World Journal of Fish and Marine Sciences 2 (3): 240-245, 2010
ISSN 2078-4589

Isolation and Identification of Diazinon Degrading Bacteria from Fresh Water: a Case Study on the Sediments of Lake Parishan in Iran

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Abstract: Diazinon is an organophosphate insecticide which is widely used in various industries. It is known as an important causative of water pollution which eventually redound death of aquatic animals. Today microorganisms are considered as a best choice to reduce environmental pollution. This study was done to identify diazinon degrading bacteria from sediments of Lake Parishan (IRAN) and to evaluate their degrading rate as well. Sampling was done from the surface sediments of Lake Parishan and its surrounding farms during summer, autumn and winter, then incubated in liquid salt medium containing diazinon for 20 days at 35°C. After growing, bacteria were transferred to solid saline medium. They were identified using biochemical tests; thereafter in order to evaluate their ability in degradation of diazinon, diagnostic tests were performed. Results showed that bacteria *Pseudomonas*, *staphylococcus*, *Bacillus*, *Corynebacterium*, *Acinetobacter*, *Alcaligenes*, *Serattia*, *Salmonella*, *Citrobacter* and *Providencia* are able to degrade diazinon. Among them *Pseudomonas* was the strongest in summer and winter, while *Citrobacter* was the most preferable bacterium in fall. It was also detected that combined population of Gram positive and Gram negative bacteria together are able to degrade diazinon faster. Therefore, the present study confirmed the application of bacteria for reducing diazinon pollution in waters considering their ease and economical application.

Key word: Diazinon · Degrading bacteria · Lake parishan · Bioremediation

INTRODUCTION

Water is the most abundant compound substrate on the surface of earth. More than 70 percent of the earth is covered by water and only two percent of it, consists of fresh water. Likewise, more than 90 percent of fresh water is located at the two poles in frozen form. In addition to this limitation of fresh water resources; nowadays; as a result of industrial development and increasing population, waters are increasingly contaminated. Basically, water is considered contaminated when its quality and composition, directly or indirectly has changed by human activities so that it is not anymore appropriate for drinking, agriculture, fishery and other purposes. Today, health officials have many concerns in connection with chemicals such as pesticides which are found in the waters [1] and enter to human body by the seafood consumption [2]. Insecticides are chemical which have been used several years ago to fight vermin. Synthetic insecticides are also used for controlling plant diseases and insects. However, today it is specified that insecticides cause destruction and problems in the ecosystem [3]. They can also cause physiological disorders in living organisms including humans. In 1995, Sherman [4] reported that phosphoric organic insecticides can cause neurological disorders. According to Zhang [5] these materials can enter the human food chain and cause cancer. FAO / WHO in 1993[6] announced that any study of pesticides in soil, water and nutrients are very important and valuable.

One of the most famous pesticides is Diazinon (O, O-diethyl O-(2-isopropyl-6-methyl-4-primidinyl) phosphorothioate) with chemical formula C12-H21-N2-O3-RS and molecular weight of 304.35 gr / mol. It is in oil liquid form and colorless and its specific gravity is 1.116 to 1.118 at 20°C. Diazinon is non-systemic

