International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-8, Issue-3, 2017, pp1684-1688
http://www.bipublication.com

Research Article

Biodegradation of diazinon using gas chromatography method

Morteza Khani[1*], Anis Radbeh[2], Zinat Mohammadi[3], Mehdi Zarei[4] and Mohammad reza Gholami[4]

[1*]Young Researchers and Elite Club, Shiraz Branch, Islamic Azad university, Shiraz, Iran

[2]Department of Biology, Shiraz Branch, Islamic Azad University, Shiraz, Fars, Iran

[3]PhD Student, plant physiology, Islamic Azad University, Eslamshahr, Eslamshahr, Iran

[4]Young Researchers and Elite Club, Marvdasht Branch,Islamic Azad University, Marvdasht, Iran

Corresponding Author:Email:mortezakhani1365@gmail.com Tell: 09369894615

ABSTRACT

Background: for many years, human have been used physical and chemical methods to eliminate pesticides such as Diazinon. However, due to its time-consuming and non-cost-effectiveness nature, nowadays, microorganisms, particularly bacteria are prioritized to remove this hazardous compound.

Materials and Methods: In the present research during two consecutive seasons of the year (fall and winter 2014) soil samples were taken in three stations from the river Kur - Marvdasht-Iran. According to standard protocol, having been extracted, soil samples were subjected to gas chromatography to measure the amount of diazinon. In order to enrich and separation of bacteria resistant to cadmium enrichment medium, MSM culture and solid nutrient agar plate were used respectively. Conventional biochemical tests were used to identify bacteria.

Results: using morphology and biochemical tests some bacteria such as Pseudomonas aeruginosa, Enterobacter, Bacillus were isolated and identified so that Pseudomonas strains accounted for the highest degradation, which is the indicators related to the upon concentration 0.2 gram per liter in 72 hours it degraded 32% diazinon followed by bacteria Pseudomonas aeruginosa (45%), Enterobacter (38%), and Bacillus (25%).

Conclusion: Isolated bacteria, especially Pseudomonas aeruginosa and Enterobacter serve as suitable candidate to eliminate diazinon in contaminated water. The bacteria growth in different concentrations of diazinon initially was increased, and in the end was reduced.

Key words: Pseudomonas aeruginosa, diazinon, gas chromatography, biodegradation.

INTRODUCTION

As a phosphorus compound, Diazinon is applied both in soil and on plant aerial organs. Diazinon is a broad spectrum insecticide and is widely applied against household and garden pests, insects, ornamental plants, pets and even mosquitoes and flies in stables. In order to eliminate pests of fruit trees such as mites, aphids, *tomato hornworm* moth, apples maggot, pears worms, cherry flies and pear psylla about 1 to 5.1 kg 40% diazinon suspended powder per thousand liters water is applied. This solution is used for spraying parks as well. Diazinon is perceived as a phosphorous compound with heterocyclic leaving group. This insecticide is characterized with high neutral pH stability, but at alkaline and acid pH is hydrolyzed. Under acidic pH, hydrolysis rate is

much more compared to alkaline one. The half-life (DT50) of diazinon is found to be 3.1,12 hours. Diazinon is hydrolyzed in mammals body, to form diethyl dithiophosphoric acid . Upon GSH, diazinon is converted to conjugate glutathione. This reaction is takes place in the housefly. At the same time, by removing ethyl agent, glutathione detoxify diazinon. The same reaction is occurred in the housefly too. On the other hand, under monooxygenases system diazinon turns into Diazoxon as one of the strongest inhibitors of cholinesterase and cholinesterase I50 (half-life) in fly is 3.5×10^{-9} moles. Diazoxon is hydrolyzed by esterase type A to release its pyrimidine derivative. Insecticides can enter water bodies and environment directly or indirectly (Lu et al., 1974) and directly penetrate to water and environment in direct way to control vermin insects. Insecticides are used for two purposes: to stop the destruction of agricultural production especially in rice fields and to control root. Widespread use of insecticides to control insects causes waster and environment contamination. For many years, human have been used physical and chemical methods to eliminate pesticides such as Diazinon. However, due to its time-consuming and non-cost-effectiveness nature, nowadays, microorganisms, particularly bacteria are prioritized to remove this hazardous compound. Enormous bacteria have been isolated for diazinon biodegradation, most of which are gram-negative belonged to Pseudomonas family. Both gram-negative and gram-positive family of bacteria has been reported as diazinon decomposers i.e. Agrobactriuom.sp (Jumesleveglia et al. 1977), Felavobactriuom (Chaudhry et al., 1988), genus Bacillus subtilis (Yasuno et al. 1965) Streptomyces (Gauger et al., 1986), actinomycetes (Collins et al. 1998) and yeasts (Digrak in 1996 and Collins 1989). Foregoing bacteria exploits diazinon as carbon and energy source.

