Microbial Degradation of the Organophosphorous Insecticide, Malathion Using the Natural Bacterial Isolate, *Pseudomonas aeruginosa*

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Abstract

Background: Pesticides are applied in agricultural fields to control pest population and to achieve crop protection. But some of them persist in the environment and cause damage to the ecosystem. Hence, their degradation becomes necessary. Microbes possess the capacity to degrade such xenobiotic compounds.

Objectives: The present study has been designed to isolate a bacterial strain effectively degrading Malathion and to test its efficiency of degradation.

Materials and Methods: The bacterial strain was isolated from soil samples collected from agricultural fields after serial dilution. Based on biochemical tests, it was identified. In 50, 100, 150 and 200 ppm concentrations of Malathion in minimal broth, parameters like orthophosphate released, pH and turbidity were measured for thirty hours. Influence of cell types and carbohydrate sources was also studied.

Results: The selected bacterial strain was identified as *Pseudomonas aeruginosa*. During treatment, both orthophosphate levels and turbidity exhibited an increase while pH showed a decline. All the supplemented carbohydrate sources enhanced degradation. Immobilized cells performed better than that of free cells in long term treatment.

Conclusion: From the above findings it can be concluded that the isolated bacterial strain could be used in the treatment of pesticide contaminants in agricultural fields.

***Keywords*:** *Malathion, Pseudomonas aeruginosa, Biodegradation, Pesticide, Bacteria.*

Introduction

Farmers are using more than 45,000 tones of different types of pesticides to meet the need of food requirements for the growing population. The advantage resulting from pesticides application is generally undisputed, but the residues of the applied pesticides stay in the environment (air, soil. ground and surface water) for variable period of time, which poses serious threats to environment and indeed can lead to acute and chronic effects on human life causing health problems or even death[1-5]. There are chemical and physical factors that influence the fate of the pesticides like biodegradation of the compounds by aerobic and anaerobic microbes isolated from soil, water and other habitats[6-9].

Malathion is a non-systemic pesticide with high selective toxicity. It is mostly applied for the control of sucking and chewing insects on crops and against mosquitoes and flies[10-13]. The biodegradation of Malathion occurs in both water and soil, and evidence supports breakdown occurring on vegetation. Microorganisms known to degrade organophosphates include *Pseudomonas* sp., *Streptomyces* sp., *Thiobacilus*

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sp., and certain fungi of the *Trichoderma* genus[14,15]. In the present study, an attempt has been made to isolate and identify a bacterial strain, which degrades Malathion and also to test its efficiency of degradation.

Materials and Methods

Pesticide, especially Malathion applied soil samples were collected from agricultural fields near Thirupuvanam, 25 kilometers away from Madurai, Tamil Nadu, India in sterile containers and immediately brought to the laboratory for analysis. The bacteria capable of degrading Malathion were isolated from the collected soil with varying concentrations of Malathion in the medium. The bacterial strain exhibiting highest tolerance to Malathion was isolated, identified and preserved for further studies. Isolated culture was maintained on nutrient agar slants and stored at 4°C. The maximum concentration of Malathion for bacterial growth was determined by the inoculation of the selected bacterial strain on minimal medium containing 50, 100, 150 and 200 ppm concentrations of Malathion. The plates were incubated at 37°C for 24 hrs. The identification and characterization of the selected bacterial strain were carried out using morphological, cultural and biochemical tests according to Bergey's manual of determinative bacteriology[16].

The organism was inoculated into minimal broth containing different concentrations of the pesticide (50, 100, 150 and 200 ppm). The flasks were incubated at room temperature and the samples were then subjected for the estimation of orthophosphate. One ml of sample was taken in a flask and 1 ml of ammonium molybdate and 3 drops of stannous chloride solution were added and kept for 10 minutes for the development of blue colour and the absorbance was recorded in a colorimeter at 650 nm. Distilled water blank was subjected in a similar manner. Similarly the standard phosphorus solution of different strengths was processed and standard curve was plotted between absorbance and the concentrations of standard phosphorus solution. The orthophosphate content of the sample was deduced by comparing its absorbance with the standard curve. pH was analyzed every 6 hours up to 30 hours of treatment for the sample containing minimal medium, culture and different concentrations of Malathion using pH meter and readings were recorded. Growth was measured as turbidity at 600 nm with 6 hours interval for 30 hours.

