

Received: 15 August 2014 • Accepted: 15 November 2014

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doi:10.15412/J.JBTW. 01040202

Diazinon degradation by *Pseudomonas aeruginosa* and *Flavobacterium* bacteria and assessing the growth Kinetics

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ABSTRACT

Each year a lot of pesticides are entered into the environment through different ways. Diazinon (O,O-DiethylO-(2-Isopropyl-6-methyl-4-pyrimidyl) Phosphorothioate) is in fact an organophosphorus that is widely employed in agriculture. Initial environmental concerns for the effect of this pesticide in injury to birds, contamination of surface water and destructive effects on aquatic species exist. Diazinon and its metabolites have been paid attention throughout the world during alarming studies on different aquatic systems. In this study, diazinon degradation by *Pseudomonas aeruginosa* and *Flavobacterium* bacteria were evaluated. For the first stage, during two consecutive seasons of the year samples were collected from the soils of three agricultural soil stations in Marvdasht city in Iran, Subsequently, counting the bacteria was done using Total Viable Plate Count (TVPC) and isolating the diazinon degrading bacteria. Eventually, Identifying diazinon degrading bacteria and the rate of decomposition by bacteria was measured using gas chromatography. Determining the growth of the isolated bacteria in different concentrations of diazinon was also performed to show the growth kinetics by studying their optical absorption. Our results showed that the *Pseudomonas aeruginosa* and *Flavobacterium* reduced the level of diazinon at level of $p \leq 0.05$. Also, the growth of the bacteria in different concentrations of diazinon has been initially an increasing trend and decreasing at the end. This results showed that *Pseudomonas aeruginosa* and *Flavobacterium* a good candidates for purification and eliminating of diazinon in contaminated soils.

Key words: *Pseudomonas aeruginosa*, *Flavobacterium*, gas chromatography, biodegradation

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1. INTRODUCTION

Diazinon, a colorless to dark brown liquid, is a thiophosphoric acid ester developed in 1952 by Ciba-Geigy, a Swiss chemical company, and later Diazinon by other companies such as Novartis and then siegnta was presented (1). Diazinon is a non-systemic organophosphate insecticide formerly used to control cockroaches, poultry, ant, silverfish and fleas in residential, non –food buildings a bait from was used to control scavenger wasps in the western U.S. Diazinon (O, O-Diethyl O-(2-Isopropyl-6-methyl-4-pyrimidyl) Phosphorothioate) is in fact an organophosphate that is widely employed in agriculture. Initial environmental concerns for the effect of this pesticide in destroying birds, contamination of surface water and destructive effects on aquatic species exist. Diazinon and its metabolites have been paid attention to throughout the world during alarming studies on different aquatic systems (2, 3).In

water, Diazinon has a limited solubility of 68.8 mg/l at 20°C (4). However, Koc experimental values are different and in 180 to 430 ml/g range (5, 6). These values indicate that Diazinon is not expected to be highly limited to soil and has a high mobility in soil on average (4). Other organophosphate pesticides include Malathion, Phorate, Bromophos and Methyl parathion. Organophosphate insecticides are esters of organic salts of phosphoric acid and its derivatives (7). These are considered as highly toxic chemicals for humans and animals and have a high sustainability in the environment (6). Pesticides inhibit the acetylcholinesterase enzyme (AchE) that results in breaking of acetylcholine (8) neurotransmitter in insects. Without the enzyme working, ACH is made in synaptic connections which results in inconsistency, seizure and finally the death of insects (9). About 1 trillion dollars is needed for cleaning the places that have been contaminated by toxic wastes in farms. This is done by traditional

methods of waste disposal like incineration and filling the land. Due to the severity of this problem and the lack of a reasonable solution, a quick and cost effective way for cleaning the environment is required (8). Biodegradation is considered as the main and most effective way for destroying pesticides in the environment. Biodegradation of pesticides is a comprehensive environmental process that is possible in different habitats including soil, sediments, surface and underground waters, wastewater sludge and similar things. All pesticides are potentially sensitive to one or more change in the life form. In this study, Diazinon degradation by *Pseudomonas aeruginosa* and *Flavobacterium* bacteria were evaluated.

2. MATERIALS AND METHODS

2.1. The used pesticide

The required Diazinon pesticide was produced with 95 percent purity level by Chemical Products made in Iran with registration number 127 and was used.