Experimental

Sampling was carried out in sterile containers during autumn and winter. For each sampling, number 9 samples were taken from the waters of the river Kur (for each station, three sampling times). Immediately, in less than 2 hours samples in flask containing ice were sent to laboratory.

Figure 1: sampling points

Enrichment and isolation of diazinon biodegrading bacteria in water and sediment samples

First, a mixed solution of diazinon was prepared in acetone and stored in biological hood to evaporate acetone (15 mm water sample.)(Xu et al.,2008) and was added to broth and 11 g sediment samples were put separately in Erlenmeyer flasks containing 251 ml, 51 ml broth mineral salt and 25 mg diazinon and incubated for 24 hours in shaking incubator. Sediment samples diluted in 151 rpm at 27 ° C and, for any dilution, 1 ml samples from each flask was inoculated to tubes containing 9 mm broth. Each dilution was characterized with 5 replications. Then tubes were kept for a week in incubator at 37 C. one tube containing 9 ml broth and diazinon in 151 rpm but in absence of bacteria were considered as control. The emergence of turbidity is a sign for the growth of bacteria. Tubes showing turbidity were cultured in mineral salt medium contain agar with 25 mg diazinon and incubated for 7 days in 31C.

Analysis of biodegradation metabolites using GC:

To monitor diazinon biodegradation by isolated bacteria, gas chromatography (GC) was used. In this method, bacterial suspension was added to a

flask containing salt medium with the desired substrate according to McFarland standard and was kept for one week in incubator at 30 C. A medium containing diazinon without microbial inoculums was prepared as a control. The culture is removed after 72 hours and sieved through milipowder filter. The resulting liquid is used to GC test. the sample is taken to GC equipped with column characterized with 30×0.25×32 µm, absorbent pheny dimethyl siloxane 5%, injector temperature 25 c and MASS type detector in which column temperature was began in 80 C and after 4 minutes was reached to a 260 C in 20 C/min gradient.

Results of experiment

Stations I, I, III were located at Kamfiruz , Markazi and Seyedan districts.

Table 4- 1 bacteria counting

winter			autumn			season
II	II	I	III	II	I	station
135*10-3	95*10-3	55*10-3	270*10-3	100*10-4	55*10-3	diazinon
195*10-3	125*10-3	90*10-3	300*10-3	250*10-3	86*10-3	control

Identification of diazinon degrading bacteria

Diazinon degrading bacteria were isolated in river Kur-Iran and the bacteria include Pseudomonas aeruginosa, Enterobacter, Bacillus.Analysis of biodegradation metabolites by GC:

The diazinon standard value

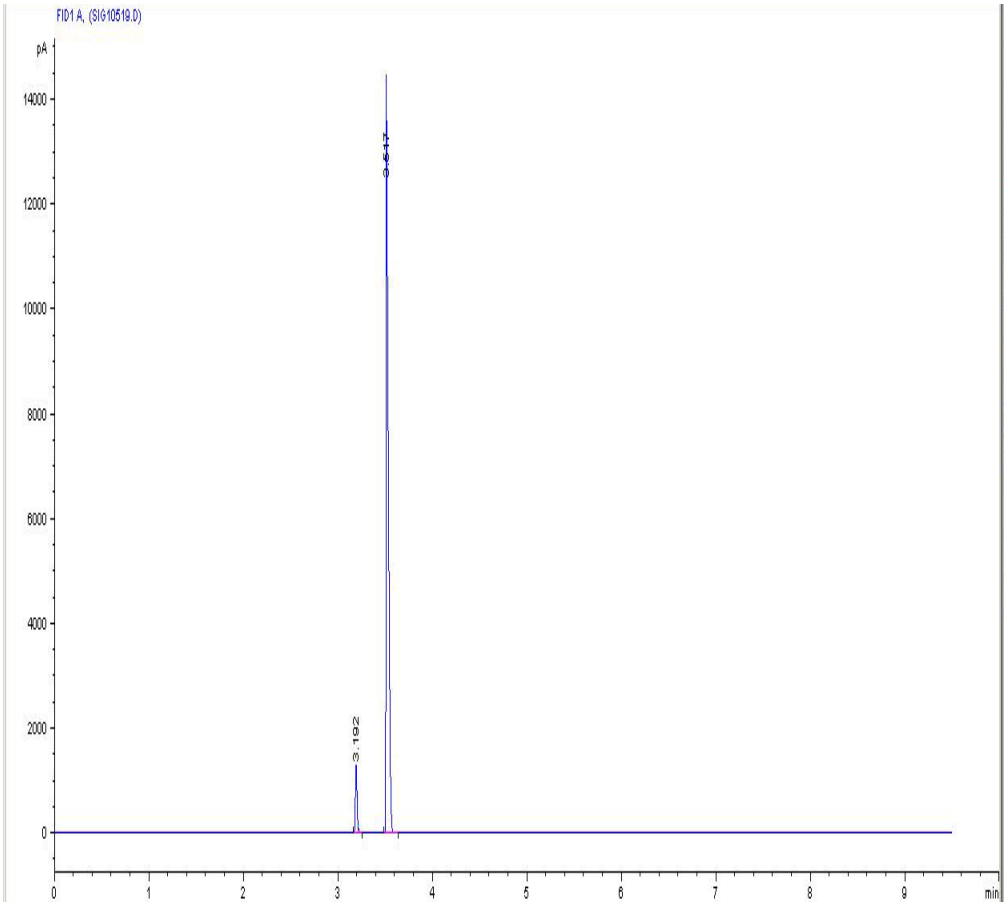

Figure 2: dizinon standard

Percent of Diazinon decomposition at a concentration of 0.2 g l by the bacteria Pseudomonas aeruginosa

Percent of Diazinon decomposition at by the bacteria Pseudomonas aeruginosa after 72 hours was 45%.

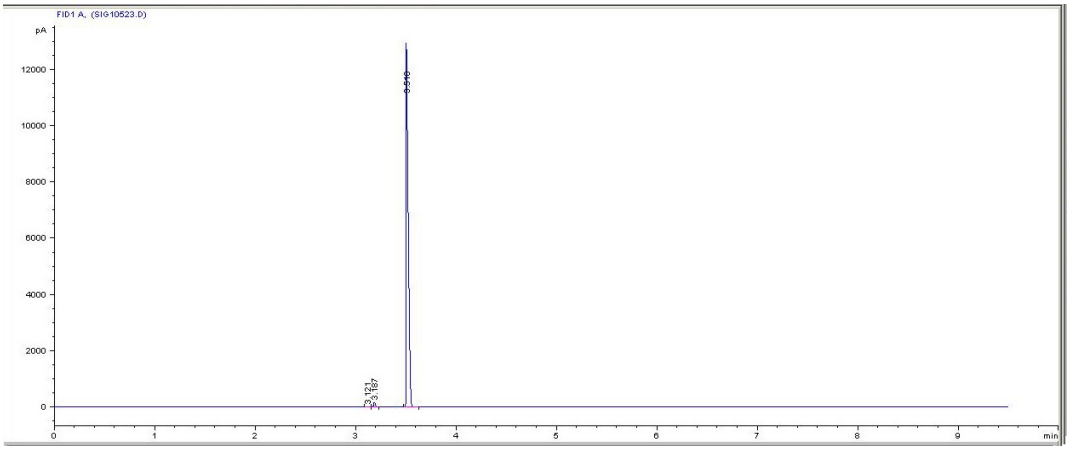

Figure 3: Diazinon degredation at a concentration of 0.2 g l by the bacteria Pseudomonas aeruginosa

Diazinon degredation at a concentration of 0.2 g l by the Enterobacter

Percent of Diazinon decomposition at by the bacteria Enterobacter after 72 hours was 38%.