The efficiency of pesticide degrading ability of the bacterium was tested by providing different carbon sources like fructose, glycerol, lactose, maltose and sucrose of 1% concentration in minimal medium containing 200 ppm concentration of Malathion. The flasks were incubated at 37°C and orthophosphate released was estimated every 6 hours up to 30 hours. The pure culture of the isolate was grown in nutrient broth and the cells were harvested by centrifugation at 10,000 rpm for 10 minutes and the cells were washed and suspended in 0.1% NaCl. Then 3.5% of sodium alginate was added to the cell suspension and mixed thoroughly without forming any air bubble in the slurry. The slurry containing the cells was extended as drops through a tube (2 mm diameter) into 4% $CaCl_2$ solution. The drops formed into spherical beads of 2 mm size. The gel beads were kept in 4% $CaCl_2$ solution at 5°C for about an hour for complete gelation. Then the beads were washed with sterile distilled water and used for Malathion degradation study[17]. Two way analysis of variance (ANOVA) was performed on the factors like orthophosphate released, turbidity, pH and influence of sugars for the two variables namely treatment period and Malathion concentration using MS-Excel latest version 2019.

Findings

Results

Bacterial strain isolated from the Malathion applied soil was identified as *Pseudomonas aeruginosa.* It exhibits positive response for biochemical tests that include Sorbitol, citrate utilization, Lysine decarboxylase, Nitrate reductase, catalase and oxidase. Orthophosphate released during the degradation of Malathion at different concentrations of Malathion (50, 100, 150, and 200ppm) by the isolate seems to be fluctuating (Fig.1). Initially the orthophosphate concentration was increasing gradually and there was a decline after 18 hours. At the end of 18 hours, orthophosphate released was high in the degradation of 150ppm concentration of Malathion. The natural isolate *P. aeruginosa* effectively degraded Malathion releasing high levels of orthophosphate. So, 150ppm concentration of the Malathion was found to be optimum for the degradation of pesticide and it was selected for further analysis. Effective degradation

of Malathion by the isolate *P. aeruginosa* was carried out at the pH range of 6-8 (Fig.2). With the increase in incubation period, pH was decreasing indicating the release of acidic intermediates. Turbidity measurements (Fig.3) divulge that there was a significant increase in the growth during the treatment period until 24 hours which indicates that the organisms effectively utilized the pesticide as the sole source of carbon and phosphorus.

Immobilized cells released orthophosphate with a constant, stable and gradual increase but in the case of free cells the concentration of orthophosphate declined after 12 hours of treatment (Fig.4). Supplementation of carbon sources to the minimal medium enhanced degradation process where *P. aeruginosa* utilized sucrose and lactose effectively for degradation of Malathion (Fig.5). In two way ANOVA for the factors such as orthophosphate released, pH and turbidity with the variables, treatment period and methyl parathion concentration, variations due to treatment period and Malathion concentration were statistically significant at 5% level.

