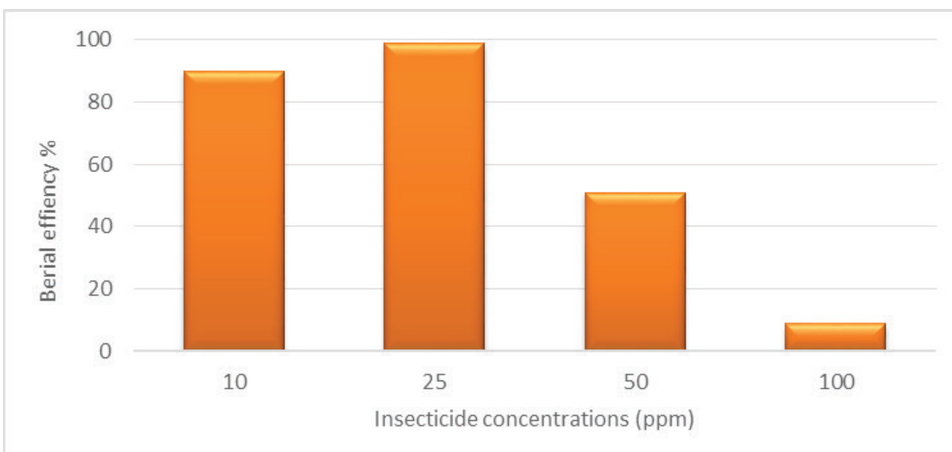

Figure 3: The effect of different insecticide concentrations on the bacterial efficiency forf the removal of insecticide from the soil.

Previous study has examined the effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium and reported the bacteria at pH greater than 6.7 was able to maintain its biodegradation of chlorpyrifos insecticide. Such pH effects was also reported by other works [(1)] However, it seems that the soil pH affects sorptive behavior of pesticide molecules as reported by previous study [(3)] Regarding the effect of temperature on the biodegradation of insecticide in general, it was reported that the biodegradation is increased with increasing the temperature up to 40 °C .[(5)] For insecticide concentration, it was found that the insecticide concentration had the greatest impact on the degradation of chlorpyrifos inseciticide as reported by previous study [(7)] but at very higher insecticide concentration (1000µg/ g) , the biodegradation of of chlorpyrifos is inhibited as reported by an early work . [(9)] On the other hands, other study has reported that that the pH variable needs to be associated with temperature for better biodegradation of chlorpyrifos insecticide [(3).] However, *A. hydrophila* bacteria has wide thermal range (5 – 50 °C) and also its ability in secretion of various enzymes that assiss to get environmental adaption . [(6)] Also, this bacteria has the ability to degrade chlorpyrifos insecticide via excreting excellular hydrolysis phosphotriesterase enzyme [(9).]

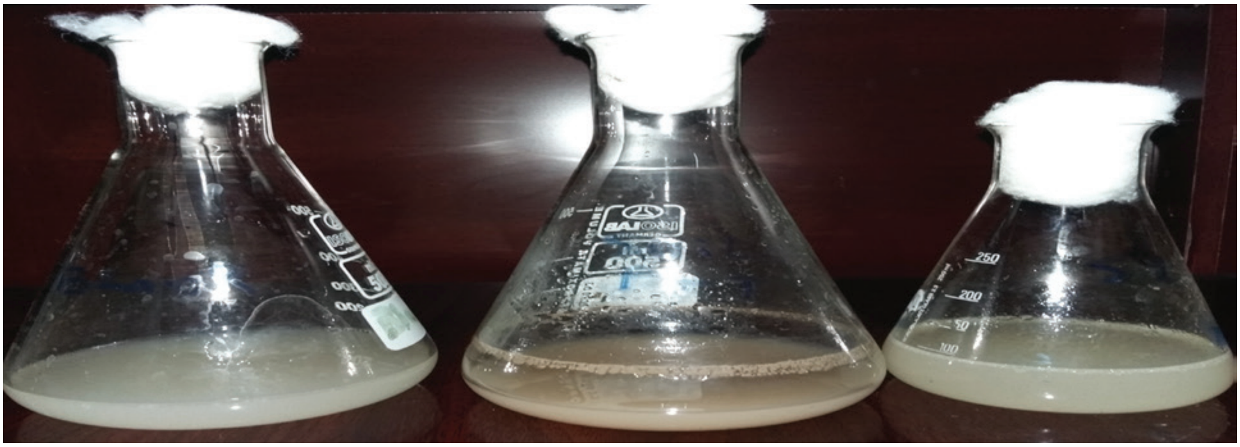

Figure 4 A picture showing the transformation of the pesticide into an emulsified solution two weeks after the experiment

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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