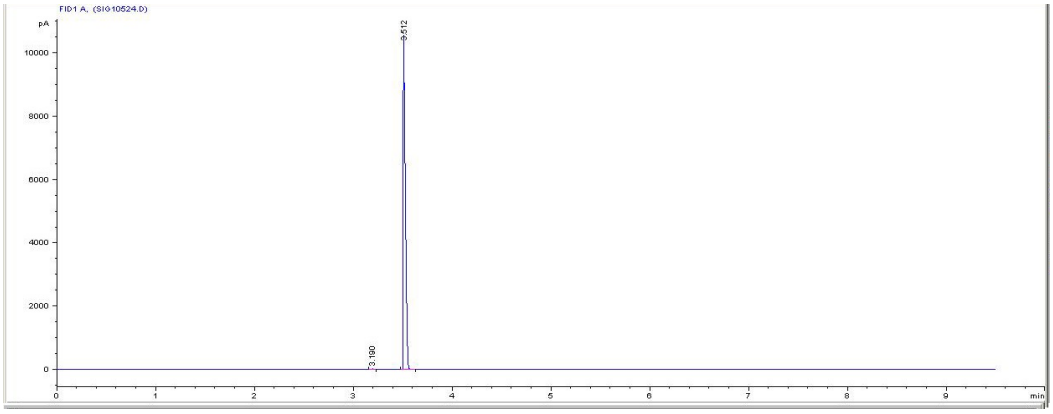

Figure 4: Diazinon degredation at a Concentration of 0.2 g l by the bacteria Enterobacter

7-4-4 percent of Diazinon degredation at a concentration of 0.2 g l by the Bacillus

Percent of Diazinon decomposition at by the bacteria Enterobacter after 72 hours was 25%.

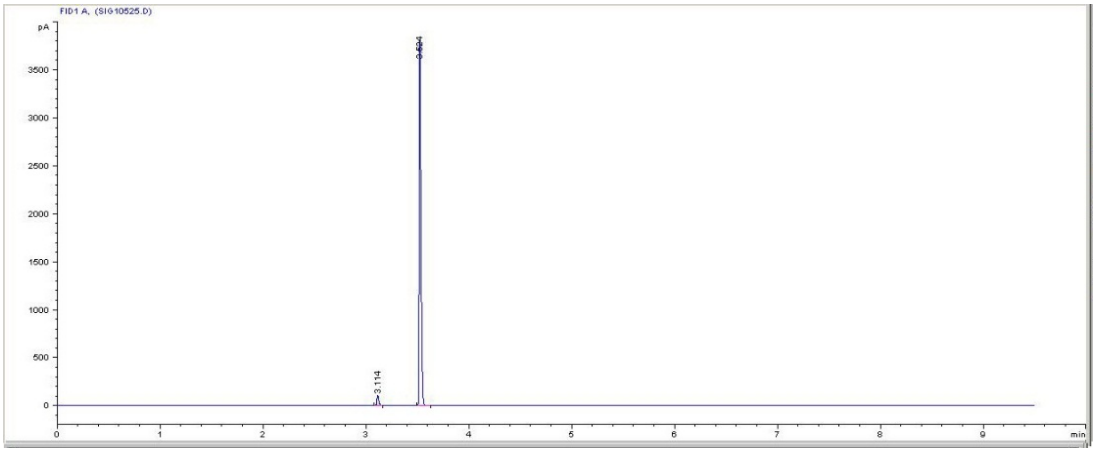

Figure 5: Diazinon degredation at a concentration of 0.2 g l by the bacteria Bacillus

CONCLUSION:

Digrak et al. 1995 proved that some organophosphate insecticides such as

Diazinon, chlorpyrifos, cationic, malathion and gusathion are susceptible to microbial degradation in Pseudomonas sp, Arthrobactersp, Flavobacteriumsp as monoculture and mixed for carbon. In the present study Pseudomonas and flavobacterium were identified as diazinon biodegrading agent and used it as carbon source. By enrichment method in 1999, Ramanathan and LalithakumariisolatedPseudomonas sp soil strains able to degrade Parathion methyl, malathion, and was Foso-diazinon. In the present study Pseudomonas was isolated. Ghassempouret a1.,2002 isolated diazinon degrading bacteria Agrobacterium sp and Flavobacteriumsp, Pseudomonas sp in soil and surface water in rice field of Gilan which used diazinon as carbon source. in this research pseudomonas was identified and isolated.