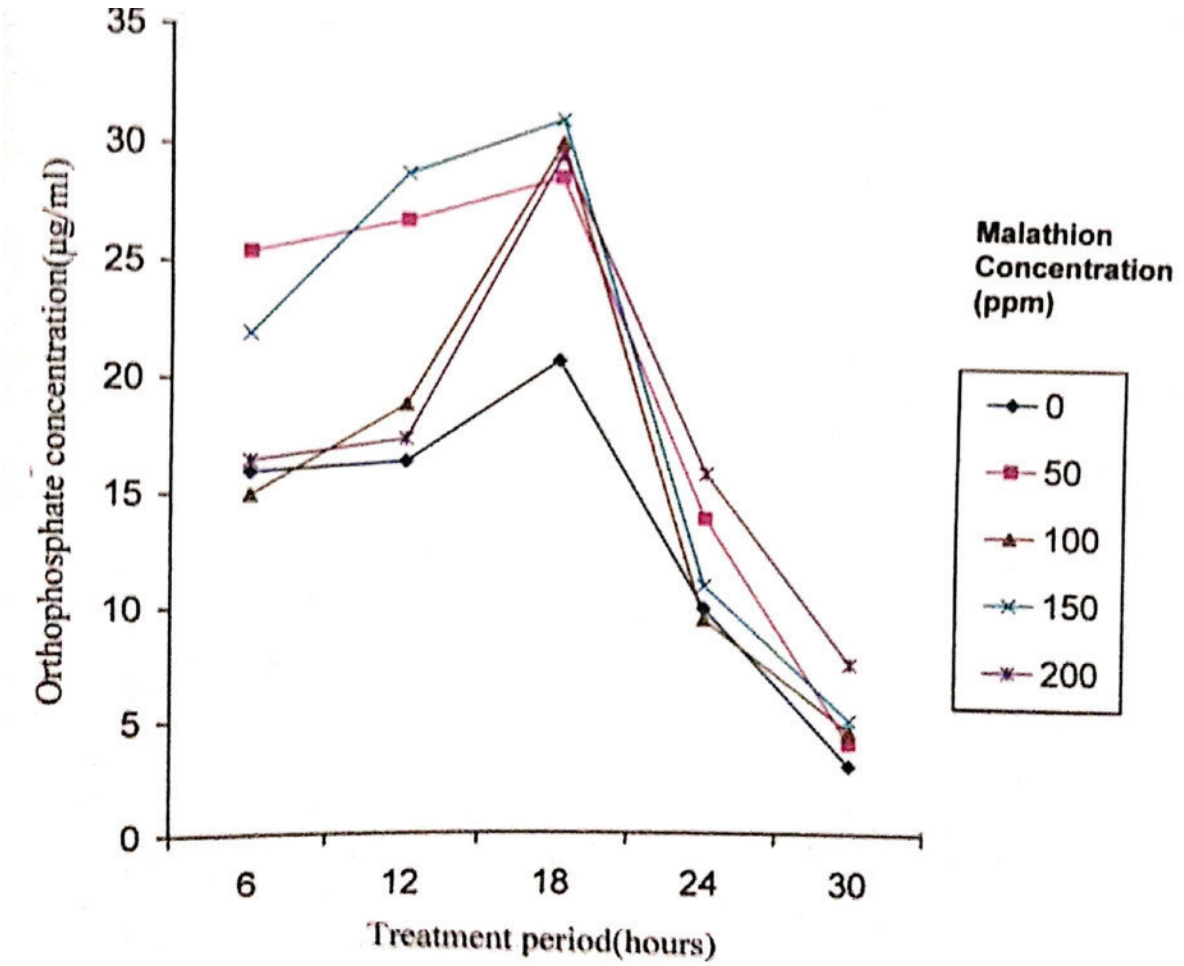

Fig. 1. Orthophosphate released during the degradation of Malathion by *P. aeruginosa*