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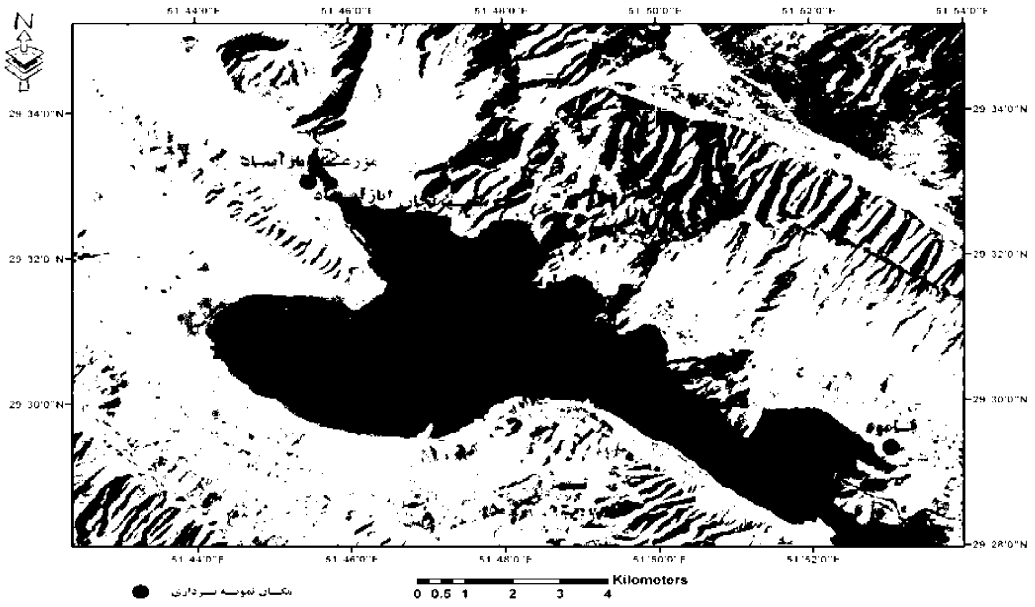

Fig. 1: Satellite map of areas of sampling

insecticide which has drawn little mite properties and is used in agriculture as pesticide of rice, fruits, sugarcane and ornamental plants, etc. [3]. In nature, insecticides are degraded by microorganisms in the soil as a physical, chemical and photochemical degrading [7 - 9]. Some studies reported that *Pseudomonas* genus [10] *E. coli* [11] and a number of soil microorganisms are capable of degrading diazinon and use it as a sole source of carbon and phosphorus [12].

One of the most exceptional and most beautiful lakes of fresh water in Iran is the Lake Parishan which is known as Moore, Parishan, Kazeroun and yon, too. This lake is located between 51°, 44‘ and 29°, 32‘ N latitude. Free water elevation is 820 meters (Figure 1). The lake basin is 266.5 square kilometers and lake water is supplied from annual rain. This study has been done to isolate and identify diazinon degrading bacteria from sediments of Lake Parishan in the three seasons of the year and evaluate their bioremediation as well.

MATERIALS AND METHODS

Sampling: Samples were collected with completely sterile containers during three season of summer, autumn and winter. Each time of sampling from each station (Ayaz Abad, Shahrenjan, Famur, Lake Parishan, Ayazabad field) three samples were collected with a total of 45 samples. Samples were kept in flask containing ice and transferred to the laboratory less than 12 hours.

Culture Media Used: Mineral Salt Medium (MSM), Mineral Salt Solution (MSS), Blood Agar, Agar-Agar, LB Medium and Mueller Hilton agar all from Merck Co. Germany were used as a media for bacteria culturing in this study.

Isolation of Bacteria in Soil and Sediment Samples: The isolation of bacteria was done as following: 10 g of each sample was added to 100 ml of medium mineral salt solution (MSS) that one liter of that is consisted of 2 g, KNO3, 0/2 g MgSO4, 7H2O, 0/1 g CaCl2, 2H2O, 0/1 g NaCl, 0/01 g Fecl3 6H2O, 10 mg Na2MoO4, 2H2O, 5 mg SnCl2, 2H2O, 10 mg H3BO3, 20 mg KBr, 5 mg BaCl2 and 8 mg EDTA-NA-Fe^{3+}. After adjusting pH of medium to 7.2 with sodium hydroxide, 25 ml insecticide diazinon was added to it. Erlenmeyer flask containing both medium and samples were shaked for 20 days at 35°C. Some of the microorganisms that were able to utilize diazinon as a sole source of phosphor and carbon grew. Samples were taken from Erlenmeyer flask and cultured on sterile solid mineral salt medium (Mineral Salt Medium) and incubated for 7 days at temperature 35°C [7]. Then the bacteria were cultured on Blood Agar medium and were kept for 24 hours at temperature 35°C to identify.