Concluding remarks

In the light of results, any Gram-negative bacteria have potential to biodegrade pesticides diazinon and analysis of strains isolated during a week, the ability of bacteria to pesticides was evaluated. Pseudomonas strains accounted for the highest degradation, which is the indicators related to the upon concentration 0.2 gram per liter in 72 hours it degraded 32% diazinon followed by bacteria Pseudomonas aeruginosa (45%), Enterobacter (38%), and Bacillus (25%).

REFERENCES:

1. Habib Abbasi, Mir Manochehr Hamedi, Tayebe Bagheri Lotfabad, et al., (2011) Biosurfactant-producing bacterium, Pseudomonas aeruginosa MA01 isolated from spoiled apples: Physicochemical and structural characteristics of isolated biosurfactant, Journal of Bioscience and Bioengineering
2. Hashemy-Tonkabony, S. E. and B. Mosstofian. 1979. Chlorinated pesticide residues in chicken egg. Poul. Sci.58:1432-1434
3. Hammoch, B. D. 1985. Regulation of juvenile hormone titer:degradation, in Comprehensive insect phsiology, biochemistry and pharmacology. Edited by Kerkut, G. A. and Gilbert, L. I. Pergamon press. Vol.7:431-472
4. Hodgson, E. and R.J. Kuhr. 1990. Safer insecticides, Marcel Dekker.
5. Hodgson, E. and R. J. Kuhr. 1990. Insecticides, Marcel Dekker
6. Irence Horne, Tara D. Sutherland, John G. Oakeshot, Robyny. Russell.2002. Cloning and expression of the phosphortriesterase gene hocA from Pseudomonas monteilii C11.148:2687-2695
7. John E. Casida and Gary B, Organophosphate Toxicology:Safety Aspects of Nonacetylcholinesterase Secondary Targets Chem. Res Toxicol 2004;17:983-992
8. Jung, F., Gee, S. j., Harrison, R., Goodrow, M., Karu, A., Braun, A.,Xiao Li, Q. and B. Hammock. 1989. Use of immunochemical techniques for the analysis for pesticides. Pestic. Sci.26:303-317
9. Katz, E. D. 1996. High performance liquid chromatography: principles and methods in biotechnology. John Wiley & Sons.
10. Maron, D. M and B. N. Ames. 1983. Revised methods for the Salmonella mutagenicity test, Mutation Research,113:173-213
11. Metin Digrak, Ferdag Kazanici.1999.Effect of some organophosphorus Insecticideson soil micro organism.25:51-58
12. Muzket, L. and N. Aharanson, 1986. High-performance liquid chromatography; In:Analysis methods for pesticides and plant growth regulators. Edited Zwieg, G. and Sherma A.P.AP.Vol.14;95-131
13. Pedigo, L. P. 1999. Entomology and pest management 3rd ed.Prentice Hall
14. Parfitt, C. H. 2000. Pesticide and Industrial chemical residues. In: Official methods of analysis of AOAK international. Vol. 1 edited by Horwitz, W. AOAK International.
15. Rechcigl, j. E and N.A. Rechcigl.2000. Insect pest management. Lewis Publishers
16. Sethunathan, N., Caballa, S. and M.D. Pathak. 1971. Absorption and translocation of diazinon by rice plants from submerged soils and paddy water and the persistence of residues in plant tissues. L. Econ. Entomol. 64 (3):571-6
17. Ravehl, segally, ieaderH, Rothschildn. Levanon D, Henisn , Ashani Y. Protection aganistabun toxicity in mice by prophylaxis with an enzyme hydrolyzing organophosphate esterase. Biochem Pharm 44:397-400
18. Supelco Chromatoghraphy Products, 1988. Solid phase extraction, Supleco, pp.335-344
19. Swannel R.J.P., Lee K, and Medonagh M.1996, Field evraluation of marine oil spill Bioremediation Microbiological Reviews.60:342-36