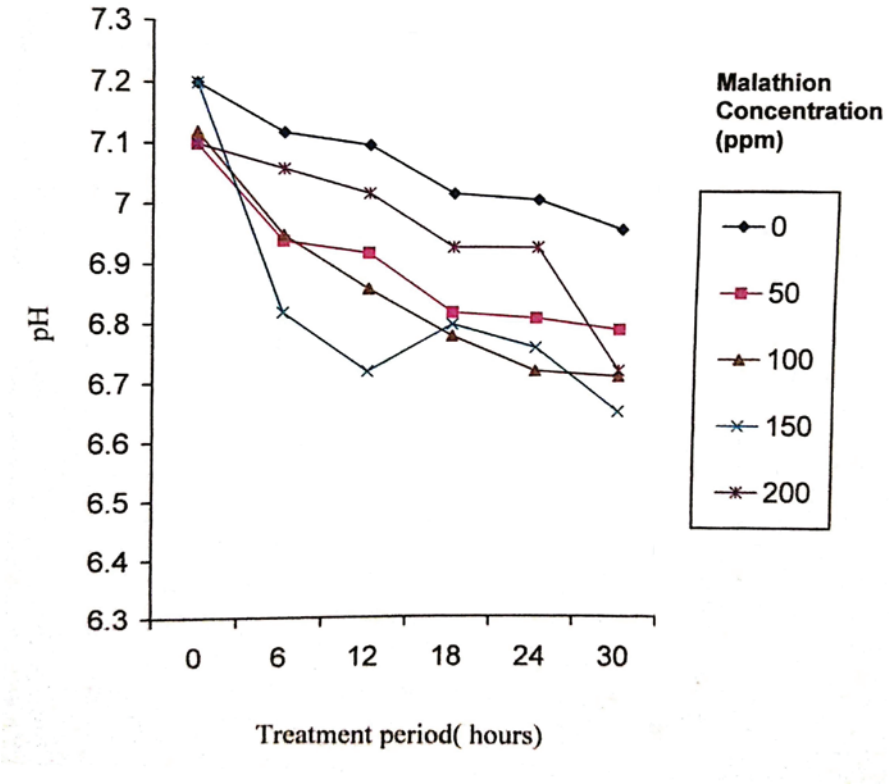

Fig.2. Changes in pH during the degradation of Malathion by *P. aeruginosa*

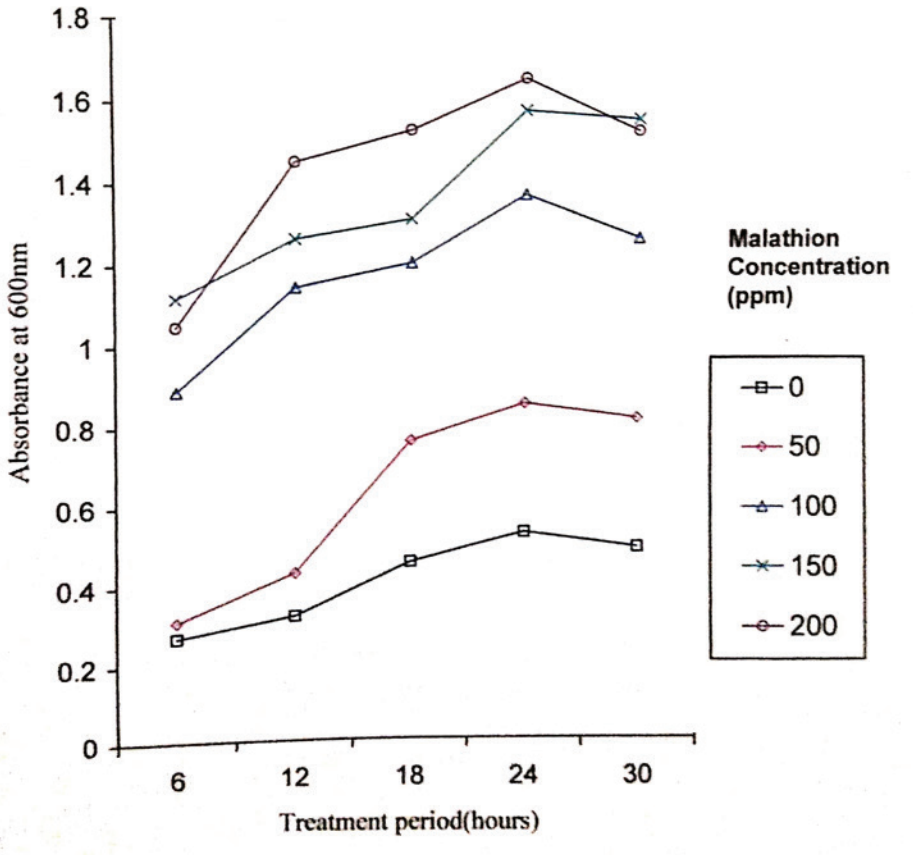

Fig.3. Turbidity during the degradation of Malathion by *P. aeruginosa*

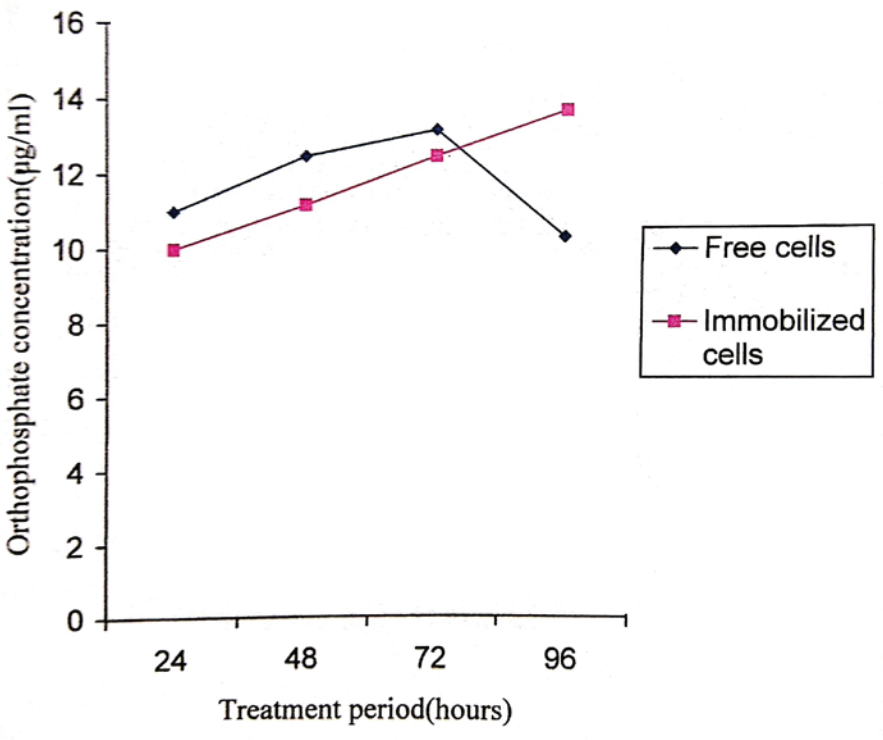