Identification of Isolated Bacteria: The standard biochemical tests of Gram staining, growth in aerobic / anaerobic conditions, movement and numerous tests of oxidase, catalase, KOH, TSI, SIM, Simon citrate, LD / OD,

urease, oxidation and fermentation along with microscopic morphology and colony shape were used to identify isolated bacteria.

Assess of Ability of Diazinon Elimination and Growth of Degrading Bacteria: For each bacterium 7 tubes containing 3 ml MSS medium was considered and the amounts of 0.7, 0.9, 1.1, 1.3, 1.5, 1.7, 1.9 ml diazinon were added to tubes. Then 25 ml of each bacterium which have already incubated for 24 hours in LB medium were cultured into separate tubes. Tubes were incubated for 20 days on shaker with regular aeration in temperature 35°C. Degradation rate of diazinon were assessed in 5 intervals of days 5,10,15,20.

Determination of the Best Combination of Bacteria Degrading Diazinon: Composites of different types of isolated bacteria such as Gram positive bacteria, Gram negative bacteria and combination of Gram positive and Gram negative bacteria together were examined. As such, to 100 ml of medium MSS containing 25 ml diazinon, 100 ml of combinations of different bacteria were inoculated and incubated on shaker for 7 days at temperature 35°C. In comparison with control samples without bacteria, the best combinations of degrading diazinon bacteria were determined.

Statistical Analysis of Number of Bacteria in Different Seasons and Stations: Analysis of variance (ANOVA) was used to analyze number of bacteria during different seasons and stations and compare their ability in degrading of diazinon.

RESULTS

Results of identification tests indicated that during summer, bacteria *Psuedomonas* (as the most powerful diazinon degrading bacteria), *Acinetobacter*, *Corynebacterium*, *Staphilococcus*, *Bacillus* and *Alcaligenes* involved in degrading of diazinon, while during autumn, bacteria *Citrobacter* (as the most powerful bacteria degrading diazinon) *Serattia*, *Salmonella*, *Bacillus* and *Providencia* were isolated. During winter, however, the isolates consisted of bacteria *Psuedomonas* (as the most powerful bacteria degrading diazinon), *Salmonella*, *Staphilococcus*, *Acinetobacter* were isolated. Figure 2 shows the level of diazinon degradation by bacteria after 20 days. As can be seen, the bacteria *Pseudomonas* and *citrobacter* degraded the concentration of 1.5 ml of diazinon.