2.2. Sample collection and determining the level of Diazinon in the samples

During two consecutive seasons of the year in Fall and Winter of 2013, samples were collected from the soils of three agricultural soil [Farm Corn, wheat, tomatoes] stations in Marvdasht city in Iran. In each season, sample collection was done three times from the soil of each station. For microbial culture, the samples were collected in sterilized glass containers and for measuring the level of Diazinon pesticide, they were collected in polyethylene container. Sample collection was done from the depth of three to four centimeters from the surface as oxygen exists in this part and after this depth the conditions becomes anaerobic. All the experiments were done by standard methods of 9030,9060A-3010A, B and with three times repetition (10). The level of Diazinon in the soil samples was determined by GC electron ionization detector device (Hewlett-Packard, HP1800A, Palo Alto, CA, USA). 1 nX volume was used for injection. The GC column used had HP-5MS cross bonds dependent on 5% PH ME siloxane and thickness of 30 m × 0.25 mm × 0.25. The rate of Helium gas flow was 0.7 ml/min. The delay of the solvent is about 3.5 minutes. In order to avoid overloading, electron ionization detector was used. Input and temperature detector were about 230 and 300° C respectively. The temperature program: from 60 to 185 in °C/min (11) increase to 209° C in 3 °C/min and (2) increase to 300 in °C/min for improving the chemical separation was used.

2.3. Counting the number of the bacteria

Surface sediment samples from 3 stations, in two

consecutive seasons such as autumn and winter in 2013, has been accomplished. In each season, three samples were taken from each station. Specimens for microbial culture in sterile glass containers and to measure Diazinon were collected in polyethylene containers. sediment sampling from depth of three to four cm has been accomplished because the oxygen carried out in the part of sediment and then there was anaerobic conditions 9030,9060 A-3010 A₂B was performed according to standard methods and all experiment according to standard methods were repeated for three times.

2.4. The method of isolating the Diazinon degrading bacteria

Viable plate count method was used to count bacteria. Beginning of each sediment sample, 10⁻¹ to 10⁻¹⁰ dilutions were prepared. In this experiment, for each sample, ten tubes containing 9 ml normal saline were prepared and then 1 gr sediment was poured in number 1 tube and mixed.

2.5. Identifying Diazinon degrading bacteria

The isolated bacteria were identified using common biochemical tests based on Bergey's Manual Systematic Bacteriology book and by warm coloring, investigation of morphological characteristics, catalase and oxidase tests, KOH and other differential tests.

2.6. Determining the growth of the isolated bacteria in different concentrations of Diazinon by studying their optical absorption

Continuous recording OD600 is used to determine the kinetics of bacterial growth. After the initial record of OD, medium cultures in 35 c were incubated for 12 hours and every hour, OD600 read and recorded. The last record OD600 was related 12 hour after inoculation with bacteria. In the third case, after 5 hours, when the bacteria reached the logarithmic phase, the metal added and every hour OD600 read.

2.7. The analysis of the statistical data

Excel software was used for drawing the charts and SPSS software was used for data analysis. Also, ANOVA and DUNCAN statistical tests were used.

3. RESULTS AND DISCUSSION

3.1. Sampling location

In this study, soil samples were taken from around Shiraz Petrochemical. Longitude, latitude and altitude of sampling Place are respectively equivalent [29°18'22.98" N 51°30'12.35"E] ... [Figure 1](#) showing the Sampling location.