Fig.4. Orthophosphate released during the degradation of 150ppm Malathion by free and immobilized cells of *P. aeruginosa*

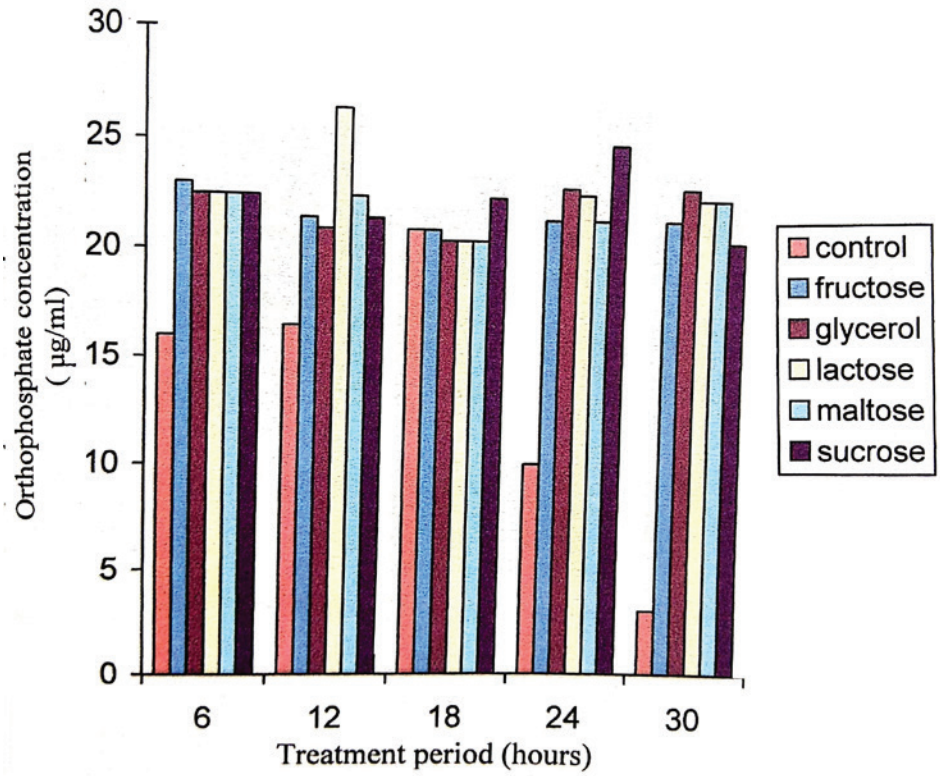

Fig.5. Orthophosphate released during the degradation of 150ppm Malathion by *P. aeruginosa* **when supplemented with various sugars of 1% concentration**