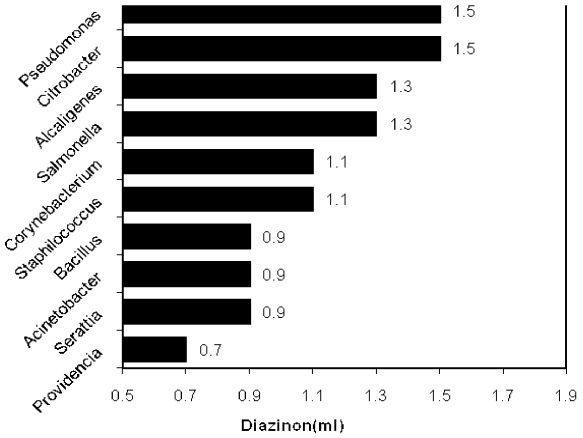

Fig. 2: The growth of bacteria in the presence of diazinon after 20 days

They degraded of 0.9, 1.1 and 1.3 ml of diazinon after 5, 10, 15 days, respectively. They, however, showed identical growth pattern. *Alcaligenes* and *salmonella* bacteria could degraded 1.3 ml of diazinon. They degraded 0.7, 0.9, 1.1 and 1.3 ml of the insecticide diazinon after 5, 10, 15 and 20 day, respectively. They showed the identical growth pattern too. *Staphylococcus* and *Corynebacterium* degraded the concentration of 0.7, 0.9 and 1.1 of the insecticide diazinon on days 10, 15, 20, respectively and showed the same growth pattern. Bacteria *Bacillus*, *Acinetobacter*, *Serattia* degraded the concentration of 0.7 and 0.9 ml of diazinon after 15 and 20 days, respectively and they showed same growth pattern. *Providencia* was the weakest bacteria in term of degrading of diazinon whit??? just 0.7 ml of diazinon after 20 days. The best combinations of bacteria which degraded and grew on insecticide diazinon were Gram-positive bacteria and Gram-negative composition after 10 days, Gram-negative bacteria composition after 12 days and Gram-positive bacteria composition during the 15 days respectively. Statistical analyses showed that mean of bacteria significantly differ among seasonal, while it is not significant in the stations. Figure 3 shows that the mean number of bacteria are significantly different (P <o.o???) in seasons so that are most in summer, followed by winter, autumn, respectively. According to Figure 4, if the numbers 0, 1, 2, 3, 4 represent stations of: Ayazabad, Ayazabad field, Shahrenjan, Famur and Lake Parishan, respectively, there is no significant difference in the mean number of bacteria in different stations, however Ayaz Abad farm had the highest mean number of bacteria. Figure 5 shows that the average number of bacteria, except in Ayaz Abad field, in all stations was highest during summer and lowest during