Figure 1. Sampling location

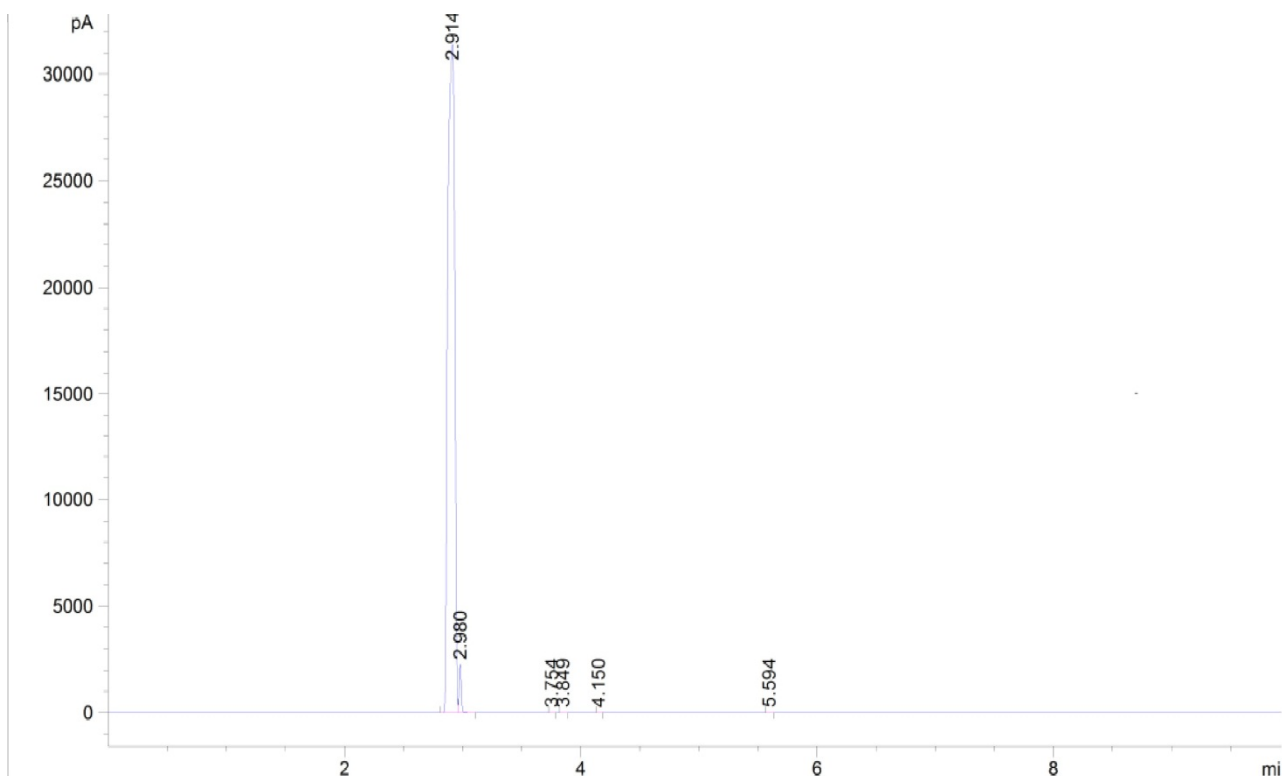


Figure 2. A measure of the different stations in the Diazinon gas chromatography

3.2. Counting of the bacteria and the level of diazinon the samples

The logarithmic mean of the number of the bacteria in the media containing Diazinon was 20×10^{-2} in comparison with control culture media 20×10^{-4} . In addition, there was a significant difference at 5% level between logarithmic value of the number of the bacteria in the media containing the substance and the media without it. The highest number of the bacteria was in station 2 with the 50×10^{-6} and the lowest number of the bacteria was in the station 3 with 40×10^{-4} . The amounts of Diazinon in the soil of different stations were not significantly different. In

addition, the highest amount of contamination was observed in the soil sample of station 1 with 660 (ppm) and the lowest amount of contamination was observed in the soil sample of station 2 with 455 (ppm) (Figure 2).

3.3. Isolation and identification and investigation of Diazinon degrading bacteria growth kinetics

Based on microbiological and biochemical texts conducted in this study, 2 resistant bacteria among the other strains of *Pseudomonas aeruginosa* and *Flavobacterium* were identified. These two separated bacteria, *Pseudomonas aeruginosa* and *Flavobacterium*, were the most powerful

strains that began growing in the lowest possible time. Considering chart 1, it is observed that the growth of *Flavobacterium* in 0.2g/l Diazinon concentration reaches its peak at hour 120 and then it has increasing trend till hour 216. *Flavobacterium* in 0.6g/l Diazinon concentration has mildly grown at hour 96 and then its decreasing trend has reached near zero till hour 216. In 0.8g/l Diazinon concentration, the growth trend of *Flavobacterium* has been quick till hour 48 and then the growth has decreased. In 1g/l Diazinon concentration, the growth trend of

Flavobacterium has been quick till hour 48 and then the growth has decreased and has reached zero. In addition, the slope of *Flavobacterium* has been lower. From these two charts (Figure 3 & Figure 4), it can be concluded that the growth trend in bacteria in different concentrations of Diazinon has been increasing initially and then at the end, the trend has been decreasing.