Discussion

Based on the morphological, physiological and biochemical characteristics of the strain isolated from Malathion applied soil, it was found to be Gram-negative motile rod. It was identified as *P. aeruginosa* on the basis of classification schemes published in Bergey's manual of systemic bacteriology[16]. It utilized Malathion as a sole source of carbon and phosphorus for growth. High concentration of orthophosphate has been released at 150ppm by *P. aeruginosa* isolate indicating the efficiency of Malathion degradation. The orthophosphate levels increased to maximum in 18 hours and later began to decline. The isolated pesticide degrading strain *P. aeruginosa* released more orthophosphate. Hydrolytic cleavage of organophosphate bond is considered as the initial step in the metabolism of organophosphates. But the hydrolytic reaction did not supply energy required for the growth of the organisms and only the degradation products from these pesticides appear to serve as energy for growth and proliferation of microorganisms[18-20]. The alteration in the pH during the growth of *P. aeruginosa* in Malathion amended minimal medium proved not only the breakdown of Malathion but also the formation of acidic intermediates. It has been reported that the alteration of pH in the medium from 7.2 to 3.0 during the growth of *Serratia marcescens* in Malathion amended medium confirmed the breakdown of Malathion and formation of acidic intermediates. Similarly, the pH of the assay mixtures showed the change in pH from 7.2 to 6.0 - 6.1 at the end of three hours, which confirmed the breakdown of Malathion and the formation of acidic intermediates. It is observed that, initially, at 0 hour, the inoculated cell population was low and then started increasing slowly until 24 and 18 hours respectively and *P. aeruginosa* isolate entered into the phase of positive acceleration. The organism utilized Malathion as a sole source of carbon and phosphorus for its growth. The bacterial cells in log phase would mean that the substrate conversion would be at its maximum. Decline phase was not achieved even after 18 hrs of incubation indicating that the nutrients were still available for the cells to grow and other environmental conditions are also favourable. A decrease in cell density was observed after log phase. It was reported that *Pseudomonas* sp. could grow in medium having Malathion (35-220 mg/l). However, the optimum concentration which supported normal bacterial growth during 24 hours was found to be 120 mg/l Malathion. Significant increase in bacterial population was observed at low concentration, while at high concentration lag phase increased but no zone of inhibition was noticed[21-24].

Immobilized pesticide-degrading bacteria can be used on a large scale for pesticide detoxification. Immobilized *P. aeruginosa* isolate was able to degrade Malathion gradually with constant increase when cornpared to free cells where degradation proceeds at a faster rate and there was a decline in cell population after 72 hours of treatment period. Addition of 1% concentration of different carbon sources enhanced the degradation of Malathion with the release of high levels of orthophosphate. Lactose, sucrose and fructose showed effective degradation of Malathion. It has been reported that when fructose was the growth substrate, the rate of demeton-S-methyl (Organophosphate) consumption by *Corynebacterium glutamicum* was greater than the rate of consumption when either acetate or glucose was the growth substrate. Addition of 1% concentration of different carbon sources indicated disaccharides enhancing the degradation of chloropyrifos with the release of orthophosphate, total phosphorus and increasing the activity of acid phosphatase when compared with that of other carbon sources[25-27].

Conclusion

This study discovered the efficiency of *Pseudomonas aeruginosa* on the biodegradation of Malathion that can be beneficial for bioremediation programmes for restoring soil quality. This study will help the researchers to uncover the critical areas of using the natural isolate, *P. aeruginosa* for biodegradation of pesticides that many researchers were not able to explore. Thus a new theory on using *P. aeruginosa* for treating pesticide polluted soil may be arrived at.

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