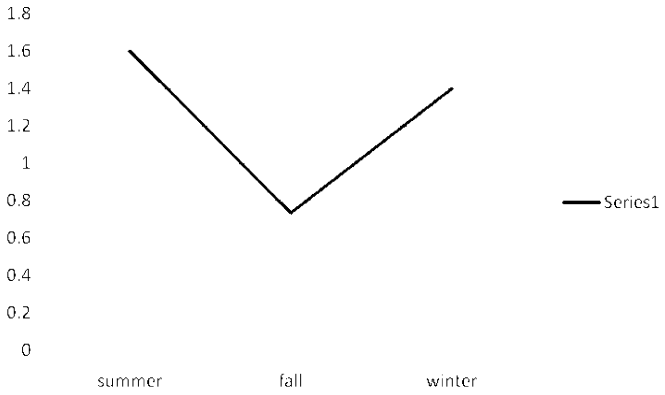

Fig. 3: The average number of bacteria in the season

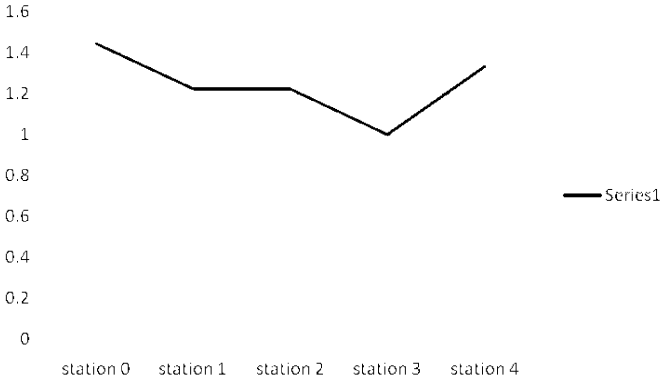

Fig. 4: The average number of bacteria in the stations

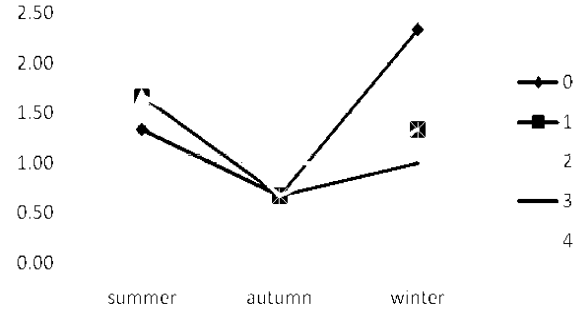

Fig. 5: The average number of bacteria in each station seasons

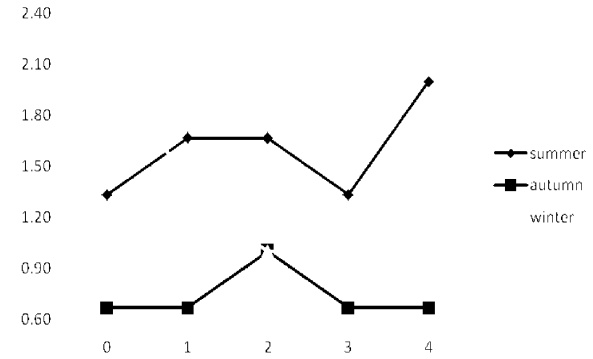

Fig. 6: The effect of seasons in the mean number of bacteria in each station

autumn. Figure 6 presents the effect of seasons on mean number of bacteria in the stations so that during summer there has been the highest and during winter the lowest average number of bacteria. During autumn, field station has had highest mean while other station had the same mean of bacteria.

DISCUSSION

Previous studies have been reported that some soil microorganism can degrade organophosphate insecticides and use them as sources of carbon and phosphorus [13-15]. Among the microorganism; *Pseudomonas aeruginosa*, *Pseudomonas fluorescencecna*, *citrobacter*, *Serattia* and *Salmonella* have been found that could degrade organophosphate insecticides [3, 16, 17].

According to previous studies, diazinon degrading bacteria have been distributed in the soils and water. They produce numerous enzymes, which can degrade chemicals such as insecticides. Bacteria that have enzymes OPAA (Organophosphateacidanhyderolase) can break the bond between O-P of diazinon and degrade it and use it as source of phosphorus and carbon [16, 18]. In this study, some microorganisms that were capable of using insecticide diazinon as a sole source of carbon, phosphorus and energy were isolated. Using Morphology and physiology o f microorganisms and biochemical tests the bacteria of *Pseudomonas aeruginosa*, *serattia*, *salmonella*, *Citrobacter*, *Alcaligenes*, *Acinetobacter*, *bacterium Corynebacterium*, *Staphylococcus*, *Bacillus* and *Providencia* were identified.

These results are in agreement with previous researched about ability of some soil microorganism like; fungi, *Alcaligenes*, *Acinetobacter*, *Corynebacterium*, *Bacillus Subtillis*; *Staphylococcus*; *Agrobacterium*; *Providencia* to degrade diazinon [10, 13, 19, 20]. Further studies are needed to determine whether other bacteria have the ability to degrade diazinon or not. The results of this study indicated that bacteria degrading diazinon have wide distribution in nature, especially in water and here in the Lake Parishan. As a conclusion, elimination of insecticide diazinon by bacteria has value of considering because of its advantage of being rapid and economic benefit.

ACKNOWLEDGEMENT

We wish to thank all colleagues for their help and cooperation in conducting of this study. We are also grateful to professor Nor-Aini Ab-Shukor from university Putra Malaysia for her help in editing and very helpful advice.