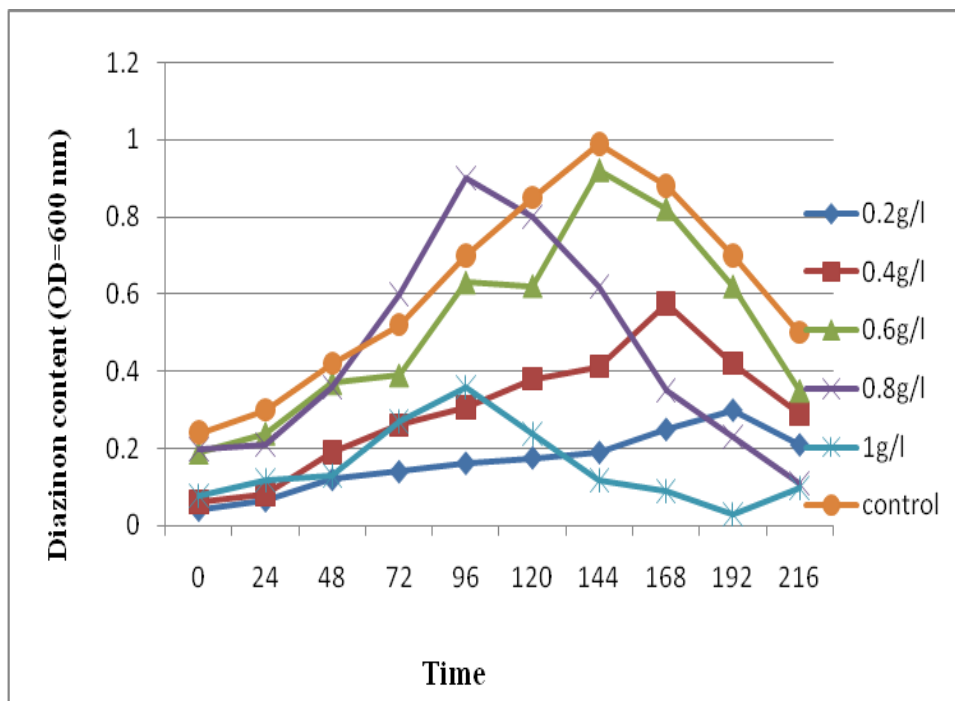


Figure 3. The curve of the *Flavobacterium* growth in different Diazinon concentrations

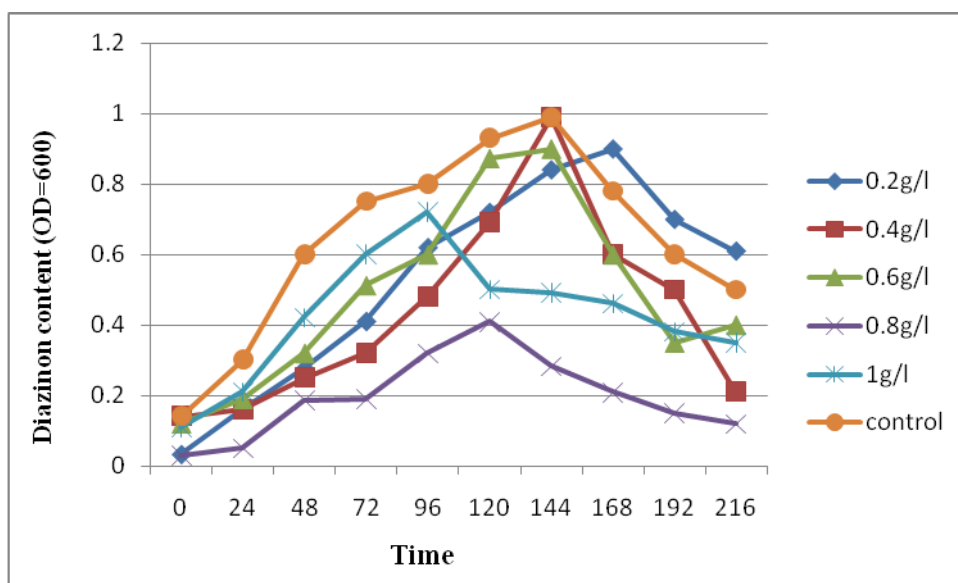


Figure 4. The curve of the *Pseudomonas aeruginosa* growth in different Diazinon concentrations

Diazinon was decomposed by *psedumonanas aeruginosa* and *flavobacterium*. the results of the experiment showed that the ability of the *psedumonanas aeruginosa* to decompose the diazinon was more than *flavobacterium*