REFERENCES

1. Magara, Y., T. Aizawa, N. Matumoto and F. Souna, 1994. Degradation of pesticides by chlorination during water purification. Water Science and Technology, 30(7): 119-128.
2. Kafilzadeh, F., 2005. Study of heavy metals basin in river kor, Research project, Azad University, Arsanjan Branch, Iran.
3. Najafi, Y.F., 2002. Isolation and Identification Degradation bacteria of Diazinon. Department Of Biology, Faculty of science Guilan University Rasht, pp: 1-9.
4. Sherman, J.D., 1995. Organophosphate pesticides-neurological and respiratory toxicity. Toxicology and Industrial Health, 11(1): 33-99.
5. Zhang, Y.R.L., J.S. Autenrieth, S.P. Bonner, J.R. Harvey and J.R. Wild, 1999. Biodegradation of neutralized Sarin. Biotechnology and Bioengineering, 64(2): 221-231.
6. FAO/WHO., 1993. Pesticide residues in food Diazinon.In Joint/WHO Meeting on Pesticide Residues, pp: 57-81.
7. Gisi, D., G. Stucki and K.W. Hanselmann, 1997. Biodegradation of the pesticide 4, 6-dinitro-ortho-cresol by microorganisms in batch cultures and in fixed-bed column reactors. Applied Microbiology and Biotechnol., 48(4): 441-448.
8. Topp, E., H. Zhu, S.M. Nour, S. Houot, M. Lewis and D. Cuppels, 2000. Characterization of an atrazine-degrading Pseudaminobacter sp. isolated from Canadian and French agricultural soils. Applied and Environmental Microbiol., 66(7): 2773-2782.
9. Singh, B.K. and A. Walker, 2006. Microbial degradation of organophosphorus compounds. FEMS Microbiology Reviews, 30(3): 428-471.
10. Cycon, M., M. Wójcik, and Z. Piotrowska-Seget, 2009. Biodegradation of the organophosphorus insecticide diazinon by Serratia sp. and Pseudomonas sp. and their use in bioremediation of contaminated soil. Chemosphere, 76(4): 494-501.
11. Timmis, K.N., P.R. Lehrbach, S. Harayama, R.H. Don, H. Mermond, J. Bas, R. Leppik, A.J. Weightman, W. Reineke and H.J. Knackmuss, 1985. Analysis and manipulation of plasmid encoded pathways for the catabolism of aromatic compounds by soil bacteria. In: Plasmids in bacteria. Plenum press, New York, pp: 719-739.

12. Drufovka, K., T. Danevcic, P. Trebse and D. Stopar, 2008. Microorganisms trigger chemical degradation of diazinon. International Biodeterioration & Biodegradation, 62(3): 293-296.
13. Çelik, S.K. and T. Aan, 1995. Degradation of some pesticides in the field and effect of processing. The Analyst, 120(6): 1739-1743.
14. Najafi, A. and M. Rajabzadeh, 2002. Monitoring of the Pesticide Diazinon in Soil, Stem and Surface Water of Rice Fields. Analytical Sci., 18(7): 1-5.
15. Zhang, R., Z. Cui, J. Jiang, J. He, X. Gu, and S. Li, 2005. Diversity of organophosphorus pesticide-degrading bacteria in a polluted soil and conservation of their organophosphorus hydrolase genes. Canadian J. Microbiol., 51(4): 337-343.
16. Horne, I., T.D. Sutherland, J.G. Oakeshott and R.J. Russell, 2002. Cloning and expression of the phosphotriesterase gene hocA from Pseudomonas monteilii C11. Microbiol., 148(9): 2687-2695.
17. Singh, B.K., A. Walker, J.A.W. Morgan and D.J. Wright, 2004. Biodegradation of Chlorpyrifos by Enterobacter Strain B-14 and its use in bioremadation of contaminated soils. Applied and Environmental Microbiol., 70(8): 4855-4863.
18. Karpouzas, D.G. and B.K. Singh, 2006. Microbial degradation of organophosphorus xenobiotics: metabolic pathways and molecular basis. Advances in Microbial Physiol., 51: 119-225.
19. Fenlon, K.A., K.C. Jones and K.T. Semple, 2007. Development of microbial degradation of cypermethrin and diazinon in organically and conventionally managed soils. J. Environmental Monitoring, 9(6): 510-515.
20. Seo, J.S., Y.S. Keum, R.M. Harada and Q.X. Li, 2007. Isolation and characterization of bacteria capable of degrading polycyclic aromatic hydrocarbons (PAHs) and organophosphorus pesticides from PAH-contaminated soil in Hilo, Hawaii. J. Agric. Food Chemistry, 55(14): 5383-5389.