(12). The results showed *psedumonanas aeruginosa* 45% and *flavobacterium* 36.2% of diazinon decomposition approximately in the 72% range. Other researchers also on the ability *psedumonanas aeruginosa* and *flavobacterium*

to analysis diazinon reported. There are limited reports for organophosphorus compounds as a source of carbon and phosphorus for bacteria but bacteria isolated in an experiment showed that ability use Diazinon as source of carbon and phosphorus. *Pseudomonas* are a heterogeneous group and a large number of them live in the soil (13). These genera of bacteria are major catabolic pathways. A superior strain isolated in this study was *Pseudomonas* (14, 15).

4. CONCLUSION

The results showed that these bacteria have organophosphorus acid anhydrolase enzyme that can be degraded dangerous compounds like diazinon. The results showed that these bacteria can be used for removing the pollution regarding diazinon.

ACKNOWLEDGMENT

No mentioned any acknowledgment by authors.

AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

REFERENCES

1. Mallick K, Bharati K, Banerji A, Shakil NA, Sethunathan N. Bacterial degradation of chlorpyrifos in pure cultures and in soil. Bulletin of environmental contamination and toxicology. 1999 Jan;62(1):48-54. PubMed PMID: 9870989. Epub 1998/12/31. eng.
2. Albanis T, Hela D, Sakellariades T, Konstantinou I. Monitoring of pesticide residues and their metabolites in surface and underground waters of Imathia (N. Greece) by means of solid-phase extraction disks and gas

- chromatography. Journal of Chromatography A. 1998;823(1):59-71.
3. Bailey HC, Deanovic L, Reyes E, Kimball T, Larson K, Cortright K, et al. Diazinon and chlorpyrifos in urban waterways in northern California, USA. Environmental Toxicology and Chemistry. 2000;19(1):82-7.
4. Howard PH. Handbook of Environmental Fate and Exposure Data: For Organic Chemicals, Volume III Pesticides: CRC press; 1991.
5. Montgomery JH. Groundwater chemicals desk reference: CRC Press; 2007.
6. Chapalamadugu S, Chaudhry GR. Microbiological and biotechnological aspects of metabolism of carbamates and organophosphates. Critical reviews in biotechnology. 1992;12(5-6):357-89.
7. Hassall KA. Biochemistry and uses of pesticides: Macmillan Press Ltd; 1990.
8. Reddy CA, Mathew Z, editors. Bioremediation potential of white rot fungi. BRITISH MYCOLOGICAL SOCIETY SYMPOSIUM SERIES; 2001.
9. Amato JR, Lukasewycz MT, Robert ED, Mount DI, Durhan EJ, Ankley GT. An example of the identification of diazinon as a primary toxicant in an effluent. Environmental Toxicology and Chemistry. 1992;11(2):209-16.
10. Bhagobaty RK, Joshi SR, Malik A. Microbial degradation of organophosphorus pesticide: chlorpyrifos (mini-review). The Internet Journal of Microbiology. 2007;4(1).
11. Smith-Greeier LL, Adkins A. Isolation and characterization of soil microorganisms capable of utilizing the herbicide diclofop-methyl as a sole source of carbon and energy. Canadian journal of microbiology. 1996 Mar;42(3):221-6. PubMed PMID: 8868228. Epub 1996/03/01. eng.
12. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: Cold spring harbor laboratory press New York; 1989.
13. Singh BK, Walker A, Morgan JAW, Wright DJ. Biodegradation of chlorpyrifos by *Enterobacter* strain B-14 and its use in bioremediation of contaminated soils. Applied and environmental microbiology. 2004;70(8):4855-63.
14. Chaudhry GR, Ali AN, Wheeler WB. Isolation of a methyl parathion-degrading *Pseudomonas* sp. that possesses DNA homologous to the opd gene from a *Flavobacterium* sp. Appl Environ Microbiol. 1988 Feb;54(2):288-93. PubMed PMID: 3355128. Pubmed Central PMCID: 202445. Epub 1988/02/01. eng.
15. Lee KS, Metcalf WW, Wanner BL. Evidence for two phosphonate degradative pathways in *Enterobacter aerogenes*. Journal of bacteriology. 1992 Apr;174(8):2501-10. PubMed PMID: 1556070. Pubmed Central PMCID: 205888. Epub 1992/04/01. eng.